Food Science

An Applied Approach

Maci Bale

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Contents

Chapter 1

Effects of Radiation Technologies on Food Nutritional Quality

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Abstract

Generally, foods are thermally processed to destroy the vegetative microorganisms for food preservation. However, only thermal treatment triggers many unwanted biochemical reactions, which leads to undesirable sensorial and nutritional effects. Therefore, a number of nontraditional preservation techniques are being developed to satisfy consumer demand with regard to nutritional and sensory aspects of foods. Ensuring food safety and at the same time meeting such demands for retention of nutrition and quality attributes has resulted in increased interest in emerging preservation techniques. These emerging food preservation technologies can extend the shelf life of unprocessed or processed foods by inactivating the enzymes, reducing the food spoiling microbial growth rate or viability without altering the food quality attributes including flavor, odor, color, texture, and nutritional value. On the emerging technology, sphere promising results have been attained by the radiation processes. Ionizing radiation has been widely used in industrial processes, especially in the sterilization of medicals, pharmaceuticals, cosmetic products, and in food processing. Nonionizing radiation has been used in surface decontamination, as on the packaging sector. Although radiation techniques, as the other traditional or emerging techniques, can impair alterations that can modify the chemical composition and the nutritional value of foods, these changes depend on the food composition, the irradiation dose and factors such as temperature and presence or absence of oxygen in the irradiating environment. The aim of this chapter is to discuss retention or loss of vitamins and minerals in several food products submitted to a radiation process (gamma irradiation, short wave ultraviolet, microwave, and pulsed electric fields).

Keywords: nutritional, nonionizing radiation, ionizing radiation, micronutrient, macronutrient, emerging technologies

1. Introduction

The first food preservation methods were developed by men in the beginning of its existence. Since ancient times, radiation has been used as a preserve food method, when used to preserve meats, fish, fruits, and vegetables from solar energy. In the drying method, the men realized that some fruits and vegetables were still edible and the meat lasted longer without spoiling, once this process consists in removal of part of the water from the food to a final concentration. The microorganisms present in food need water for their vital functions, and by reducing the water content, it is possible to decrease considerably the microbiological growth in the food [1].

The growing demand from the consumers for high quality products reveals the need to apply of new food preservation techniques that provide microbiological safety in the production, increasing the commercial validity, and still provide minimal biochemical alterations, promoting the maintenance of the nutritional and sensorial quality of the products [2].

Some of the currently proposed methods inhibit microbial growth, even in some cases, they cause enzyme inactivation, without the considerable increase of the product temperature. Moreover, these processes promote little damage to pigments, flavor compounds, and vitamins; and in contrast to conventional processes that apply high temperatures, the original sensory and nutritional characteristics of the food are maintained, with no significant loss of fresh product quality [3].

Throughout the centuries, the food preservation techniques have been improving with the increase of scientific development. These methods include freezing, drying, canning, pickling preparation, pasteurization, fermentation, cooling, controlled atmosphere storage, and application of preservatives, and among the emerging technologies, the highlight is the radiation technique [4].

The terminology radiation refers to the physical processes of emission and propagation of energy, either by means of wave phenomena, or by the kinetic energy from the particles. In a simpler way, radiation is energy that spreads from one point to another in space or in a material environment. Irradiation is the process of applying this energy to a material, such as food, with the purpose of sterilizing or preserving them through the destruction of microorganisms, parasites, insects, and other pests [5, 6].

There are various forms of radiant energy emitted in various ways that belong to the electromagnetic radiation spectrum, and these include radio waves, microwaves, infrared radiation, visible light, ultraviolet, X-rays, and gamma rays. These forms of energy differ in the wavelength, frequency, force of penetration, and other effects that they exert on biological systems. As can be observed in **Figure**, in general, it can be said that in the electromagnetic spectrum, there are two types of radiation: ionizing and nonionizing [7].

Of all the bands of the electromagnetic spectrum, the best known is the narrow band of visible light, shown in the expanded form in the **Figure**. The human brain recognizes these wavelengths as the different colors ranging from violet to red. The sunlight, as the human being is able to see with the naked eye, is the sum of multiple electromagnetic waves with different

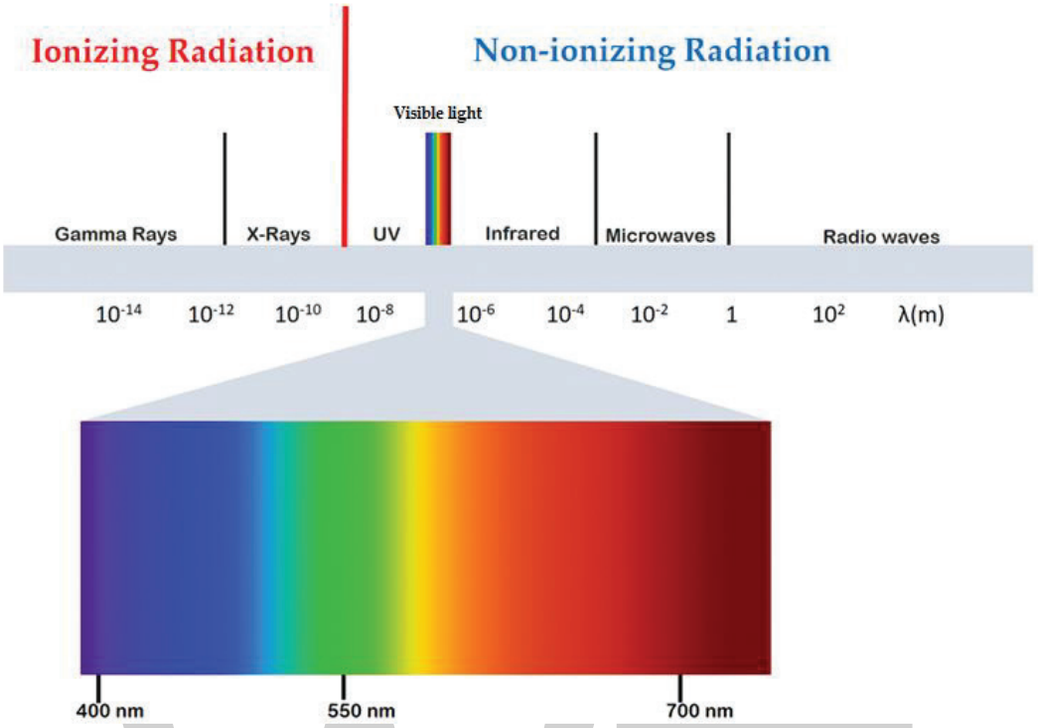

Figure. Electromagnetic spectrum.

wavelengths in the visible range. Sir Isaac Newton was the first to realize this fact in the late 1660s by passing the sunlight into a rainbow using a prism and turning it back into white light with a second prism [8].

2. Nonionizing radiation

In search for new and better food preservation methods, the research has turned to the possibility of using radiations in different ranges of frequencies, such as nonionizing radiations. This type of radiation does not have sufficient energy to break molecular bonds or remove electrons from atoms; in other words, perform ionization. The types of frequencies characterized as nonionizing are ultraviolet, infrared, radiofrequency, laser, microwave, and visible light [9, 10]. However, the use of nonionizing radiation has low interest as a food preservation method, in contrast to ionizing radiations [7].

2.1. Microwave radiation

Electromagnetic energy in the microwave region (300 MHz–300 GHz) has been extensively studied as an alternative energy source for sterilization. Microwave radiation has enormous applications in our daily routine, from communication systems, medical applications, to the level of industrial application [11, 12].

Microwave radiation does not carry enough energy to ionize food molecules or to generate free radicals [13]. The basic principle of the process is the baking by molecular vibration that

penetrates superficially in the foods, and the depth of penetration ranges from 2 to 4 cm. The process makes the food vibrates and consequently heating the water, fat, and sugar molecules. However, microwave heating leads to uneven distribution of the temperature in the product. In addition, the products do not show nonenzymatic darkening and surface crust formation, which might be advantageous in some applications [10].

The use of microwave energy has been reported in the literature as a method used for improving microbiological quality and extending shelf life. The use of microwave irradiation resulted in the conclusion that microbial cell death occurs due to the heat produced by the irradiation and due to the electric field created by the microwaves, which promotes a change in the secondary and tertiary structure of the microorganism proteins [12, 14]. Currently, microwave energy has been applied in the pharmaceutical industry, and in drying, thawing and sterilization processes in the food industry.

Despite the limitations of this technology, the application of microwave energy has advantages in the decontamination of utensils and in the process time, which entails the reduction of energy costs and also eliminates the chemicals commonly used in some of the conventional methods. All these reasons make this technology economically and environmentally viable, to be implemented in industrial systems, provided that its limitations are reviewed, such as the lack of uniformity in the distribution of heating in systems without temperature and power control [14, 15].

2.2. Ultraviolet radiation

Ultraviolet (UV) radiation was discovered in 1801 by the German scientist Johan Ritter, who perceived an invisible form of light beyond violet capable of oxidizing silver halides, which was named ultraviolet light at the end of the nineteenth century [16]. The UV-light occupies a wide wavelength range in the nonionizing region of the electromagnetic spectrum, between X-rays (100 nm) and visible light (400 nm) [17]. The wavelength of UV-light is divided into three bands—UV-A, UV-B, and UV-C.

The long (UV-A, 400–320 nm) and middle (UV-B, 320–280 nm) wavelengths are present in sunlight and have some germicidal value. However, the short wavelengths or UV-C (280–100 nm) have high germicidal capacity and do not naturally exist, having to be produced by the conversion of electric energy [18].

The UV-light can be applied as a continuous or pulsed mode. The continuous mode is the conventional method, the light being emitted continuously without interruption. In pulsed UV-light mode, the UV-light is released as intermittent pulses using a capacitor, which allow to increase the energy intensity per pulse. Therefore, the pulsed mode is more effective for microbiological inactivation and the most used method [19].

The use of UV-light as a nonchemical disinfection method is increasingly gaining acceptance by its germicidal power. The equipment used in the UV method has a low maintenance cost, besides being considered an “eco-friendly” technology that eliminates the need to use many chemical treatments, while is able to guarantee high levels of security [20].

UV-light is a physical process that offers several advantages, such as it does not produce by-products that could change the characteristics of the food, does not generate chemical residues, and it is a cold process, dry, simple, effective, and low cost in relation to other sterilization processes, besides not generating any type of ionizing radiation [21].

UV-light has been applied in water purification and has been used in beverage industries; in industries, it has been applied in final cleaning, in the rinsing water of CIP (Clean-in-Place) systems and in the disinfection of packaging surfaces and storage tanks [20].

2.3. Pulsed electric field

The pulsed electric field (PEF) technology, also known as high-intensity electric field pulses (HELP), refers to the application of high voltage pulses (20–80 kV cm^{-1}) in a product situated between two electrodes [22]. The inactivation of pathogenic and spoilage bacteria, yeasts, and some enzymes related to food quality have been demonstrated by several authors, although

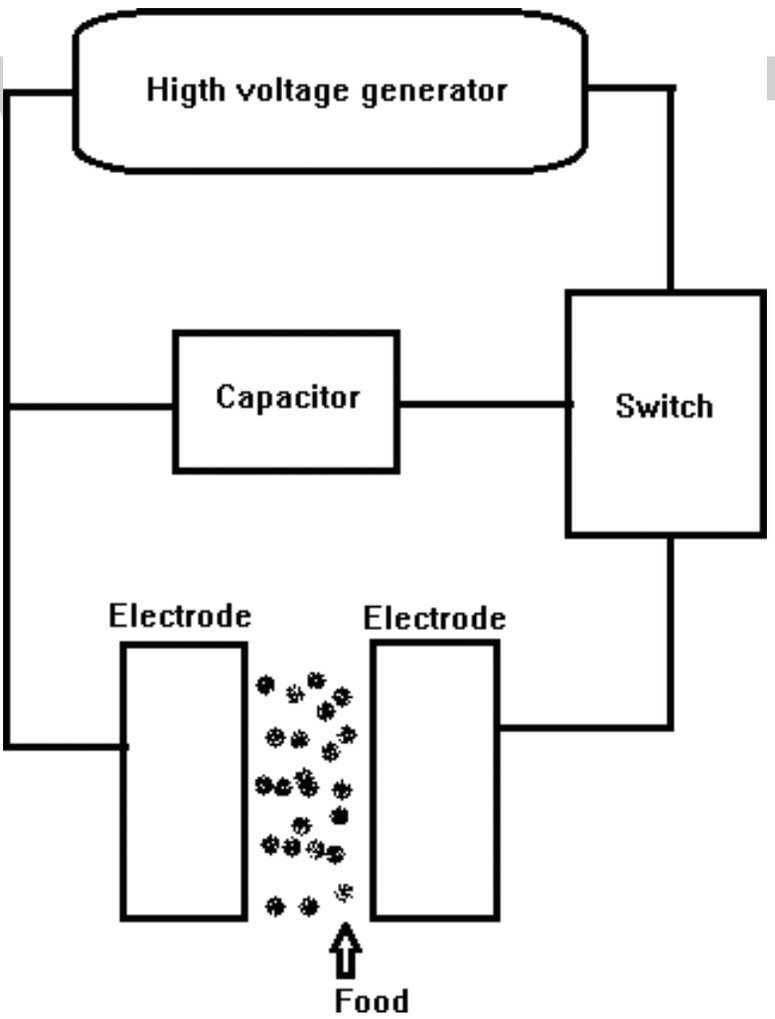

Figure. Outline of pulsed electric field.

bacterial spore is not eliminated through this treatment [23, 24]. Regarding to this limitation, other methods are used in combination with irradiation technologies for the elimination of bacterial spores.

The technology consists of subjecting the product to repeated high-intensity fields with short-time electrical pulses, (ms or μs), with the purpose of causing the inactivation of enzymes and destruction of microorganisms [25]. The outline of pulsed electric field technology is shown in **Figure**.

Regarding food quality attributes, PEF is considered superior to conventional heat treatment because it avoids or reduces changes in sensory and physicochemical properties [26]. Many studies have reported the advantage of the application of PEF for the conservation of the micronutrient content of foods, once that is expect the thermolabile vitamins to be conserved, since the electric pulse method does not constitute a heat treatment. There are several factors that influence the results from the pulsed electric field on the microorganisms. These factors are related with the parameters of the process (time, field intensity, temperature, and number of pulses), to the characteristics of the product, and the characteristics of the microorganisms present in the product [27].

The application of PEF for food preservation has shown to be a promising alternative to traditional thermal methods. Although there are not products processed by pulsed electric fields on the market, the great number of works related to such technology and its advantages claim to believe that soon the market will experience the emergence of this and other nonthermal food technologies [28, 29].

3. Ionizing radiation

Nowadays, the types of radiations commonly used in the treatment of foods are the ionizing radiations. Ionizing radiation is classified as ionizing because its energy is high enough to displace electrons from atoms and molecules and to convert them into electrical charges, called ions [5].

Ionizing radiation is transmitted by high-energy particles (alpha, protons, electrons, and neutrons) or electromagnetic waves (X-rays and gamma rays). Only electromagnetic radiation (X-rays and gamma rays) and electron-beam radiation are the two forms of ionizing radiations applied on food among the various existing forms of ionizing radiation. While the propagation of electromagnetic radiation is in the form of waves, the radiation by emission of electrons (corpuscular radiation) is based on the transmission of energy by the acceleration of the electrons [30, 31].

The irradiation of food, physical method of treatment, is based on submit of the product, already packaged or in bulk, to controlled doses of ionizing radiation for sanitary, phytosanitary and/or technological purposes [30].

3.1. Gamma radiation

The frequency of the gamma rays is above 1019 Hz, which implies in wavelengths below 10–12 m. Radiation equipment is composed of one high energy radiation source (isotope

source) to produce gamma rays, or less frequently, by an equipment which emits high electron-beam energy [32, 33]. Gamma radiation is produced from the excited nucleus of radioactive elements, such as Cobalt 60 (^{60}Co) and Cesium 137 (^{137}Cs) [34, 35].

The process of irradiation by gamma rays has several advantages: low heat generation, low energy requirements, irradiation of packaged or frozen products, and cause changes in the food nutritional value similar or inferior to other methods of conservation [36]. Nevertheless, as disadvantages of the gamma radiation are the high initial cost and difficulty in establishing the necessary doses, because certain doses can cause the death of some insects, however, others still can be found alive [37]. Despite all the benefits, a number of barriers still persist and prevent irradiated foods from being widely marketed, mainly related to the cost of their use and the resistance from the consumers due to a lack of information on food induced radioactivity [38].

In order to ensure the quality of a food, from the microbiological point of view and the nutritional quality, several methods are available for the food industries, including the use of radiation. In view of the requirements of consumers for assurance of microbiological safety and maintaining the natural characteristics of the product, the present chapter focuses on nutritional variation in food products submitted to radiation processes, such as gamma irradiation, UV-C, microwave, and pulsed electric fields.

4. Effects of radiation on food nutritional composition

The radiation techniques have been widely studied and, like most food processing techniques, can induce some changes that are able to modify the chemical and nutritional characteristics on foods. These changes are dependent on some factors such as the radiation dose, the constitution of the irradiated food, the type of packaging, and how it was processed, besides the variables of the process as temperature and oxygen saturation on the atmospheric [39].

Activists and consumers have been questioned about the nutritional value of foods submitted to these processing techniques. The main questions and hypotheses about the use of irradiation are related to the excessive denaturation of nutrients, the conception that there is generation of toxic substances in irradiated foods, and they became radioactive [39, 40]. However, research conducted since the 1950s does not confirm these hypotheses of radioactivity in food, demonstrating there is no induction [21, 41]. Thus, the radiation methods used in food are considered safe and effective, according to several agencies such as the Food and Drug Administration (FDA) and the United Nations (UN) [42, 43].

Among the advantages cited by studies related to food irradiation are the minimal changes caused in food constituents [44]. According to research, macronutrients—carbohydrates, proteins, and lipids—are relatively stable when they come in contact with doses of ionizing radiation up to 10 kGy [39, 45].

Regarding to micronutrients, in particular vitamins are perceived since they are sensitive to any processing technique. Thus, vitamins are unstable in food and dependent on specific

parameters such as the cooking time or time of exposure to the method, pH, temperature, and among others. The water-soluble vitamins are labile to any process technique, and the fat-soluble vitamins are destroyed by the radiation methods [46–48].

4.1. Macronutrients

When food is irradiated, its components may be subject to significant changes. Among the existing macronutrients, the carbohydrates are less sensitive to radiation. Some of the glycosidic bonds linking the monosaccharides break when exposed to radiation, reducing the degree of polymerization and subsequently increasing the viscosity of polysaccharide solutions.

Regarding proteins, the amino acid chains can be altered in the presence of water, due to electron transfer, and might accelerate the denaturation protein process by altering the secondary and tertiary structures before destroying the amino acid chains. Nevertheless, the denaturation is less intense than in a thermal processing. In the case of lipids, in the presence of oxygen, radiation processes can accelerate oxidation, and other reactions such as the production of free radicals, the formation of hydrogen peroxides, and the destruction of compounds as carboxylic acids and antioxidants. Thus, radiation processes are not recommended for products with a high fat content [49, 50].

The effects of UV-C on the composition of macronutrients in eggs were evaluated, demonstrating that cholesterol is oxidized to cholesterol oxides in contact with UV-C light, presenting reduction in doses higher than 5.910 J cm^{-2}. In addition, UV-C light seems to be able to generate vitamin D3 (cholecalciferol), from the precursor 7-dehydrocholesterol. However, further testing may be required to identify oxidation products and to evaluate the production of vitamin D3 [51]. Unlike the results found for UV-C light applied in eggs, in pasteurization, cholesterol levels remained almost unchanged in the temperature range of 110–120°C, and less than 10% was oxidized after 80 hours of heating, corroborating with the fact that cholesterol is quite resistant to heat up to that temperature. However, cholesterol levels are extensively oxidized by exceeding the temperature range [52]. In relation to the protein content, it was observed that in the presence of oxygen, the total amount of protein decreased, occurring an aggregation of the proteins, and after the UV-C treatment, this situation indicates that some sulfhydryl groups were oxidized to the formation of new disulfide bonds [51].

Analyzing the effects of UV-C light on cow's milk and comparing the results with the pasteurization treatments, it is possible to visualize there was no change in fat concentration. On the protein content, there were no significant changes when compared to the samples treated with ultraviolet against the raw milk [53]. Therefore, it is suggested that ultraviolet radiation is an alternative to traditional heat treatments, for better preserving the nutrients, unlike pasteurization that causes nutrient loss. The effects of gamma radiation on milk proteins demonstrate that solubility decreases, probably due to the denaturation and agglomeration of proteins [54].

A study was conducted on the effects of agricultural production systems (organic and conventional), UV-C radiation and different types of drying (greenhouse and lyophilization) in grape waste and flour formulated from the wastes. The results showed, regardless of the

method, there was no interference in the fiber contents, but it was observed that the samples that were produced by the method of organic cultivation with application of UV-C light showed a higher protein content [55].

The effects of different thermal treatment methods were conducted in beef and chicken burgers cooked in microwave, conventional oven, and fry in oil. Samples of the two types of burgers submitted to microwave process obtained the highest loss of moisture, weight, and greater degree of retraction. In the chicken burger, the loss of moisture resulted in higher percentages of fat, protein, and ashes. In the beef hamburger, the highest percentage of proteins and ashes were observed in the microwave treatment, while the highest percentage of lipids was found in the oil frying treatment [56].

Studies on the application of UV-C light to tilapia fillets demonstrated that this type of treatment was not able to increase lipid oxidation due to the low fat content in this type of food. Regarding the protein content, UV-C light provided an increase in the carbonyl content with changes directly related to the doses of applied radiation, presenting a pro-oxidant action [57]. Action that can be explained by the capacity of stimulus of generation and reactivity of oxygen species, causing the protein oxidation, raises carbonyl formation [51]. In the literature, the oxidative effect of proteins and lipids has a direct connection and might vary according to the protein and lipid composition and pro-oxidant and antioxidant compounds of a food [58, 59, 60, 61]. Therefore, these chemical modifications promoted by UV-C light depend on the nutritional composition and the dose to be used [62].

4.2. Micronutrients

At commercial doses, gamma irradiation causes no higher nutrient loss than the other methods used in food processing. The destruction of vitamins from the use of ionizing radiation applying doses of up to 60 kGy does not differ from the degradation generated from the cooking process [63]. The degradation of vitamins presents varied sensitivity to gamma radiation, since this level depends on the doses used and the state of matter from the food analyzed. The fat-soluble vitamins in descending order of sensitivity to gamma radiation are Vitamin E > Vitamin A > Vitamin D > Vitamin K. While the order of sensitivity of water-soluble vitamins is thiamine > ascorbic acid > pyridoxine > riboflavin > cobalamin > nicotinic acid [50, 64]. However, studies related to the effects of radiation on the content of vitamins are still inconclusive. In order to minimize nutrition losses and negative effects on sensory quality, some factors are applied, such as the use of low doses (less than 10 kGy) of gamma radiation and controlled conditions of process such as temperature and presence of oxygen [49, 50].

Among the nonthermal technologies used in food, UV-light treatment presents great commercial potential as an alternative for thermal treatments in liquid foods, such as pasteurization. However, as well the other methods used in food preservation, UV-light causes changes in the vitamin content. UV-light treatment has been shown to be able to significantly alter vitamin content in milk samples when compared to the traditional pasteurizing process. The vitamins present in the cow's milk in descending order of sensitivity are C > E > A > B2. However, the number of sample passes through the UV-C system and the initial vitamin concentration in a sample are important factors to affect the level of loss in vitamin content [65].

The minerals present in foods (i.e., iron, phosphorus, and calcium) are not affected by technologies that employ radiation [42]. No more data were reported in the literature relating the effects of irradiation on vitamins and minerals, and more studies are needed.

4.3. Antioxidant activity

The relationship between the treatments that use radiation and the antioxidant content of foods is still inconclusive. Several studies discuss that radiation treatments have demonstrated the decrease or increase in antioxidant activity of foods, and these variations are related to the type of treatment, the dose of radiation used, the exposure time, and the composition and state of matter of the raw material [66].

With positive effect, it is proven that the ultraviolet radiation, when applied in appropriate conditions, is able to increase the antioxidant capacity of fruits and vegetables, being a technology with commercial potential to be used in post-harvest stages in order to extend shelf life and increase quality, such as papaya, grape, and pineapple [67, 68]. Supposedly, UV-C radiation is able to increase phenolic content and antioxidant enzymatic activities as a defense mechanism against oxidative stress, thus increasing the antioxidant activity of vegetables [67, 69, 70].

In commercial doses, it is not possible to obtain conclusive answers regarding the effect of gamma radiation on the content of the antioxidant activity in foods. However, most of the studies mention gamma radiation as a conservation method related to the increase or maintenance to the potential of the antioxidant activity of foods such as, soybean, olive, starch, and safflower powder [66, 71–74]. However, it is possible to find studies that report the reduction of antioxidant activity when the radiation process is mediated with another parameter, as changes in moisture and application of different doses of gamma radiation in mung bean [75].

4.4. Food allergens

Food processing methods using radiation, ionizing, and nonionizing can be used to reduce the potential of food allergenicity. The UV-light, gamma irradiation, and ultrasound have the capacity to reduce the allergenicity of food proteins from the formation of protein aggregation, protein crosslinking, and/or amino acid sequence alteration. Studies carried out with the application of gamma radiation on cow's milk proteins have demonstrated the reduction of the allergenicity and antigenicity of the proteins when submitted to the irradiation process [54]. However, to date, the processes that involve radiation are not capable of completely inactivating the allergenic compounds [76].

5. Conclusions

The technologies that involve radiation are pointed out as promising methods to be used in food preservation. Some methods used cause the elimination or decrease the growth of microorganisms, without significant nutritional losses. When applied in a controlled manner, the radiation processes promote equal or inferior damages in the nutritional quality of the food when compared to the conventional processes. At commercial doses of up to 10 kGy,

macronutrients and minerals are stable against radiation techniques. Vitamins have a higher degree of sensitivity than macronutrients when exposed to treatments that use some type of radiation. However, nutrient degradation can be reduced by using lower radiation doses and using an oxygen saturated atmosphere.

Acronyms and abbreviations

CIP	clean-in-place
FDA	Food and Drug Administration
HELP	technology, also known as high-intensity electric field pulses
PEF	pulsed electric field
UN	United Nations
UV	ultraviolet

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References

[1] Tezotto-Uliana JV, Silva PPM, Kluge RA, Spoto MHF. Radiação gama em produtos de origem vegetal. Revista Virtual de Química. 2015;**7**:267-277. DOI: 10.5935/1984-6835.20150013

[2] Leinstner L, Gorris LGM. Food preservation by hurdle technology. Trends in Food Science & Technology. 1995;**6**:41-45. DOI: 10.1016/S0924-2244(00)88941-4

[3] Vega-Mercado H et al. Non-thermal food preservation: Pulsed electric fields. Trends in Food Science & Technology. 1997;**8**:151-157. DOI: 10.1016/S0924-2244(97)01016-9

[4] Polizel GG. O uso da radiação no controle microbiológico dos alimentos de origem animal [monografia]. Rio de Janeiro: Universidade Castelo Branco; 2006

[5] Franco BDGM, Landgraf M. Microbiologia dos Alimentos. São Paulo: Atheneu; 1996. p. 182

[6] Spolaore AJG, Germano PML, Germano MIS. Irradiação de Alimentos. In: Germano PML, Germano MIS, editors. Higiene e Vigilância Sanitária de Alimentos. 2nd ed. São Paulo: Livraria Varela; 2001. pp. 421-442

[7] Gava A. Princípios de Tecnologia de Alimentos. 1st ed. São Paulo: Nobel; 1984. p. 284

[8] Silva AVR. Nossa estrela: o sol. 1st ed. São Paulo: Editora Livraria da Física; 2006. p. 176. ISBN-13: 9788588325654

[9] Freitas TP, Mendez NRM. Radiações Não Ionizantes: Causas, Efeitos e Prevenções [Internet]. 2005. Disponível em: http://www.unirevista.unisinos.br. [Acessado em: 20-05-2018]

[10] Ordoñez JA. Tecnologia de Alimentos. 1st ed. Porto Alegre: Artmed; 2005. p. 294

[11] Paulraj R, Behari J. Single strand DNA breaks in rat brain cells exposed to microwave radiation. Mutation Research. 2006;**596**(1-2):76-80

[12] Banik S, Bandyopadhyay S, Ganguly S. Bioeffects of microwave: A brief review. Bioresource Technology. 2003;**87**:155-159. DOI: 10.1016/S0960-8524(02)00169-4

[13] Lassen A, Ovesen L. Nutritional effects of microwave cooking. Nutrition & Food Science. 1995;**95**:8-10. DOI: 10.1108/00346659510088654

[14] Aymerich T, Picouet PA, Monfort JM. Decontamination technologies for meat products. Meat Science. 2008;**78**:114-129. DOI: 10.1016/j.meatsci.2007.07.007

[15] Bélanger JM et al. Remarks on various applications of microwave energy. Journal of Microwave Power and Electromagnetic Energy. 2008;**42**:24-44. DOI: 10.1080/08327823.2007.11688597

[16] BALL DW. The electromagnetic spectrum: A history. Spectroscopy. 2007;**3**:14-17

[17] Bintsis T, Litopoulou-tzanetaki E, Robinson R. Existing and potential applications of ultraviolet light in the food industry—A critical review. Journal of the Science of Food and Agriculture. 2000;**80**:637-645. DOI: 10.1002/(SICI)1097-0010(20000501)80:6<637::AID-JSFA603>3.0.CO;2-1

[18] Souza SRP, Fischer FM, Souza JMP. Suntanning and risk of cutaneous melanoma: A literature review. Revista de Saúde Pública. 2004;**38**:588-598. DOI: 10.1590/S0034-89102004000400018

[19] Krishnamurthy K, Demirci A, Irudayaraj JM. Staphylococcus aureus inactivation using pulsed UV light for continuous milk treatment. In: ASAE Annual Meeting; 17-20 July 2005; Florida. pp. 1-11. DOI: 10.13031/2013.19590

[20] Howarth C. UV Disinfection Shows Promise [Internet]. 2007. Disponível em: http://www.hanovia.com. [Acessado em: 17-05-2018]

[21] Guerrero-Beltrán JA, Barbosa-Cánovas GV. Review: Advantages and limitations on processing foods by UV light. Food Science and Technology International. 2004;**3**:137-147. DOI: 10.1177/1082013204044359

[22] Góngora-Nieto MM, Sepúlveda DR, Pedrow P, Barbosacánovas GV, Swanson BG. Food processing by pulsed electric fields: Treatment delivery, inactivation level, and regulatory

aspects. Lebensmittel-Wissenschaft & Technologie. 2002;**35**:375-388. DOI: 10.1006/fstl.2001.0880

[23] Álvarez I, Pagán R, Condón S, Raso J. The influence of process parameters for the inactivation of Listeria monocytogenes by pulsed electric fields. International Journal of Food Microbiology. 2003;**87**:87-95. DOI: 10.1016/S0168-1605(03)00056-4

[24] Damar S, Bozoglu F, Hizal M, Bayindirli A. Inactivation and injury of *Escherichia coli* O157:H7 and *Staphylococcus aureus* by pulsed electric fields. World Journal of Microbiology and Biotechnology. 2002;**18**:1-6

[25] Soliva-Fortuny R et al. Effects of pulsed electric fields on bioactive compounds in foods: A review. Trends in Food Science & Technology. 2009;**20**:544-556. DOI: 10.1016/j.tifs.2009.07.003

[26] Qin BL, Pothakamury UR, Vega H, Martin O, Barbosacanovas GV, Swanson BG. Food pasteurization using high intensity pulsed electric fields. Journal of Food Technology. 1995;**49**:55-60

[27] Binoti ML et al. Pulsed electric field. Ciência Rural. 2012;**42**. DOI: 10.1590/S0103-84782012005000025

[28] Binoti ML, Ramos AM. Conservação de alimentos: Uma visão mais saudável. HU Revista. 2015;**41**:171-179

[29] Cortés C, Esteve MJ, Frigola A. Color of orange juice treated by high intensity pulsed electric fields during refrigerated storage and comparison with pasteurized juice. Food Control. 2008;**19**:151-158. DOI: 10.1016/j.foodcont.2007.03.001

[30] Agência Nacional de Vigilância Sanitária (ANVISA). RDC n° 21, de 26 de janeiro de 2001. Aprova o Regulamento técnico para irradiação de alimentos. Diário Oficial [da] República Federativa do Brasil, Brasília, DF. [Internet]. 26 Jan, 2001. Disponível em: http://www.anvisa.gov.br/legis/resol/21_01rdc.htm. [Acesso em: 12-06-2018]

[31] Grandison AS. Irradiation. In: Brennan JG, editor. Food Processing Handbook. Weinheim: Wiley-VCH Verlag; 2006. pp. 147-171. ISBN: 3-527-30719-2

[32] Lima LS. Radiação gama. Revista de Ciência Elementar. 2014;**2**:1-2

[33] Jay JM. Microbiologia de Alimentos. 6th ed. Porto Alegre: Artmed; 2005. p. 711. ISBN: 9788536305073

[34] Barros EA. Produção de bebida mista de extrato hidrossolúvel de soja e suco de uva submetida a diferentes doses radiação gama [tese]. Botucatu: Universidade Estadual Paulista, Botucatu; 2016

[35] Franco BDGM, Landgraf M. Microbiologia dos alimentos. São Paulo: Atheneu; 2008. p. 182

[36] Fellows PJ. Tecnologia do Processamento de Alimentos–Princípios e práticas. Porto Alegre: Artmed; 2006. p. 602. ISBN 978-85-363-0652-0

[37] Hallman GJ. Ionizing gradiation quarantine treatments against tephritic fruit flies. Postharvest Biology and Technology. 1999;**16**:93-106. DOI: 10.1016/S0925-5214(99)00012-5

[38] Ornellas CBD, Gonçalves MPJ, Silva PR, Martins RT. Atitudes do consumidor frente à irradiação de alimentos. Ciência e Tecnologia de Alimentos. 2006;**26**:211-213. DOI: 10.1590/S0101-20612006000100033

[39] Dionísio AP, Gomes RT, Oetterer M. Ionizing radiation effects on food vitamins: A review. Brazilian Archives of Biology and Technology. 2009;**52**:1267-1278. DOI: 10.1590/S1516-89132009000500026

[40] Guedes AMM, Novello D, Mendes GMP, Cristianini M. Tecnologia de ultravioleta para preservação de alimentos. Boletim do Centro de Pesquisa de Processamento de Alimentos. 2009;**27**:59-70

[41] Fan X, Sokorai KJB. Effect of ionizing radiation on furan formation in fresh fruits and vegetables. Journal of Food Science. 2008;**73**:79-83. DOI: 10.1111/j.1750-3841.2007.00622.x

[42] Food and Drug Administration. Code of Federal Regulations Part 179. Irradiation in the production, processing and handling of food. Federal Register. 2012;**77**:71316

[43] World Health Organization. Safety and Nutritional Adequacy of Irradiated Food; 1994

[44] Brendan AN, Meixu G. Irradiation of fluid foods. In: Novel Thermal and Non-Thermal Technologies for Fluid Foods. 1st ed. Dublin: Academic Press; 2012. pp. 167-183. DOI: 10.1016/C2009-0-61145-4

[45] Crawford LM, Ruff EH. A review of the safety of cold pasteurization through irradiation. Food Control. 1996;**7**:87-97. DOI: 10.1016/0956-7135(96)00004-7

[46] Lima KSC et al. Efeito da irradiação ionizante γ na qualidade pós-colheita de cenouras (*Daucus carota l.*) cv. Nantes. Ciência e Tecnologia de Alimentos. 2001;**21**:202-208

[47] Silva PT, Lopes MLM, Valente-Mesquita VL. Effect of different processing methods on ascorbic acid content in orange juice used to make cakes, puddings and jelly. Ciência e Tecnologia de Alimentos. 2006;**26**:678-682

[48] Satpute M, Annapure U. Approaches for delivery of heat sensitive nutrients through food systems for selection of appropriate processing techniques: A review. Journal of Hygienic Engineering and Design. 2013;**4**:71-92. Record Number: 20133394689

[49] Woodside JV. Nutritional aspects of irradiated food. Stewart Postharvest Review. 2015; **3**:1-6. DOI: 10.2212/spr.2015.3.2

[50] Harder MNC, Arthur V, Arthur PB. Irradiation of food: processing technology and effects on nutrients: Efect of ionizing radiation on food components. In: Encyclopedia of Food and Health. Elsevier; 2016. pp. 476-481. DOI: 10.1016/B978-0-12-384947-2.00405-0

[51] Souza PM, Briviba K, Müller A, Fernández A, Stahl M. Cyto-genotoxic and oxidative effects of a continuous UV-C treatment of liquid egg products. Food Chemistry. 2013; **138**:1682-1688. DOI: 10.1016/j.foodchem.2012.11.105

[52] Vicente SJV, Sampaio GR, Ferrari CKB. Oxidation of cholesterol in foods and its importance for human health. Food Reviews International. 2012;**28**:47-70. DOI: 10.1080/87559129.2011.594972

[53] Oliveira PHB. Efeitos do tratamento do leite por radiação ultravioleta (UV) em comparação à pasteurização [Dissertação]. Juiz de Fora: Universidade Federal de Juiz de Fora; 2012

[54] Lee JW et al. Effects of gamma radiation on the allergenic and antigenic properties of milk proteins. Journal of Food Protection. 2001;**64**:272-276. DOI: 10.4315/0362-028X-64.2.272

[55] Lopez LD, Pinto EP, Börger BR, KFC K, Lucchetta L, Tonial IB. Interferência do sistema de cultivo, radiação UV-C e método de secagem na qualidade da farinha de subprodutos de uva. Científica. 2017;**4**:347-354. DOI: 10.15361/1984-5529.2017v45n4p347-354

[56] Borba CM, Oliveira VR, Montenegro KR, Hertz PF, Venzke JG. Avaliação físico-química de hambúrguer de carne bovina e de frango submetidos a diferentes processamentos térmicos. Alimentos e Nutrição–Brazilian. Journal of Food and Nutrition. 2013;**24**:21-27

[57] Monteiro MLG et al. Impact of UV-C light on the fatty acid profile and oxidative stability of nile tilapia (*Oreochromis niloticus*) fillets. Journal of Food Science. 2017;**82**:1028-1037. DOI: 10.1111/1750-3841.13685

[58] Wongwichian C, Klomklao S, Panpipat W, Benjakul S, Chaijan M. Interrelationship between myoglobin and lipid oxidations in oxeye scad (*Selar boops*) muscle during iced storage. Food Chemistry. 2015;**174**:279-285. DOI: 10.1016/j.foodchem.2014.11.071

[59] Babakhani A, Farvin KHS, Jacobsen C. Antioxidative effect of seaweed extracts in chilled storage of minced atlantic mackerel (*Scomberscombrus*): Effect on lipid and protein oxidation. Food and Bioprocess Technology. 2016;**9**:352-364. DOI: 10.1007/s11947-015-1630-9

[60] Utrera M, Parra V, Estévez M. Protein oxidation during frozen storage and subsequente processing of different beef muscles. Meat Science. 2014;**96**:812-820. DOI: 10.1016/j.meatsci.2013.09.006

[61] Figueirêdo BC, Bragagnolo N, Skibsted LH, Orlien V. Inhibition of cholesterol and polyunsaturated fatty acids oxidation through the use of annatto and bixin in high-pressure processed fish. Journal of Food Science. 2015;**80**:1646-1653. DOI: 10.1111/1750-3841.12964

[62] Rizzotti L, Levav N, Fracchetti F, Felis GE, Torriani S. Effect of UV-C treatment on the microbial population of white and red wines, as revealed by conventional plating and PMA-qPCR methods. Food Control. 2015;**47**:407-412. DOI: 10.1016/j.foodcont.2014.07.052

[63] Guimarães IC et al. Physicochemical and microbiological quality of raspberries (*Rubus idaeus*) treated with different doses of gamma irradiation. Food Science and Technology. 2013;**33**(2):316-322. DOI: 10.1590/S0101-20612013005000040

[64] Taipina MS et al. The effects of gamma irradiation on the vitamin E content and sensory qualities of pecan nuts (*Carya illinoensis*). Radiation Physics and Chemistry. 2009;**78**:611-613. DOI: 10.1016/j.radphyschem.2009.03.019

[65] Guneser O, Yuceer YK. Effect of ultraviolet light on water- and fat-soluble vitamins in cow and goat milk. Journal of Dairy Science. 2012;**95**:6230-6241. DOI: 10.3168/jds.2011-5300

[66] Hussain PR et al. Influence of postharvest gamma irradiation treatment on the content of bioactive compound and antioxidant activity of fenegreek (*Trigonella foenum-graceum L.*) and spinach (*Spinacia oleracea L.*) leaves. Innovative Food Science & Emerging Technologies. 2016;**33**:268-281. DOI: 10.1016/j.ifset.2015.11.017

[67] Rivera-Pastrana DM et al. Effect of UV-C irradiation and low temperature storage on bioactive compounds, antioxidant enzymes and radical scavenging activity of papaya fruit. Journal of Food Science and Technology. 2014;**51**(12):3821-3829. DOI: 10.1007/s13197-013-0942-x

[68] Jiang T. Influence of UV-C treatment on antioxidant capacity, antioxidant enzyme activity and texture of postharvest hiitake (*Lentinus edodes*) mushrooms during storage. Postharvest Biology and Technology. 2010;**56**:209-251. DOI: 10.1016/j.postharvbio.2010.01.011

[69] Sheng K. Comparison of postharvest UV-B and UV-C treatments on table grape: Changes in phenolic compounds and their transcription of biosynthetic genes during storage. Postharvest Biology and Technology. 2018;**128**:74-81. DOI: 10.1016/j.postharvbio.2018.01.002

[70] Sari LK, Setha S, Naradisorn M. Effect of UV-C irradiation on postharvest quality of 'Phulae' pineapple. Scientia Horticulturae. 2016;**213**:314-320. DOI: 10.1016/j.scienta.2016.09.049

[71] Popovic MB et al. Enhancement of antioxidant and isoflavones concentration in gamma irradiated soybean. The Scientific World Journal. 2013:383574. DOI: 10.1155/2013/383574

[72] Aouidi F et al. Gamma irradiation of air-dried olive leaves: Effective decontamination and impact on the antioxidative properties and on phenolic compounds. Food Chemistry. 2011;**127**:1105-1113. DOI: 10.1016/j.foodchem.2011.01.109

[73] Mukhtar R et al. γ- Irradiation of oat grain—Effect on physici-chemical, structural, thermal, and antioxidant properties of extracted starch. International Journal of Biological Macromolecules. 2017;**104**:1313-1320. DOI: 10.1016/j.ijbiomac.2017.05.092

[74] Esmaeili S et al. Effect of gamma irradiation under various atmospheres of packing on the microbial and physicochemical properties of turmeric powder. Radiation Physics and Chemistry. 2018;**148**:60-67. DOI: 10.1016/j.radphyschem.2018.02.028

[75] Verma M, Sharma P, Gour VS, Kothari SL. Moisture-mediated effects of γ-irradiation on antioxidant properties of mung bean (*Vigna radiate L.*) cultivars. Innovative Food Science and Emerging Technologies. 2016;**34**:59-67. DOI: 10.1016/j.ifset.2016.01.012

[76] Ekezie FC, Cheng J, Sun D. Effects of nonthermal food processing technologies on food allergens: A review of recent research advances. Trends in Food Science & Technology. 2018;**64**:12-25. DOI: 10.1016/j.tifs.2018.01.007

Chapter 2

Agrifood By-Products as a Source of Phytochemical Compounds

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Abstract

In last years, food by-products and waste valorization practices have gained importance because these processes are sustainable and can increase the profit for local economies. Many compound families of phytochemicals like carotenoids, tocopherols, glucosinolates and phenolic compounds can be obtained through plant by-products coming from agroindustries, such as citric peels, tomato wastes or wine pomace. A number of novel methods like pressured liquid, microwaves or supercritical CO_2 are being used for the extraction of compounds, affecting them in different ways. Phytochemicals obtained can be used in cosmetics, medical uses and dietary supplements or reused in agrifood industries among others, as natural pigments, antioxidants or antimicrobials.

Keywords: phytochemical, valorization, by-products, extraction methods, antioxidant, antimicrobial

1. Introduction

It is estimated that one-third of all food produced for human consumption is lost or wasted. The annual global volume of food wastage is estimated to be 1.6 Gtonnes. It seems clear that a reduction of food wastage would have a substantial positive effect on natural and societal resources and that food wastage represents a missed opportunity to improve global food security and to mitigate environmental impact generated by agriculture [1].

Nowadays, the agricultural processing industries produce substantial quantities of by-products, which are currently generally treated as waste of industry. This practice is not only a waste of

resource, but also causes environmental pollution and they gradually permeant and release odors. The valorization of agricultural and food residues via green chemistry technologies is among the most important objectives of contemporary chemical research. The environmental benefits reduce waste production, reduce energy demand economical grows, thanks to the significant economic value of many bioproducts new revenues made available to farmers and job creation.

2. Classification of phytochemicals and sources

Phytochemicals are natural chemical compounds, which are found in foods derived from plants. In these, phytochemical substances act as systems of natural defenses for their plants, protecting them from infections and microbial invasions and guests giving them color, aroma and flavor. Phytochemicals are not considered essential for our organism; however, most of them have beneficial properties for health. The main sources of these compounds in foods are fruits, vegetables, legumes, whole grains, nuts, seeds, mushrooms, herbs and spices.

There are more than 2000 phytochemicals in plants, which can be grouped according to their structural characteristics in four large groups: terpenoids, phenolic compounds, nitrogen compounds and sulfur compounds.

2.1. Terpenoids

Terpenoids also referred as isoprenoids are a large and diverse family of organic compounds similar to terpenes. Terpenoids are made up of five-carbon isoprene units, assembled and modified in many different ways, always based on the skeleton of the isopentane. Majority of terpenoids have multicyclical structures, which differ from each other not only in functional group but also in their basic carbon skeletons.

These compounds are found in all kinds of living beings, and are biosynthesized in plants, where they are important in numerous biotic interactions [2]. In plants, the terpenoids fulfill many primary functions: some carotenoid pigments are formed by terpenoids; they are also part of the chlorophyll and gibberellin hormones among others. Steroids and sterols are produced from terpenoids precursors.

Terpenoids of the plants are widely used for their aromatic qualities. They play an important role in traditional medicine and herbal remedies, and their possible antibacterial effects and other pharmaceutical uses are being investigated. They are present, for example, in the essences of eucalyptus, the flavors of clove and ginger. The biosynthesis of terpenoids in plants is through the mevalonic acid pathway.

Terpenoids family comprises very different compounds, of which, the most interesting in food can be classified in the following subgroups:

Mono- and sesquiterpenoids: are the chief constituents of the essential oils; these are the volatile oils obtained from the sap and tissues of certain plants and trees. The essential oils

have been used in perfumery from the earliest times. The main terpenoids shown in nature are: myrcene, ocimene, citral, geraniol, eugenol, carvacrol, linanool, citronellal, carvone, limonene, terpinenes, menthol and menthone, the carane group and the pinane group, among others [3]. The main by-product source of essential oils is the citrus juice processing residues.

Tetraterpenoids (carotenoids): the carotenoids are a large group of pigments widely in animal and vegetable kingdoms; they produce colors ranging from yellow to deep red. Chemically, carotenoids are divided into two groups: the carotenes, which are hydrocarbons, and the xanthophylls, their oxygenated derivatives. Among the carotenes, very soluble in no-polar solvents, are α, β and γ-carotenes, and lycopene. The xanthophylls may present as acids, aldehydes or alcohols. Examples of these compounds are the cryptoxanthin, fucoxanthin, lutein and zeaxanthin. Currently, a high proportion of carotenoids is obtained synthetically, since it is cheaper; however, increasingly those of natural origin are used more [4]. Carotenes can be obtained from orange and reddish sources, as carrot waste or tomato pomace, while xanthophylls can be extracted from spinach residues [5].

2.2. Phenolic compounds

Polyphenols or phenol compounds are organic compounds whose molecular structures contain at least one phenol group; an aromatic ring at least joined a hydroxyl group. Many are classified as secondary metabolites of plants, products synthesized in plants, which possess the biological characteristic of being secondary products of their metabolism. They are generally synthesized by one of two biosynthetic pathways: the path of the shikimic acid or via malonic acid (or two, e.g., flavonoids).

Phenolic compounds in plants are a heterogeneous group of products with more than 10,000 compounds. Some are soluble in solvents organic, others are glycosides or carboxylic acids and therefore soluble in water, and others are very large and insoluble polymers.

This group also plays a very heterogeneous range of roles in plants, roles that are generally attributed to the by-products of plants: many are products of defense against herbivores and pathogens; others provide mechanical support to the plant, other they attract pollinators or dispersers of fruits, some of them absorb the ultraviolet radiation or act as allelopathic agents. In humans, these compounds are well known to possess healthy benefits, and the interest in food phenolics has increased due to their antioxidant and free radical-scavenging abilities [6]. Polyphenols can be structurally classified into several groups: flavonoids, lignans, stilbenes, and phenolic acids.

Flavonoids are one of the most studied families, since about 4000 different structures are known. Being a numerous group, they can be subdivided into: flavonols, flavanones, flavanols, flavones, and isoflavones anthocyanidins. Flavonoids have antioxidant and other living-body modulating activities [7]. For its part, phenolic acids can be classified as hydroxybenzoic acids and hydroxycinnamic acids, which are derived from non-phenolic molecules of benzoic and cinnamic acid, respectively. Phenols are present in numerous products, and collection has been studied in by-products of the obtaining of different oils, wine, juices, coffee, as well as saved cereal among others.

Group	Subgroup	Sources
Terpenoids	Mono- and sesquiterpenoid	Citric peels
	Carotenoids	Carrot, tomato and spinach wastes
Phenolic compounds	Flavonoids	Onion skin waste, citric peels, grape skins, olive leaf, soybean flour
	Lignans	Sesame cake
	Stilbenes	Wine pomace
	Phenolic acids	Pomegranate and grape by-products, rice bran
Nitrogen alkaloids	—	Cocoa and coffee wastes
Organosulfur compounds	Glucosinolates	Cauliflower and broccoli by-product
	Allyl sulphides	Garlic

Table. Classification of phytochemical and main sources of obtainment in agroindustrial by-products.

2.3. Nitrogen alkaloids

They are a large family of secondary metabolites of plants, which chemically have three characteristics in common: they are soluble in water, contain at least one atom of nitrogen and have biological activity [8]. In plants, alkaloids seem to offer protection from insects and herbivores. In humans, the alkaloids produce physiological and psychological responses, most of them because of their interaction with neurotransmitters. The alkaloids are classified according to the rings present in the molecule. Some of the alkaloids present in foods are of interest as piperine, caffeine and theobromine, from the pepper, coffee and cocoa, respectively.

2.4. Organosulfur compounds

The organosulfur compounds or thiols are chemical compounds containing sulfur in their structure. They are present in garlic and vegetables of genus cruciferous (cabbage, turnips and members of the mustard family). They include the glucosinolates and allyl sulphides.

Glucosinolates are metabolites characteristic of the *Brassicales* order. Many of us are familiar with the characteristic pungent flavor of wasabi and mustard. Chemically, glucosinolates are organic anions with an amino acid-derived side chain and oxime moiety with an S-linked thioglucose and an O-linked sulfate group [9]. Allyl sulphides are a very important phytochemical group present in garlic.

Main sources of obtainment of these compounds in agrifood by-products are summarized in **Table**.

3. Extraction processes

Numerous methods of extraction have been developed for obtaining phytochemical compounds. The use of one or other will depend on both type of matrix (solid or liquid) and

type of molecule extracted, as well as the use that will be given to extract thus obtained. The conventional techniques used include maceration, infusion, decoction or Soxhlet extraction among others [10]. However, since the main objective of the extraction is the reuse of waste and reduce environmental impact, green extraction techniques are necessary for this purpose. The objective of these techniques is to improve extraction performance, decreasing the use of organic solvents or the energy used. These techniques include:

3.1. Ultrasound-assisted extraction (UAE)

Ultrasound is a mechanical wave that propagates in an elastic medium and their frequency is above the audible sounds, this is more than 20,000 Hz. The ultrasound-assisted extraction is a technique that uses ultrasonic waves to agitate an immersed sample in an organic solvent. The major effects of ultrasound in a liquid medium are attributed to the cavitation phenomena, which comes from the physical processes that create, enlarge, and implode microbubbles of gases dissolved in the solvent [11].

Since the extraction is carried out in a medium, the temperature, time and solvent-type can affect not only the extraction yield but also the composition of the extract and should thus be taken into consideration. Furthermore, after extraction, compounds with organic solvent are separated from the matrix by centrifugation or filtration like in a conventional extraction.

The greatest advantage of using this technique against conventional techniques is that this technology shortens the total time of procedure, together with a decrease of consumed energy and pollution. For that, the procedures using ultrasound assistance have a production cost and a functioning cost much lower than the cost for conventional procedures with a high purity final product. On the other hand, as the temperature reached is not very high, sonication is suitable for extracting thermolabile compounds [12]. However, and despite the fact that this technology can be easily integrated into the industry, its use implies initial costs, which range between 10,000 and 200,000 euros [11].

The application of this technology for the extraction of phytochemicals from by-product has been widely researched, but its application differs according to the various matrices and analytes to extract. Thus, terpenoids have been obtained by UAE from different by-products: The industrial extraction of terpenoids limonin, nomolin and obakunone were performed from lemon seeds with UAE, obtaining high extraction yields of limonoids [13]. In case of lycopene, tomato paste processing wastes are an important source of obtainment; the UAE has demonstrated to decrease the solvent-solid ratio and to require less time and lower temperature than conventional organic solvent extraction [14]. Papaya processing waste is also studied to lycopene extraction. In this case, results indicated that UAE was the most effective extracting method among the conventional and Soxhlet method [15]. In addition, the extraction of carotenoids without solvent also can be improved with the use of ultrasound, as is the case with the extraction of carotenoids from pomegranate peel using sunflower oil [16].

Different wastes have been used for the ultrasound-assisted extraction of phenolic compounds. Mandarin peel has been utilized for the extraction of flavonoids and hesperidin comparing the results with maceration extraction. These showed greater extraction efficiency

and lower times of extraction with UAE method [17]. On the other hand, the pomace formed in the wine or juice making from blueberry contains many phenolic and other bioactive compounds. These can be retrieved through ultrasound-assisted extraction obtaining the same compounds than in a conventional solvent extraction but with higher yields of extraction [18]. Spent coffee grounds are a valuable source of phenolic compounds, of which, the main are chlorogenic and protocatechuic acids. These can be extracted with an ultrasound-assisted solid-liquid extraction with mild temperatures and short times [19].

For its part, isothiocyanates extraction has been optimized from cauliflower by-products using ultrasound-assisted extraction. These extracts can be added to an apple beverage until 10% preserving well the sensorial properties to obtain a new functional drink [20].

3.2. Microwave-assisted extraction (MAE)

Microwave-assisted extraction (MAE) is a relatively new extraction technique that combines microwave irradiation and traditional solvent extraction. Microwave energy can be used to improve the extraction of compounds soluble in specific fluids (liquids or gases) as a result of changes in the cell structure caused by electromagnetic waves. It provides rapid and selective techniques with best recoveries than the obtained in conventional extraction processes [21]. Also offers other advantages over common technologies, such as lower energy consumption, volume of solvent and toxicity of the solvents used and, in general, less waste. Another advantage of MAE is that the quicker heating occurring inside the solids where the dissolution of the extract components takes place.

In general, the use of microwave is widespread at laboratory scale. However, its use in the industry offers some disadvantages such as the high initial cost, maintenance, or safety aspects [22]. Moreover, since its use is based on the warming of the matrix and many of the phytochemicals of interest are thermolabile, its use for this purpose is limited. However, there are numerous studies that have been carried out using MAE, both in the pre-treatment of the sample and to facilitate the extraction. For example, the extraction of β-carotene and other carotenoids contained in carrot residues has been studied using intermittent microwave radiation. The thermal degradation caused by MAE in β-carotene was investigated using for that the measurement of antioxidant activity [23]. For the extraction of phenolic compounds from dried waste grape skins, MAE achieves a savings of 83% with respect to conventional in extraction time [24]. Microwave-assisted extraction has also been investigated for the extraction of phenolics from other different matrix as *Eucalyptus robusta* [25] or green tea leaves [21].

The combined use of microwave-assisted and ultrasound-assisted extraction has also been developed for the extraction of phytochemicals. For example, it has been proposed the extraction of essential oils, polyphenols and pectin from the orange peels waste in a solvent-free process [26].

3.3. Enzymatic-assisted extraction (EAE)

A procedure widely used to improve the efficiency of the extraction of compounds from a plant matrix is based on the enzymatic assisted extraction (EAE) [27]. This procedure is

based on prior treatment of the matrix with the corresponding enzyme followed by a process of extraction solvent [28]. This non-traditional and environmentally friendly technology improve the yield of target compounds while reduces the use of solvents in the process. This is possible because enzymes can catalyze reactions in aqueous solutions under mild conditions. The use of enzymes for the extraction is based on their ability to degrade cell walls and membranes, thereby increasing cell wall permeability and enabling targeted compounds release into the medium.

This involves the use of hydrolytic enzymes to alter the cell, mainly composed of large polymer walls highly complex, such as cellulose, hemicellulose, lignin, and pectin [27]. Specific use of enzymes can increase use pre-treatment effects or decrease the amount of solvent, as well as increase the yield of extracted compounds. Enzymes such as cellulases, pectinases or hemicellulases are widely used in juice processing and clarification of beer for cell wall degradation. When matrix have been previously treated with enzymes, it is gotten the cell walls break, thus increasing the performance of the extraction of bioactive compounds [28].

The use of EAE can be tested and optimized on the laboratory scale, and the use of common food-grade enzymes makes it a low-cost technique for extraction purposes. However, some technical limitations on the industry application are a higher relative cost for processing and the difficulty to optimize the process at industrial scale [10].

EAE has been tested for improve the extractions of carotenoids from tomato peels [29]. Therefore, the use of hydrolytic enzymes can improve the recovery of bound and free phenols from pomegranate peels [30]. EAE has been used successfully for the recovery of polyphenols in citrus peel and ginger [31] winemaking by-products [32]; underutilized watermelon rind [33] or cauliflower outer leaves [34]; among others.

3.4. Supercritical fluid extraction (SFE)

It is a technique that uses a solvent in supercritical conditions. A substance at a pressure higher than its critical pressure and temperature higher than its critical temperature is known as supercritical fluid. Supercritical fluids have properties intermediate between a gas and a liquid, favoring its penetration in different matrices, and therefore the solubilization of solutes, having the possibility of extracting thermolabile compounds. Nowadays, the most important application of SFE to food industry is the extraction of caffeine from coffee.

The supercritical fluid extraction consists of four stages [10]: pressurization step, temperature adjustment stage, extraction stage and separation step.

Advantages of using this technology are the easy separation between solvent and matrix that avoids to increase the temperature to remove the solvent; the possibility to work with non-toxic solvent and the reuse of this, and a relatively low-cost obtainment of high pressure or temperature. However, its practical application is limited to processes that are not affected by the relatively high temperature necessaries and the initial costs are high.

In the majority of cases, the supercritical fluid used is CO_2, for being generally recognized as safe (GRAS). The application of supercritical CO_2 has high performances for the extraction of nonpolar substances. However, the CO_2 under critical conditions is a poor solvent for polar compounds. This limitation can be solved by adding co-solvents that alter the polarity of the CO_2, but they may become contained, requiring a subsequent separation operation.

SFE has been used for the extraction of lycopene β-carotene from tomato peels [35] and carrot peels [36]. For its part, the recovery of phenol by SFE has been tested in apple pomace [37], cacao pod husk [38], mango by-products [39] or sour cherry pomace [40].

3.5. Pressurized liquid extraction (PLE)

The so-called pressurized solvent extraction is a technique that combines solvent extraction at temperatures (50–200°C) and high pressures (1500–2000 psi) to quickly and efficiently extract compounds from solid matrices. The use of liquid solvents at high temperatures and pressures improves the performance of the extractions since the solubility is increased and the mass transfer is improved and the rupture of the composite-matrix superficial equilibrium is facilitated [41]. PLE can be considered as a green extraction process especially when a non-toxic solvent is used.

A wide range of organic nonpolar to polar solvent and their mixtures have been used in pressurized liquid extraction of phytochemical. However, it is a technology that modifies solvent properties. This fact makes water suitable for the extraction to polar and nonpolar organic compounds.

PLE could be carried out in static and dynamic modes. Static extraction is considered more efficient because of the greater penetration of the solvent into the pores of the source [10]. The advantages of this method with respect to conventional ones are the short extraction times, the yields obtained and the reduced usage of solvents. However, this method is not suitable for thermolabile compounds because the high temperature can alter the structure of these. For that, the use of this technique for the extraction of phytochemical is very limited.

Some researches have been carried out with pressurized liquid for the extraction of carotenoids from shrimp waste [42] or phenolic acid in potato peels [43].

3.6. Other technologies

In addition to the above, other extraction technologies have been developed as the instant controlled pressure drop or pulsed electric field. The latter has been tested for the extraction of phenolic compounds from orange peel [44] or grape pomace [45] and alkaloids form potato peels [46]. Finally, there are numerous investigations in which the combination of these techniques has been used to take advantage of a possible synergy between them. Thus, for example, enzymes-assisted extraction often complemented with ultrasonic extraction or supercritical fluid extraction.

An overview of the improvement of all these methods can be observed in **Table**.

Extraction method	Improvement of method
Ultrasound-assisted extraction (UAE)	Cavitation phenomena, which comes from the physical processes that create, enlarge, and implode microbubbles of gases dissolved in the solvent
Microwaves-assisted extraction (MAE)	The quicker heating occurring inside the solids where the dissolution of the extract components takes place
Enzyme-assisted extraction (EAE)	Ability of enzymes to degrade cell walls and membranes, increasing cell wall permeability
Supercritical fluid extraction (SFE)	Supercritical fluids have properties intermediate between a gas and a liquid, favoring its penetration in different matrices
Pressurized liquid extraction (PLE)	The solubility is increased and the mass transfer is improved
Combined extraction processes	The possible synergic effect of techniques

Table. Overview of the improvement of the main green extraction techniques.

4. Industry phytochemical residue applications

As stated in previous sections, phytochemicals are present in foods of plant origin. The food industry is considered a source of extraction of phytochemical compounds, which can be obtained from waste generated in the processing of fruit and vegetables. This fact is beneficial for food industry producers, as it generates an economic advantage [47].

It is worth mentioning that in its elaboration process, the less handling and processing of food generate a more retain of these compounds. For this reason, processes like a cereal refining, intense and long heating, cocking in broths which are then discarded and some of its compounds are lost, reduce the food phytochemical content. In nature, phytochemicals are in many foods, but in the future, the development of bioengineering will allow us to create plants with more concentration of these compounds.

In reference to its possible applications, the great phytochemicals properties allow us to open a wide range of possibilities. For example, due to its antioxidant activity, these could be used in creams, functional foods and fortified foods formulation, or due to its antimicrobial activity, these could be used in food conservation and medicine. In the following sections, we see some of the most important applications in detail.

4.1. Phytochemicals in medicine and pharmacy

The phytochemical application in medicine and pharmacy is directly related with the phytotherapy (use of plant-derived medications in the treatment and prevention of disease). The large quantity of properties that the plants has allowed us to apply them for treatments of diseases, and this is due to their phytochemical content. Numerous trials and pharmacological studies of specific phytotherapeutic preparations exist but, in some countries, phytotherapy is viewed as a form of traditional medicine.

For example, against respiratory diseases are effective the consumption of essentials oils of *Eucalyptus globulus Labill.* [48], *Origanum vulgare* L. [49], *Pinus* sp. [50] or *Thymus vulgaris* [51], which due to its high concentration of tannins, phenolic acids, terpenes and flavonoids, among others, cause an antiseptic and expectorant action.

Against circulatory diseases, the application of *Vitis vinifera* L. due to its high concentration of polyphenols, mainly resveratrol, in its fruits generate a lot of antioxidant activity and its anthocyanins do a veinotonic and vasoprotective action [52].

Other plant species which are of great importance is *Camellia sinensis* from which green tea is obtained. Some studies have reported the use of this specie rich in catechins against cancer chemoprevention, hypercholesterolemia, atherosclerosis, Parkinson's disease, Alzheimer's disease, and other aging-related disorders [53].

Concretely, an agroindustry residue, olive leaf, is a great source of phytochemicals. These contain a high concentration of oleuropein, tyrosol and hydroxytyrosol, three important phenolic compounds which have bioactive properties like antioxidant capacity [54]. The European Food Safety Association (EFSA) determined officially that oleuropein present in olive leaf extract generate a better glucose tolerance in humans [55]. These are some applications and uses in medicine and pharmacy of phytochemical compounds present in some plants but the number or these is increased day by day.

4.2. Phytochemicals in food industry

The food and agricultural products industries are an important source of phenolics-rich by-products, which have been a good source of natural antioxidant. The application of these by-products in other foods like oil, fish and meat has shown antioxidant values similar to synthetic antioxidant, particularly the flavonoids and hydroxycinnamic acids [56]. The way to consume these by-products and add to our diet is directly, for example, the consumption of plants in tea form, or indirectly, for example, like an alimentary additive or supplement.

Another application of phytochemicals in food is the creation of active packaging for them. The films and coatings of different origin (proteins, polysaccharides, etc.) incorporate a wide variety of essential oils or plant extracts with the aim of creating active packaging with antimicrobial/antioxidant properties that improve the conservation of various types of food, for example, for fruit conservation [57], meat [58] and fish [59].

As we have already mentioned, the consumption of phytochemicals and adding these to our diet generate a lot of benefits to our health due to the large number of biological activities that possess.

4.3. Other phytochemical application fields

A great number of phytochemicals bioactivities open a wide range of possibilities and applications of these important food plants compounds. Other field is the cosmetic industry due to the antioxidant properties of phytochemical compounds. The reactive oxygen species generate

cell damage, and this can generate to appear early signs of aging. If plant extract or directly phytochemicals compounds are adding to cosmetic products these can combat these toxic substances to cells and prevent the appearance of wrinkles, skin blemishes, and so on [60].

Currently, plant extracts rich in phytochemicals are also being evaluated in the oral cavity to prevent the development of microorganisms and prevent the appearance of dental caries and plaque. Specifically, the green tea extract has been evaluated against one of the main microorganisms that develop oral infections, Streptococcus mutants, obtaining great antimicrobial activity when applying the ethanolic extract of *C. sinensis* [61]. These results are of great importance because they show us that plant extracts rich in phytochemical compounds can be applied, for example, to dental prosthesis or toothpaste to prevent buccal infections.

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References

[1] The Food and Agricultural Organization (FAO). Food Wastage Footprint: Impacts on Natural Resources. Summary Report. Rome, Italy: The Food and Agricultural Organization (FAO); 2013

[2] Goodwin TW. Distribution of carotenoids. Chemistry and Biochemistry of Plant Pigments. 1976;**1**:225-261

[3] Singh G. Terpenoids. In: Chemistry of Terpenoids and Carotenoids. Vol. 3. Discovery Publishing House; 2007

[4] Badui Dergal S. Química de los alimentos. 4th ed. Pearson Education; 2006. 736 p

[5] Benito de Valle-Prieto M, Delgado-Adámez J, Gil MV, Martillanes S, Franco, MN, Martín-Vertedor D. Virgin olive oil enriched with lutein-zeaxanthin from *Spinacia oleracea*. Journal of Oleo Science 2017;**66**(5):463-468. DOI: 10.5650/jos.ess16189

[6] Martillanes S, Rocha-Pimienta J, Cabrera-Bañegil M, Martín-Vertedor D, Delgado-Adámez J. Application of phenolic compounds for food preservation: Food additive and active packaging. In: Phenolic Compounds-Biological Activity. InTech; 2017. pp. 39-58. DOI: 10.5772/66885

[7] Terahara N. Flavonoids in foods: A review. Natural Product Communications. 2015; **10**(3):521-528

[8] Aniszewski T. Alkaloids: Chemistry, Biology, Ecology, and Applications. 2nd ed. Elsevier; 2015. 496 p

[9] Kopriva S. Advances in Botanical Research, Glucosinolates. 1st ed. Vol. 80. Academic Press; 2016. 364 p

[10] Carciochi RA, D'Alessandro LG, Vauchel P, Rodríguez MM, Nolasco SM, Dimitrov K. Valorization of agrifood by-products by extracting valuable bioactive compounds using green processes. In: Ingredients Extraction by Physicochemical Methods in Food. Academic Press; 2017. pp. 191-228. DOI: 10.1016/B978-0-12-811521-3.00004-1

[11] Rostagno MA, Prado JM, editors. Natural Product Extraction: Principles and Applications. Vol. 21. Royal Society of Chemistry; 2013. 398 p

[12] Romdhane M, Gourdon C. Investigation in solid–liquid extraction: Influence of ultrasound. Chemical Engineering Journal. 2002;**87**(1):11-19

[13] Yu H, Wang C, Deng S, Bi Y. Optimization of ultrasonic-assisted extraction and UPLC-TOF/MS analysis of limonoids from lemon seed. LWT-Food Science and Technology. 2017;**84**:135-142. DOI: 10.1016/j.lwt.2017.05.059

[14] Kumcuoglu S, Yilmaz T, Tavman S. Ultrasound assisted extraction of lycopene from tomato processing wastes. Journal of Food Science and Technology. 2014;**51**(12):4102-4107. DOI: 10.1007/s13197-013-0926-x

[15] Li AN, Li S, Xu DP, Xu XR, Chen YM, Ling WH, et al. Optimization of ultrasound-assisted extraction of lycopene from papaya processing waste by response surface methodology. Food Analytical Methods. 2015;**8**(5):1207-1214. DOI: 10.1007/s12161-014-9955-y

[16] Goula AM, Ververi M, Adamopoulou A, Kaderides K. Green ultrasound-assisted extraction of carotenoids from pomegranate wastes using vegetable oils. Ultrasonics Sonochemistry. 2017;**34**:821-830. DOI: 10.1016/j.ultsonch.2016.07.022

[17] Nipornram S, Tochampa W, Rattanatraiwong P, Singanusong R. Optimization of low power ultrasound-assisted extraction of phenolic compounds from mandarin (*Citrus reticulata* Blanco cv. Sainampueng) peel. Food Chemistry. 2018;**241**:338-345. DOI: 10.1007/s12161-014-9992-6

[18] He B, Zhang LL, Yue XY, Liang J, Jiang J, Gao XL, Yue PX. Optimization of ultrasound-assisted extraction of phenolic compounds and anthocyanins from blueberry (*Vaccinium ashei*) wine pomace. Food Chemistry. 2016;**204**:70-76. DOI: 10.1016/j.foodchem.2016.02.094

[19] Al-Dhabi NA, Ponmurugan K, Jeganathan PM. Development and validation of ultrasound-assisted solid-liquid extraction of phenolic compounds from waste spent coffee grounds. Ultrasonics Sonochemistry. 2017;**34**:206-213. DOI: 10.1016/j.ultsonch.2016.05.005

[20] Amofa-Diatuo T, Anang DM, Barba FJ, Tiwari BK. Development of new apple beverages rich in isothiocyanates by using extracts obtained from ultrasound-treated cauliflower by-products: Evaluation of physical properties and consumer acceptance. Journal of Food Composition and Analysis. 2017;**61**:73-81. DOI: 10.1016/j.jfca.2016.10.001

[21] Pan X, Niu G, Liu H. Microwave-assisted extraction of tea polyphenols and tea caffeine from green tea leaves. Chemical Engineering and Processing: Process Intensification. 2003;**42**(2):129-133. DOI: 10.1016/S0255-2701(02)00037-5

[22] Ciriminna R, Carnaroglio D, Delisi R, Arvati S, Tamburino A, Pagliaro M. Industrial feasibility of natural products extraction with microwave technology. ChemistrySelect. 2016;**1**(3):549-555. DOI: 10.1002/slct.201600075

[23] Hiranvarachat B, Devahastin S. Enhancement of microwave-assisted extraction via intermittent radiation: Extraction of carotenoids from carrot peels. Journal of Food Engineering. 2014;**126**:17-26. DOI: 10.1016/j.jfoodeng.2013.10.024

[24] Pedroza MA, Amendola D, Maggi L, Zalacain A, De Faveri DM, Spigno G. Microwave-assisted extraction of phenolic compounds from dried waste grape skins. International Journal of Food Engineering. 2015;**11**(3):359-370. DOI: /10.1515/ijfe-2015-0009

[25] Bhuyan DJ, Van Vuong Q, Chalmers AC, van Altena IA, Bowyer MC, Scarlett CJ. Microwave-assisted extraction of Eucalyptus robusta leaf for the optimal yield of total phenolic compounds. Industrial Crops and Products. 2015;**69**:290-299. DOI: 10.1016/j.indcrop.2015.02.044

[26] Boukroufa M, Boutekedjiret C, Petigny L, Rakotomanomana N, Chemat F. Bio-refinery of orange peels waste: A new concept based on integrated green and solvent free extraction processes using ultrasound and microwave techniques to obtain essential oil, polyphenols and pectin. Ultrasonics Sonochemistry. 2015;**24**:72-79. DOI: 10.1016/j.ultsonch.2014.11.015

[27] Boulila A, Hassen I, Haouari L, Mejri F, Amor IB, Casabianca H, Hosni K. Enzyme-assisted extraction of bioactive compounds from bay leaves (*Laurus nobilis* L.). Industrial Crops and Products. 2015;**74**:485-493. DOI: 10.1016/j.indcrop.2015.05.050

[28] Özkan G, Bilek SE. Enzyme-assisted extraction of stabilized chlorophyll from spinach. Food Chemistry. 2015;**176**:152-157. DOI: 10.1016/j.foodchem.2014.12.059

[29] Prokopov T, Nikolova M, Dobrev G, Taneva D. Enzyme-assisted extraction of carotenoids from Bulgarian tomato peels. Acta Alimentaria. 2017;**46**(1):84-91. DOI: 10.1556/066.2017.46.1.11

[30] Mushtaq M, Sultana B, Anwar F, Adnan A, Rizvi SS. Enzyme-assisted supercritical fluid extraction of phenolic antioxidants from pomegranate peel. The Journal of Supercritical Fluids. 2015;**104**:122-131. DOI: 10.1016/j.supflu.2015.05.020

[31] Manasa D, Srinivas P, Sowbhagya HB. Enzyme-assisted extraction of bioactive compounds from ginger (*Zingiber officinale* roscoe). Food Chemistry. 2013;**139**(1-4):509-514. DOI: 10.1016/j.foodchem.2013.01.099

[32] de Camargo AC, Regitano-d'Arce MAB, Biasoto ACT, Shahidi F. Enzyme-assisted extraction of phenolics from winemaking by-products: Antioxidant potential and inhibition of alpha-glucosidase and lipase activities. Food Chemistry. 2016;**212**:395-402. DOI: 10.1016/j.foodchem.2016.05.047

[33] Mushtaq M, Sultana B, Bhatti HN, Asghar M. RSM based optimized enzyme-assisted extraction of antioxidant phenolics from underutilized watermelon (*Citrullus lanatus* Thunb.) rind. Journal of Food Science and Technology. 2015;**52**(8):5048-5056. DOI: 10.1007/s13197-014-1562-9

[34] Huynh NT, Smagghe G, Gonzales GB, Van Camp J, Raes K. Enzyme-assisted extraction enhancing the phenolic release from cauliflower (*Brassica oleracea* L. var. *botrytis*) outer leaves. Journal of Agricultural and Food Chemistry. 2014;**62**(30):7468-7476. DOI: 10.1021/jf502543c

[35] Kehili M, Kammlott M, Choura S, Zammel A, Zetzl C, Smirnova I, … Sayadi S. Supercritical CO_2 extraction and antioxidant activity of lycopene and β-carotene-enriched oleoresin from tomato (*Lycopersicum esculentum* L.) peels by-product of a Tunisian industry. Food and Bioproducts Processing. 2017;**102**:340-349. DOI: 10.1016/j.fbp.2017.02.002

[36] de Andrade Lima M, Charalampopoulos D, Chatzifragkou A. Optimisation and modelling of supercritical CO_2 extraction process of carotenoids from carrot peels. The Journal of Supercritical Fluids. 2018;**133**:94-102. DOI: 10.1016/j.supflu.2017.09.028

[37] Ferrentino G, Morozova K, Mosibo OK, Ramezani M, Scampicchio M. Biorecovery of antioxidants from apple pomace by supercritical fluid extraction. Journal of Cleaner Production. 2018;**186**:253-261. DOI: 10.1016/j.jclepro.2018.03.165

[38] Valadez-Carmona L, Ortiz-Moreno A, Ceballos-Reyes G, Mendiola JA, Ibáñez E. Valorization of cacao pod husk through supercritical fluid extraction of phenolic compounds. The Journal of Supercritical Fluids. 2018;**131**:99-105. DOI: 10.1016/j.supflu.2017.09.011

[39] Meneses MA, Caputo G, Scognamiglio M, Reverchon E, Adami R. Antioxidant phenolic compounds recovery from *Mangifera indica* L. by-products by supercritical antisolvent extraction. Journal of Food Engineering. 2015;**163**:45-53

[40] Woźniak Ł, Marszałek K, Skąpska S. Extraction of phenolic compounds from sour cherry pomace with supercritical carbon dioxide: Impact of process parameters on the composition and antioxidant properties of extracts. Separation Science and Technology. 2016;**51**(9):1472-1479. DOI: 10.1080/01496395.2016.1165705

[41] Kaufmann B, Christen P. Recent extraction techniques for natural products: Microwave-assisted extraction and pressurised solvent extraction. Phytochemical Analysis. 2002;**13**(2): 105-113. DOI: 10.1002/pca.631

[42] Quan C, Turner C. Extraction of astaxanthin from shrimp waste using pressurized hot ethanol. Chromatographia. 2009;**70**(1-2):247-251. DOI: 10.1365/s10337-009-1113-0

[43] Luthria DL. Optimization of extraction of phenolic acids from a vegetable waste product using a pressurized liquid extractor. Journal of Functional Foods. 2012;**4**(4):842-850. DOI: 10.1016/j.jff.2012.06.001

[44] Luengo E, Alvarez I, Raso J. Improving the pressing extraction of polyphenols of orange peel by pulsed electric fields. Innovative Food Science and Emerging Technologies. 2003;**17**:79-84. DOI: 10.1016/j.ifset.2012.10.005

[45] Boussetta N, Vorobiev E, Reess T, De Ferron A, Pecastaing L, Ruscassie R, Lanoisellé JL. Scaleup of high voltage electrical discharges for polyphenols extraction from grape pomace: Effect of the dynamic shock waves. Innovative Food Science and Emerging Technologies. 2012;**16**:129-136. DOI: 10.1016/j.ifset.2012.05.004

[46] Hossain MB, Aguiló-Aguayo I, Lyng JG, Brunton NP, Rai DK. Effect of pulsed electric field and pulsed light pre-treatment on the extraction of steroidal alkaloids from potato peels. Innovative Food Science & Emerging Technologies. 2015;**29**:9-14. DOI: 10.1016/j.ifset.2014.10.014

[47] da Silva LMR, de Figueiredo EAT, Ricardo NMPS, Vieira IGP, de Figueiredo RW, Brasil IM, Gomes CL. Quantification of bioactive compounds in pulps and by-products of tropical fruits from Brazil. Food Chemistry. 2014;**143**:398-404. DOI: 10.1016/j.foodchem.2013.08.001

[48] Juergens UR, Dethlefsen U, Steinkamp G, Gillissen A, Repges R, Vetter H. Anti-inflammatory activity of 1.8-cineol (eucalyptol) in bronchial asthma: A double-blind placebo-controlled trial. Respiratory Medicine. 2003;**97**(3):250-256. DOI: 10.1053/rmed.2003.1432

[49] Teixeira B, Marques A, Ramos C, Serrano C, Matos O, Neng NR, Nunes ML. Chemical composition and bioactivity of different oregano (*Origanum vulgare*) extracts and essential oil. Journal of the Science of Food and Agriculture. 2013;**93**(11):2707-2714. DOI: 10.1002/jsfa.6089

[50] Mimoune NA, Mimoune DA, Yataghene A. Chemical composition and antimicrobial activity of the essential oils of Pinus pinaster. Journal of Coastal Life Medicine. 2013;**1**(1): 54-58. DOI: 10.1002/cbdv.201000185

[51] Sienkiewicz M, Łysakowska M, Denys P, Kowalczyk E. The antimicrobial activity of thyme essential oil against multidrug resistant clinical bacterial strains. Microbial Drug Resistance. 2012;**18**(2):137-148. DOI: 10.1089/mdr.2011.0080

[52] Bhat KP, Kosmeder JW, Pezzuto JM. Biological effects of resveratrol. Antioxidants and Redox Signaling. 2001;**3**(6):1041-1064

[53] Zaveri NT. Green tea and its polyphenolic catechins: Medicinal uses in cancer and non-cancer applications. Life Sciences. 2006;**78**(18):2073-2080. DOI: 10.1016/j.lfs.2005.12.006

[54] Martín-Vertedor D, Garrido M, Pariente JA, Espino J, Delgado-Adámez J. Bioavailability of bioactive molecules from olive leaf extracts and its functional value. Phytotherapy Research. 2016;**30**(7):1172-1179. DOI: 10.1002/ptr.5625

[55] EFSA Panel on Dietetic Products, Nutrition and Allergies (NDA). Scientific opinion on the substantiation of a health claim related to olive (*Olea europaea* L.) leaf water extract and increase in glucose tolerance pursuant to article 13 (5) of regulation (EC) no 1924/2006. EFSA Journal. 2014;**12**(5):3655

[56] Martínez-Valverde I, Periago MJ, Provan G, Chesson A. Phenolic compounds, lycopene and antioxidant activity in commercial varieties of tomato (*Lycopersicum esculentum*). Journal of the Science of Food and Agriculture. 2002;**82**(3):323-330. DOI: 10.1002/jsfa.1035

[57] Sánchez-González L, Pastor C, Vargas M, Chiralt A, González-Martínez C, Cháfer M. Effect of hydroxypropylmethylcellulose and chitosan coatings with and without bergamot essential oil on quality and safety of cold-stored grapes. Postharvest Biology and Technology. 2011;**60**(1):57-63. DOI: 10.1016/j.postharvbio.2010.11.004

[58] Gill AO, Delaquis P, Russo P, Holley RA. Evaluation of antilisterial action of cilantro oil on vacuum packed ham. International Journal of Food Microbiology. 2002;**73**(1):83-92. DOI: 10.1016/S0168-1605(01)00712-7

[59] Ojagh SM, Rezaei M, Razavi SH, Hosseini SMH. Effect of chitosan coatings enriched with cinnamon oil on the quality of refrigerated rainbow trout. Food Chemistry. 2010;**120**(1):193-198. DOI: 10.1016/j.foodchem.2009.10.006

[60] Aburjai T, Natsheh FM. Plants used in cosmetics. Phytotherapy Research. 2003;**17**(9): 987-1000. DOI: 10.1002/ptr.1363

[61] Tahir A, Moeen R. Comparison of antibacterial activity of water and ethanol extracts of *Camellia sinensis* (L.) Kuntze against dental caries and detection of antibacterial components. Journal of Medicinal Plants Research. 2011;**5**(18):4504-4510

Chapter 3

Ultrasound Application to Improve Meat Quality

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Abstract

High-intensity ultrasound offers an alternative to traditional methods of food preservation and is regarded as a green, versatile, popular, and promising emerging technology. Ultrasound generates acoustic cavitation in a liquid medium, developing physical forces that are considered the main mechanism responsible for the observed changes in exposed materials. In meat, ultrasound has been successfully used to improve processes such as mass transfer and marination, tenderization of meat ,and inactivation of microorganisms. It is also an alternative to traditional meat aging methods for improving the quality properties of meat. Moreover, the combination of ultrasonic energy with a sanitizing agent can improve the effect of microbial reduction in foods. This review describes recent potential applications of ultrasound in meat systems, as well as physical and chemical effects of ultrasound treatments on the conservation and modification of processed meat foods.

Keywords: ultrasound, cavitation, emerging technology, minimal processing, meat quality

1. Introduction

Evolution of food processes is driven by changes in consumer preferences and the need to produce safe and high-quality foods. Nonthermal or intermediate technologies have great potential to achieve the characteristics desired by both the industry and consumers, especially regarding the desire to avoid altering the flavor or nutritional content during production. These technologies, which include the use of high pressure, electrical pulses, microfiltration,

and ultrasonication, are especially designed for economy, simplicity, and energy efficiency. Ultrasound is an acoustic energy [1], and therefore, it is a nonionizing, noninvasive, and non-polluting form of mechanical energy [2]. These properties lead to a wide range of applications in the food industry. It is considered an emerging method with a great potential to control, improve, and accelerate processes without damaging the quality of food and other products [3, 4]. A low-power and high-frequency method is used to monitor the composition and physicochemical properties of food components and products during processing. Therefore, it contributes to control the properties that improve food quality. In recent years, research studies have been focused on assessing the effects of ultrasound on processes including mass transfer or marinating, meat tenderizing, crystallization, freezing, drying, degassing, filtration, foam production and reduction, emulsification, homogenization, and inactivation of microorganisms and enzymes [2]. Ultrasound has also been employed to optimize physicochemical characteristics, preparation processes for meat products, microbiological content, and sensory characteristics in fresh and processed meat [5]. Although ultrasound waves have been used to improve a wide variety of characteristics for a variety of matrices and processes, the appropriate conditions for scaling ultrasonic methods up to industrial levels have been established for a relatively small number of processes [4].

As emphasized by Chemat et al.[6], a key goal of ultrasound research is to study and analyze both desirable and undesirable degradation phenomena in foods resulting from ultrasonic treatment (e.g., ultrasonic processing may affect the texture and chemical composition of foods). For this reason, many research questions in the meat sector are yet to be elucidated. Although multiple reports have been published, still inconsistent results have been reported, maybe because of the specific nature of meat tissues and various factors of ultrasound application possibly involved in, affecting food properties. This review aims to identify the effect of ultrasound on the major quality characteristics of fresh meat. We believe the results will help establish a methodology to enable the scaling-up of ultrasonic technology to the industrial level.

2. Ultrasound overview

Ultrasound is a form of energy generated by a longitudinal mechanical wave whose vibration frequency is greater than 20,000 cycles per second (20 kHz), which is above the audible limit for humans. Sound is considered a pressure wave with one-dimensional propagation. The speed of an ultrasonic pulse depends on the acoustic properties of the medium, and the speed of sound propagation is greater in solids than in liquids and higher in liquids than in gases [7]. In an ultrasound system, the electrical energy is transformed into vibrational energy, which is mechanical energy [8] that has been transmitted through a sonicated medium. Part of the input energy is lost through conversion to heat, and the rest can produce cavitation. A fraction of the cavitation energy produces chemical, physical, or biological effects, while other fractions are reflected and consumed in the reemission of sound. Ultrasound ranges from 20 kHz to 10 MHz and is divided into three categories: (1) high-power (>5 Wcm^{-2}or 10–1000 Wcm^{-2}) and low-frequency (20–100 kHz); (2) medium-power and intermediate-frequency (100 kHz–1 MHz); and (3) low-power (<1 Wcm^{-2}) and high-frequency (1–10 MHz) [9]. Three different methods

are used to apply ultrasound to products: (a) direct application; (b) coupling to the device, and (c) immersion in an ultrasound bath [2].

Low-intensity, high-frequency ultrasound has analytical applications that provide information about the physicochemical properties of foods such as composition, structure, and condition [10]. Furthermore, unlike conventional analytical techniques, it is noninvasive and nondestructive [11] and the measurements are fast, automated, and easy to use in both laboratories and production lines. High-power ultrasound, also known as high-intensity ultrasound, may cause changes in the physical, chemical, or mechanical properties of foods. In the field of biochemistry, ultrasound was initially used to rupture cell walls, releasing their contents. Subsequent studies showed that high-power ultrasound can be used to activate the immobilized enzymes by increasing the rate of transport of substrates to enzymes [12].

3. Power ultrasound

Power or high-intensity ultrasound has emerged as a new and complementary technology with a high number of potential applications. Its effects are primarily mechanical: alternate cycles of expansion and compression are produced, causing the growth or formation of new bubbles in the medium [13]. When they reach a volume in which they can no longer absorb more energy, the bubbles implode violently, causing microcurrents and the collapse of liquid molecules, a phenomenon known as cavitation. The quantity of energy released by the cavitation depends on the kinetics of the bubble's growth and collapse. This energy increases with increasing surface tension at the bubble interface and decreases with increasing vapor pressure of the liquid [14]. The results of ultrasound in liquid media depends on variables such as the characteristics of the treatment medium (viscosity, surface tension, vapor pressure, nature and concentration of dissolved gas, presence of solid particles, and temperature), efficiency of the ultrasound generator (frequency and input power), and the size and geometry of the treatment container [15].

4. Applications in food

Ultrasound has potentially a wide range of applications in the food industry. Researchers have identified various areas in which ultrasound can be used effectively, such as in the modification and control of crystallization processes, liquid food degassing, enzyme inactivation, drying, filtration, and oxidation induction. [12].Ultrasound methods have also been used in emulsion preparation in fruit and vegetable dehydration, enzyme inhibition, microbial inactivation, and crystallization of fats and sugars. Another example of a successful application of ultrasound technology is acoustic drying. It can be performed at lower temperatures than those used in conventional methods because the heat transfer between a solid surface and a liquid surface increases by approximately 30–60%, reducing the probability of oxidation or degradation of the material [15]. In addition, studies have shown that ultrasound is an effective method for food freezing, and the acceleration of ice nucleation and freezing leads to better control of the crystal size distribution in frozen products [16]. Ultrasound can not only

increase the speed of freezing fresh foods, but also improve the quality of frozen products. Currently, the pasteurization and conventional thermal sterilization are the most commonly used techniques for removing the threat posed by microorganisms in food products. Heat treatment destroys the vegetative microorganisms and some spores; however, its effectiveness depends on the treatment temperature and time. The magnitude, temperature, and time of treatment are also proportional to nutrient loss, development of undesirable flavors, and deterioration of functional properties of food products [17, 18]. Studies have shown that high-power ultrasound substantially reduces microbial loads because cavitation disrupts cell walls, resulting in the destruction of living cells and thereby contributing to food preservation. Unfortunately, scarce is still known about the mechanism of inactivation.

5. Applications in meat

The use of ultrasound in the meat industry, which began with the evaluation of live cattle fat and muscle, has been conducted since the 1950s. Nowadays, low-intensity ultrasound is routinely used to improve quality, taste, and tenderness, which represent the most important quality attributes in consumer satisfaction.

Many recent studies have reported potential uses of high-intensity ultrasound on fresh meat. Applications have been published with interesting advantages in freezing [19], thawing [20], meat brining [14], cooking [2], bacterial inhibition [21], and tenderizing [22]. The resulting changes of the application of ultrasound to fresh meat are summarized in **Table**.

Sample	Application (intensity/ freq/time)	Effect of ultrasound	Authors
Beef (*longissimus thoracis* and *lumborum,* and *semimembranosus*)	62 W cm^{-2}, 20 kHz, 15 s	No effect on mastication force, sensory traits, solubility of collagen or myofibrillar proteolysis.	Lyng et al. [58]
Semimembranosus pre- and post-rigor	10 W cm^{-2}, 2.6 MHz, 2 x15 s	Larger sarcomeres, Z-line disruption, increased calcium. No effect on collagen.	Got et al. [30]
Beef (*semimembranosus*)	2 W cm^{-2}, 25 kHz, 1 or 2 min	Lower loss of water after cooling, thawing, and heating. No effect on pH. Higher water holding capacity.	Dolatowski et al. [32]
Beef (*semimembranosus*) matured for 24, 48, 72 or 96 h at 2°C	2 W cm^{-2}, 45kHz, 2 min	No effect on meat color. Increased free calcium. Changes in protein structure. Improved WHC at 4 d *postmortem*.	Dolatowski and Stadnik [16]
Beef (*semimembranosus*) 24 h postmortem and matured for 24, 48, 72 or 96 h at 2°C	2 W cm^{-2}, 45kHz, 2 min	No effect on pH or color. Reduced hardness.	Stadnik and Dolatowski [35]

Sample	Application (intensity/ freq/time)	Effect of ultrasound	Authors
Beef (*semimembranosus*) 24 h postmortem and matured for 24, 48, 72 or 96 h at 2°C	2 W cm^{-2}, 45 kHz, 2 min	Acceleration of aging process. Fragmentation of protein structures. Increase WHC.	Stadnik et al. [31]
Beef longissimus lumborum et thoracis and semitendinosus aged up to 8.5 days	12 W cm^{-2} , 24 kHz, for up to 240 s	Reduced WBS force and hardness. Increased pH. No interaction between ultrasound and aging. No changes in meat color and drip loss. Ultrasound reduced cook and total loss.	Jayasooriya et al. [34]
Hen breast meat stored for 0, 1, 3, or 7 d at 4°C	12 W cm^{-2}, 24 kHz, 15 s period	Reduced shear force. No change in cooking loss.	Xiong et al. [53]
Beef (*semitendinosus*)	1500 W, 40 kHz, 10, 20, 30, 40, 50, or 60 min	No effect on brightness and red color. Decreased the tendency to yellow. Decreased the muscle fiber diameter. No effect on heat-insoluble collagen. Weaken collagen stability.	Chang et al. [61]
Pork *biceps femoris* *24 h post mortem*	150 W, 1 MHz and 500 W, 25 kHz, 40 min	Ultrasound did not change in shear force. Ultrasound combined with actinidin decreased shear force more than actinidin alone.	Jørgensen et al. [60]
Beef (*semimembranosus*) 24 h postmortem and matured for 24, 48, 72 or 96 h at 2 °C	2 W cm^{-2}, 45kHz, 2 min	Slightly less stable color. No change in oxidative stability at 4 d storage.	Stadnik et al. [31]
Beef *semitendinosus*	40 kHz, 11 w cm-2 for 0, 60 and 90 min.	Increases luminosity and reduces redness up to 8 d of storage. No effect on water holding capacity of meat. Decreased coliforms and psychrophilic bacterial load.	Caraveo et al. [21]
Beef *longissimus thoracic* and deep *pectoralis* Matured 14 d at 2°C Cooked at 62°C or 70°C	1000 W, 20 kHz, 0, 5 or 10 min	Faster cooking, higher water retention, decreased cooking loss, shear force and soluble collagen. Higher sensory tenderness.	Pohlman et al. [44]
Holstein bulls (*longissimus lumborum)*	20 kHz, 100 and 300 W for 10, 20 or 30 min	Improved meat tenderness, decreased shear force, filtering residue and textural parameters.	Barekat and Soltanizadeh [55]

Sample	Application (intensity/ freq/time)	Effect of ultrasound	Authors
Beef *longissimus dorsi*	40 kHz, 11 W cm-2 for 60 min.	Reduces shear force. Produces more tender and juice meat. No effect on meat color.	Peña-González et al. [22]
Chicken breast and soybean gels, 4°C to 8°C	450 W, 20 kHz, 0, 3, 6, 9 or 12 min (4 or 2 s pulses)	More viscoelastic gel Improved WFB and textural properties Homogeneous fine network microstructures	Zhao et al. [54]
Chicken breast	22 W cm^{-2} 40 kHz, 15 or 30 min	Increased mass transfer and higher meat weight	Leal-Ramos et al. [62]
Pork *longissimus dorsi*	100 W and 20 kHz, 45 min	Increased salt gain and water loss. Higher mass transfer at higher ultrasound intensity.	Cárcel et al. [14]
Pork *longissimus dorsi*	2-4 W cm^{-2}, 20 kHz, 30, 90 or 180 min	Higher salt diffusion. Diffusion coefficient increases with ultrasound intensity.	Siró et al. [15]
Pork *longissimus dorsi*	40 kHz; 37.5 W dm^{-3}, 15, 30, 45, 60, 90 or 120 min	Higher salt and water diffusion.	Ozuna et al. [63]
Pork *longissimus thoracis and lumborum*	4.2, 11 or 19 W cm^{-2}, 20 kHz, 10, 25 or 40 min	No effect on water holding capacity and structure of meat. Higher mass transfer and protein extraction. Myosin denaturation at higher intensities.	McDonnell et al. [59]
Pork meat and skin surface	High-intensity ultrasound, 0.5 to 2 s	Less skin and surface bacteria	Morild et al. [80]
Chicken breast	Ultrasonic bath, 20 min	No effect on water retention capacity, shear force and cooking loss. No changes in *Salmonella* and *E. coli*.	Smith et al. [43]
Chicken wing surface	2.5 W cm^{-2}, 40 kHz, 3 or 6 min	Microorganism reduction. Higher reduction with higher time. *E. coli* more sensible to ultrasound.	Kordowska-Wiater and Stasiak [82]
Pure culture suspensions	20 kHz, 3, 6 or 9 min, 20, 40 and 60 °C	Bacteria inactivation is higher at higher time and temperature.	Herceg et al. [84]
Chicken carcasses	SonoSteam®	*Campylobacter* and viable total count reduction.	Musavian et al. [83]

Table. Effects of ultrasound on meat.

6. Effects on meat quality properties

The majority of quality parameters assessed 24 h *postmortem* in meat is physicochemical in nature [23]. The potential of hydrogen (pH), water holding capacity (WHC), drip loss (DL), color (L*, a*, b*, C*, and h*), and shear force are quality indicators for the raw meat. Physicochemical characteristics are evaluated to control the quality, assess the efficiency of production and treatment processing, compare results between laboratories, and aid research [24, 25]. The effect of a number of nutritional, breed, and production factors such as genetics, management, and slaughter on specific quality attributes should be considered when meat quality is analyzed [26]. Key markers of meat quality, including raw materials, which have a strong impact on the industry, are pH, water holding capacity, texture, and oxidative stability. In addition, aroma, color, flavor, and tenderness are the most important sensory components to the meat-consuming population [27]. When considering the use of emerging technologies in meat, both the mechanisms of action and the effects on food transformation, preservation, and integrity must be known. Ultrasound application may be an efficient alternative technology to increase meat tenderness. It is used in meat processing and preservation as a complementary or assistive technology [28].

6.1. Potential of hydrogen (pH)

pH is one of the most important indicators of raw meat quality, because it directly affects protein stability and properties. As reported in the literature, all product quality attributes depend on the ultimate pH. Water holding capacity, drip loss, and color are among the most important product quality attributes [29]. pH greatly affects the quality and functionality of muscle proteins, and decrease in pH reduces their water holding capacity, leading to economic losses. On the other hand, increases in pH increase the water holding capacity of the meat because of changes in the electrical charges within muscle proteins that occur when the pH is above the isoelectric point. Ultrasound effect on pH has been analyzed in various studies and conditions. Some authors have reported that initial pH of meat can be increased as a result of ultrasound treatment (2.6 MHz, 10 W/cm^2) [30] prior to *rigor mortis*, with no differences in final pH, while other reported small differences [31] or no differences in pH because of ultrasound [32–35].In a study, bovine muscles (*longissimus lumborum et thoracis* and *semitendinosus*) sonicated (24 kHz, 12 Wcm^{-2}) for a maximum of 4 min and subsequently stored them for 8 days led to increase tenderness and pH without a significant correlation between ultrasound and aging time [34]. The increase of pH was attributed to the release of ions from the cellular structure into the cytoplasm or to changes in protein structure, which could lead to changes in the position of ionic functionalities that could lower the muscle pH.

6.2. Water holding capacity (WHC)

Water holding capacity (WHC) may be defined as the ability of meat or muscle proteins to immobilize their own or added water during an applied force [25]. A decrease in pH causes a shrink of the network of polypeptide chains, which decreases the water holding capacity.

Therefore, WHC is directly related to pH. The speed to reach the final pH also affects the WHC. When the drop in pH is relatively rapid, the changes in myofibrillar and sarcoplasmic proteins result in a decreased water holding capacity [29]. Some meat characteristics linked to WHC include color, texture, firmness, juiciness, and tenderness. Meat WHC is affected by factors including *rigor mortis*, ATP loss, and changes in the myofibrillar structure partly associated with proteolytic activity. Many other characteristics, including drip loss, are closely related to or depend on WHC.

Approximately, three-quarter of meat is water, and about 10% of the water in live animal muscle is bound to muscle proteins compared to the 5–10% of water located in small channels between adjacent cells, or extracellular space. However, most of the water content is located in spaces between thin and thick filaments of myofibrils. In any muscle, WHC is minimal at low pH. Because of aging, it tends to increase owing to protein degradation and changes in electric charges induced by intramolecular reorganization [23]. When the WHC is low, moisture or weight loss during storage is greater owing to surface evaporation and exudation of cuts because the WHC is related to several physicochemical characteristics of the protein and myofibrillar components. WHC is the main indicator of the suitability of a given meat for preparing a product. The effects of ultrasonic treatment on WHC of meat are variable. Variation of effects on WHC is described on the next section, due to the relation between WHC and drip loss.

6.3. Drip loss (DL)

The release of water droplets from the muscle originated from the extracellular water is known as DL. It is the easiest water content to extract. DL depends on the state of contraction after *rigor mortis* because of reduction in the filamentary space and changes in the cellular membrane, which causes the release of water to the extracellular space in the form of drops through the cutting surfaces [35]. These drops consist of an aqueous red solution that largely contains proteins and water-soluble minerals, some of which are highly nutritious. Drip loss is strictly related to pH and WHC. When WHC increases, DL decreases and vice versa [36]. Several factors increase the WHC during meat aging, including pH, Z-line disintegration by protease activity, and changes in membrane permeability with diffusion and ionic redistribution, which results in substitution of divalent ions and weakening of intermolecular forces between protein chains. DL is primarily an economic problem for retailers, because weight losses during cutting cause accumulation of liquid around the product, which leads to consumer rejection [37, 38]. WHC is a key indicator of meat quality that affects the economic sector. Therefore, analyzing the effect of ultrasound on WHC is important [39]. Assessment of DL is used to identify the best conditions for the refrigeration, freezing, packaging, and storage conditions of meat. Consequently, DL measurements also make possible to determine WHC. The effects of ultrasonic treatments on WHC and drip loss are highly variable. Some authors report that ultrasound increases the rates of meat exudation and water loss [40, 41]. Whereas, other authors found no effect on the water holding capacity [42] or drip loss [21, 34] in beef (24–40 kHz, 11–12 W cm^{-2}). In contrast, some reports indicate that ultrasonicated meat has a higher WHC [16, 40, 43], similar to that of meat at an advanced *postmortem* stage. This could be explained by structural changes in myofibrillar proteins caused by ultrasound; the above is confirmed by microstructure photographs [31]. Recently, when high-intensity ultrasound is applied during brining of beef, higher WHC was found, possibly by a higher diffusion of salt into the tissue, which can increase the capacity to hold the water before cooking

the meat [44, 45]. The variability seen in the literature could result from differences in the ultrasound methods; the authors employed various times and intensities, which hindered direct comparison.

6.4. Color

Color is a key factor in meat quality because it is the first sensory characteristic assessed by the consumer [46]. In red meat, a bright red color is related to freshness and therefore consumer rejection or acceptance [47]. Meat color results from the quantity and chemical state of myoglobin in the muscle. Deoxymyoglobin and myoglobin are responsible for the purple color of fresh meat. When meat is exposed to air for several minutes, deoxymyoglobin is oxygenated into oxymyoglobin, which is responsible for a cherry red color in meat. When meat is exposed to air for several hours or days, it turns brown due to the oxidation of oxymyoglobin into metmyoglobin. Meat contains other pigments, some derived from external sources, sometimes in insignificant amounts, which commonly indicate deterioration. Meat color and exterior appearance may be associated with aging time, shelf life, hardness, and juiciness. Some studies suggest that ultrasound has no effect on meat color because the heat generated is insufficient to denature proteins and pigments [48, 49]. Conversely, in an assessment of the effect of ultrasound (22 W/cm^2) on meat, it has been found that the color changed to a lighter, less red, and more yellow-orange color (greater hue angle), which was less bright than control meat [43]. Ultrasound accelerates total changes in color, limits the formation of oxymyoglobin, and slows down the formation of metmyoglobin [48]. Nevertheless, when meat is cooked, meat panelists do not detect differences between ultrasonicated and control meat [22].

6.5. Tenderness

Tenderness in meat is determined by its texture. Tenderness is one of the most important attributes of meat quality because it is perhaps the most appreciated feature by consumers. Inconsistencies in this characteristic have been considered one of the major problems that the meat packing industry faces [50]. Tenderness is affected by the composition, structural organization, and integrity of the skeletal muscle. The two structural components that determine the intrinsic muscle strength are myofibrillar proteins and connective tissues [51] and the nature of these two components makes difficult to achieve tenderness. Tenderness depends on the size of the longitudinally arranged fiber bundles in muscles, which are delimited by the connective tissue septa forming the perimysium [25]. Myofibrillar tenderness can be controlled by manipulating conditions *pre-* and *postmortem*. Some methods and procedures used to increase tenderness include electrical stimulation, pressurization, calcium infusion, enzymatic treatment, and marination. All these methods are invasive, cause deformation, and affect the appearance of meat. In addition, some methods may contaminate the meat (e.g., brine injection with unclean needles). Currently, aging is the foremost industrial process used to increase the tenderness of meat. Aging tenderization mechanism is well known nowadays, consisting of biochemical processes driven by endogenous proteases. Nevertheless, aging is a time-consuming process, and it can be variable among animals. Therefore, various physical methods, such as electrical stimulation and chemical methods, have been used trying to improve tenderness.

Numerous studies have been conducted to develop methods to improve tenderness. Among these, ultrasound application methods have been used at various sonication times, frequencies, and intensities. Most authors agree that ultrasound increases meat tenderness [22, 40, 48] and shortens the aging period without compromising other quality parameters [16, 33]. The potential of low-frequency, low-intensity ultrasound application to improve meat tenderization is remarkable. Several authors report an important reduction of shear force after treatment with ultrasound [52]. Benefits of ultrasound treatment on beef have been observed in *longissimus lumborum* and *semitendinosus* (24 kHz and 12 W/cm^2 for 240 s) [34], *M. Semimembranosus* (45 kHz and 2 W/cm^2 for 2 min) [48], and *semitendinosus* (40 kHz, 1500 W for 10, 20, 30, 40, 50 or 60 min) [40]. Benefits to the texture of poultry (24 kHz, 12 W/cm^2 for 4 min after 7 d of storage) [53, 54] and pork (2.5–3 W/cm^2 for 180 min) [15] are also reported. It has also been observed a significant decrease in the shear force of *Bovine L. dorsi* with the application of ultrasound, both, fresh and aged [22]. More recently, it was reported a reduction of shear force values in muscle semitendinosus when it was ultrasonicated and aged for 3–7 d [45]. The effect was mainly attributed to an increase of desmin and troponin-T degradation, and myofiber fracture along Z-lines and I-bands.

It is suggested that acoustic cavitation may induce mechanical rupture of myofibrillar protein structures [31], fragmentation of collagen macromolecules, migration of proteins, minerals and other compounds, thereby accelerating proteolysis or protein denaturation. High-intensity ultrasound can cause degradation of cells and some subcellular components, because periodic oscillation of acoustic pressure softens cell membranes. Research has also evinced tissue disruption in the migration of proteins, minerals, and other components; accelerating enzymatic activity and degradation of collagen macromolecules when meat is exposed to high-intensity ultrasound [11, 55]. In addition to tenderize, high-intensity ultrasound can also improve meat sensory properties [22]. After applying ultrasound, the quantity of ATP available in muscles at pre-*rigor* stage may change [49], accelerating the start of *rigor mortis* [56]. Indirectly, ultrasound may induce tenderization because of the activation of proteolysis by the release of lysosome cathepsins and/or intracellular calcium ions that activate calpains. This mechanism may lead to a weaker cellular structure [48] through protein denaturation, which in turn causes muscle tissue disruption that results in increased tenderness [40] and a shortened aging period [57]. It should be noted that some reports also indicate that ultrasound has no effect on shear force when it is applied at 62 W/cm^2 [58], 22 W/cm^2 [43], 4–19 W/cm^2 [59], or 150 and 500 W [60]. The data available thus far indicate that ultrasound does indeed exert a key effect on meat tenderization, although the application parameters must be established before the method can be scaled to industrial levels. The effect of ultrasound on the physicochemical characteristics and *semitendinosus* muscle collagen has been studied [61]. Their results suggest that ultrasound affects denaturation and aggregation of collagen fibers in the extracellular space. These changes contribute to benefit the quality and texture of the meat. Besides, meat luminosity and tendency to redden remained unaffected.

6.6. Marination to improve meat quality

High-intensity ultrasound application during meat marination has been frequently studied. Meat marinades may contain salt in two forms: dry or wet [14]. High-intensity ultrasound application resulted favorable for salt diffusion when used in wet marinades. The effect

of power ultrasound on pork during wet marination depends on the ultrasound intensity applied [62]. Ultrasound causes bubble formation that hits the tissue, which may lead to microinjection of brine into the sample. This effect may help to explain the observed increase of NaCl content in the ultrasonicated meat [14, 63].

Ultrasonic treatment (low-intensity and low-frequency) and the use of vacuum caused favorable microstructural changes in pork loins marinated in sodium chloride [15] and these effects are highly dependent on the intensity of ultrasound treatment. Some of the critical factors in food processing warrant consideration because ultrasound generates rapid changes in temperature and pressure (109°C/s) over short time periods. Furthermore, cavitation generates shock waves, which contribute to this effect. Factors that modulate the effects of ultrasound application include time of exposure, processing volume, and sample composition [12, 14].

6.7. Microbiological properties

Bacteria are the most important microorganisms in food processing. While most are harmless and many are beneficial, some indicate the likely presence of contamination and deterioration and may cause diseases. While thousands of bacterial species have been identified, all are unicellular and fall under three basic forms: spherical, rod-shaped, and spiral. Some rod-shaped bacteria can take two forms: latent spores and active vegetative cells. The vegetative cells form spores under adverse conditions to survive. Most sporulating bacteria that grow in the presence of air belong to the *Bacillus* genus, and most of those that grow only in the absence of air belong to the genus *Clostridium*.

Meat is susceptible to the growth of some pathogenic microorganisms such as *E. coli, Campylobacter jejuni, Salmonella spp., Staphylococcus aureus,* and *Listeria monocytogenes*, which recurrently affect the properties of the meat and present serious problems during packing, processing, and storage. Several methods are used to avoid microbial growth in meat. The most commonly used methods involve heating, dehydration, and addition of preservatives [64]. The most common types of mesophilic bacteria that are pathogenic to humans include *Staphylococcus aureus*, *Salmonella*, and *Listeria*. Although it may survive without damage in the intestinal tract of humans, salmonella is a common cause of food poisoning. Another common mesophilic bacterium, *Listeria monocytogenes*, is more often distributed through contaminated foods such as raw meats or unpasteurized cheeses [64]. Animals, including humans, may transport *Listeria*, but it primarily threatens those with weakened immune systems. Some *E. coli* strains found in human feces are pathogenic, causing infection and disease. These are called enteropathogenic bacteria.

Staphylococcus is nonsporulated bacteria without mobility, but because they are resistant to drying, they are easily dispersible by dust particles through air and surfaces [65]. *S. aureus* is usually found in the skin and in mucous membranes of humans and other animals. It is almost always present in small quantities in raw meats and foods extensively handled by humans. Maintaining food that is completely free of contamination with *Staphylococcus* is often difficult or impossible. Pasteurizing or cooking destroys the organism but not its toxin [66].Meat is one of the most perishable foods consumed by humans—it is easily damaged by bacteria. One of the most commonly used preservation methods is refrigeration, including freezing. However, certain bacteria are able to grow at 4°C; these are collectively known as psychrophiles. This

group includes some pathogens such as *Yersinia enterocolitica*, *Listeria monocytogenes*, non-proteolytic strains of *C. botulinum*, and some strains of enterotoxigenic *E. coli* and *Aeromonas hydrophila*. Several other organisms that can cause foodborne diseases and grow at refrigeration temperatures include: *Vibrio parahaemolyticus*, *Bacillus cereus*, *Staphylococcus aureus,* and some *Salmonella* strains [64].

When refrigeration is extended, *Pseudomonas*, *Acinetobacter*, and *Moraxella* species may grow and damage fresh meat [67]. Gram-negative organisms are known to survive less frequently compared to their Gram-positive counterparts [68–70]. However, recent studies have shown higher survival rates among Gram-negative bacteria, especially *Pseudomonas* species, which account for the majority of bacteria responsible for refrigerated meat deterioration [67]. Refrigerated foods, such as processed meat, should be stored as close as possible to 0°C. However, in most cases, they remain close to 4–8°C. This fluctuation in temperature reduces the useful life of the products and can lead to major public health problems. The fresh meat industry must incorporate as many treatments as possible that reduce the microbial population and minimize reproduction. Some of these treatments include heat, acidification, preservatives, reduced water activity, and packaging under modified atmospheres. Although modified atmospheres are included as a potential barrier, it should be noted that reduced oxygen atmospheres can actually favor anaerobic pathogens. For many products, the modified atmosphere actually helps improve product quality rather than safety.

Yeast and molds grow on most foods, equipment, and building surfaces with small amounts of nutrients and moisture [71]. Because bacteria grow faster, they greatly outgrow yeasts and molds in most foods. Fungi and yeasts grow well in low-pH, humid, and temperature environments with high concentrations of salt and sugar. Therefore, they can pose a problem in dry foods, such as dried meat and salted fish [72].

Effective microbial destruction is of paramount importance for food processing; a single report of microbial contamination could question the reputation of a manufacturer and jeopardize their future success. To minimize the bacterial load of a product, the manufacturer must reduce the initial contamination, inactivate microorganisms present in the food, and implement procedures to prevent or slow the growth of microbial populations that have not been inactivated. Conventional methods of bacterial inactivation involve thermal treatments, such as pasteurization. These treatments generally result in undesirable flavors and the loss of nutrients. Ultrasonic treatment has been used to inactivate bacterial populations [73]. This is due to cavitation effects: pressure changes produced by the ultrasonic waves cause microbiological inactivation [3, 73]. The microbiological damage resulting from the application of various ultrasound wave amplitudes depends on factors such as contact time with the microorganism, microorganism type, food quantity, composition, and treatment temperature [74]. Microbial resistance varies among microorganisms, i.e., some are more susceptible than others to the ultrasound process. Studies have shown that larger or longer cells are more susceptible to ultrasound because they have more a larger contact surface and are therefore more exposed to the pressure produced by cavitation [75]. Gram-positive bacteria are less susceptible to ultrasound compared to Gram-negative bacteria, although results have shown that rod-shaped (bacillus) microorganisms tend to be more susceptible than cocci [76].

Gram-positive bacteria are likely less susceptible to ultrasound because of their thicker cell walls, which contain an adhesive peptidoglycan layer [77, 78]. In general, microorganisms that produce spores exhibit a greater resistance to heat and ultrasound [74, 75].

A considerable amount of data on the impact of ultrasound on microbial inactivation is available. One study demonstrated the effects on the microbiological environment of bacterial suspensions by inoculating the skin of broilers with *Salmonella*; the Salmonella population decreased with ultrasound treatment in peptone at 20 kHz for 30 min [16]. Studies have shown that the intensity of traditional heat treatments can be reduced by 50% when they are combined with power ultrasound. For this reason, a new method for antimicrobial treatment could feature the combined effects of pressure and ultrasound (manosonication), ultrasound and heat (thermosonication), or ultrasound, heat, and pressure (manothermosonication) [79]. These are likely the best microbial inactivation methods because they are more energy-efficient and effective in inhibiting microorganisms than conventional methods. The effectiveness of ultrasonic treatments requires prolonged exposure to high temperatures, which may deteriorate functional properties, sensory characteristics, and the nutritional content of foods [73]. In combination with heat, ultrasound can accelerate the rate of food sterilization, thereby decreasing the necessary duration and intensity of heat treatment and the resulting damage.

The inactivation of *Salmonella typhimurium, Salmonella derby, Salmonella infantis, Yersinia enterocolitica,* and a pathogenic strain of *Escherichia coli* was studied in inoculated samples treated for 0.5–2.0 s. The total viable bacterial counts decreased by 1.1 log CFU cm^{-2} after a 1-second treatment and by 3.3 log CFU cm^{-2} after a 4-second treatment [80]. The reduction of the population in the skin was significantly greater than that in the meat, although no significant differences were observed between the types of bacteria. However, the study by Smith *et al.* [81] stands out. They reported no effect after ultrasound on *Salmonella* or on *E. coli* in marinated chicken, likely because ultrasound alone is not fully effective in bacterial inhibition.

Some authors [82] have studied the elimination of Gram-negative bacteria (*Salmonella anatum, Escherichia coli, Proteus* sp., and *Pseudomonas fluorescens*) on the surface of chicken skin after ultrasonic treatment (40 kHz and 2.5 Wcm^{-2} for 3 or 6 min) in water and in 1% aqueous lactic acid. Sonication in water alone or lactic acid solutions for 3 min resulted in a decrease in the number of microorganisms on the surface of the skin of 1.0 CFU cm^{-2}. Other reports show that treating chicken carcasses in the process line with steam and ultrasonic treatments significantly reduces the population of *Campylobacter* in contaminated poultry. The total viable content decreased by approximately three logarithmic units when steam and ultrasound were applied immediately after slaughter [83]. Ultrasound treatments combined with lactic acid may be a suitable method for decontaminating poultry carcass skins.

Ultrasound effects depend on frequency, amplitude, time, and temperature [84] as it was demonstrated on the inactivation of suspensions containing *Escherichia coli, Staphylococcus aureus, Salmonella* sp., *Listeria monocytogenes*, and *Bacillus cereus* treated with a 12.7-mm ultrasound probe at 20 kHz and 60, 90, and 120 mm amplitudes for 3, 6, and 9 min at 20, 40, and 60°C. These three parameters affected the inactivation of bacteria in pure cultures. The results showed increased microbial inactivation for longer treatment periods, particularly when they were combined with high temperature and amplitude.

It has been observed that treating fresh beef with a power ultrasound method decreased its bacterial load, particularly of coliforms and psychrophilic bacteria, when a frequency of 40 kHz and intensity of 60 W/cm^2 were applied for 60 and 90 min. Meat treated for the longer period showed the largest reduction of microorganisms during storage [21].

7. Conclusions

Selected and potential applications of ultrasound mainly in the field of food preservation and product modification were discussed. High-intensity ultrasound generates acoustic cavitation in a liquid medium, developing physical forces that are considered the main mechanism responsible for the observed changes in exposed materials. These forces include acoustic streaming, cavitation, shear, micro-jet, and shockwaves. The quantity of energy released by the cavitation depends on many factors such as treatment medium and ultrasound frequency. Ultrasound has a wide range of applications in the food industry. It can be used as a processing aid in extraction, crystallization, freezing, emulsification, filtration, and drying. Applications of ultrasound in meat have been reported with interesting advantages in freezing, thawing, meat brining, and tenderizing. Ultrasound has also been shown to improve physicochemical characteristics, preparation processes for meat products, microbiological content, and sensory characteristics in fresh and processed meat. Acoustic cavitation may induce the mechanical rupture of the myofibrillar protein structure with significant effect on collagen characteristics and meat textural properties. High-intensity ultrasound reduces microbial loads in meat, resulting in the destruction of living cells and this effect remains during cold storage. Like most innovative food processing technologies, high-power ultrasonics needs to be developed and scaled up for each application.

8. Conflict of interest

The authors declare that they have no interest or benefit arising from the direct applications of this chapter.

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References

[1] Hoover DG. Ultrasound. Journal of Food Science. 2000;**65**:93-95

[2] Chemat F, Zill-e H, Khan MK. Applications of ultrasound in food technology: Processing preservation and extraction. Ultrasonics Sonochemistry. 2011;**18**:813-835

[3] Awad TS, Moharram HA, Shaltout OE, Asker D, Youssef MM. Application of ultrasound in analysis, processing and quality control of food. A review. Food Research International. 2012;**48**:410-427

[4] Gallego-Juárez JA. High-power ultrasonic processing recent developments and prospective advances. Physics Procedia. 2010;**3**:35-47

[5] Alarcón-Rojo AD, Janacua H, Rodríguez JC, Paniwnyk L, Mason TJ. Power ultrasound in meat processing meat. Meat Science. 2015;**107**:86-93

[6] Chemat F, Rombaut N, Meullemiestre A, Turk M, Périno-Issartier S, Fabiano-Tixier A, Vian M. Review of green food processing techniques. Preservation, transformation, and extraction. Innovative Food Science and Emerging Technologies. 2017;**41**:357-377

[7] Kasaai MR. Input power-mechanism relationship for ultrasonic irradiation: Food and polymer applications. Natural Science. 2013;**5**:14-22

[8] Berlan J, Mason T. Sonochemistry from research laboratories to industrial plants. Ultrasonics. 1992;**30**:203-212. DOI: 10.1016/0041-624X(92)90078-Z

[9] Ashokkumar M, Mason TJ.Sonochemistry. In: Kirk-Othmer Encyclopedia of Chemical Technology. New York, NY: John Wiley & Sons; 2007

[10] Demirdöven A, Baysal T. The use of ultrasound and combined technologies in food preservation. Food Reviews International. 2009;**25**:1-11

[11] Jayasooriya SD, Bhandari BR, Torley P, Darcy BR. Effect of high power ultrasound wave son properties of meat: A review. International Journal of Food Properties. 2004;(2): 301-319

[12] Knorr D, Zenker M, Heinz V, Dong-Un L. Applications and potential of ultrasonics in food processing. Trends in Food Science and Technology. 2004;**15**:261-266

[13] McClements DJ. Advances in the application of ultrasound in food analysis and processing. Trends in Food Science and Technology. 1995;**6**:293-299

[14] Cárcel JA, Benedito J, Bon J, Mulet A. High intensity ultrasound effects on meat brining. Meat Science. 2007;**76**:611-619

[15] Siró I, Vén C, Balla C, Jónas G, Zeke I, Friedrich L. Application of an ultrasonic assisted curing technique for improving the diffusion of sodium chloride in porcine meat. Journal of Food Engineering. 2009;**91**:353-362

[16] Dolatowski ZJ, Stadnik J, Stasiak D. Applications of ultrasound in food technology. Acta Scientiarum Polonorum Technologia Alimentaria. 2007;**6**:89-99

[17] Mason TJ, Paniwnyk L, Lorimer JP. The uses of ultrasound in food technology. Ultrasonics Sonochemistry. 1996;**3**:S253-S260

[18] Ercan S, Soysal Ç. Use of ultrasound in food preservation. Natural Science. 2013;**5**:5-13. DOI: 10.4236/ns.2013.58A2002

[19] Zheng L, Sun DW. Innovative applications of power ultrasound during food freezing processes — A review. Trends in Food Science and Technology. 2006;**17**:16-23

[20] Miles CA, Morley MJ, Rendell M. High power ultrasonic thawing of frozen foods. Journal of Food Engineering. 1999;**39**:151-159

[21] Caraveo O, Alarcón-Rojo AD, Renteria A, Santellano E, Paniwnyk L. Physicochemical and microbiological characteristics of beef treated with high-intensity ultrasound and stored 4°C. Journal of the Science of Food and Agriciculture. 2015;**95**:2487-2493. DOI: 10.1002/jsfa.6979

[22] Peña-González EM, Alarcón-Rojo AD, Rentería A, García I, Santellano E, Quintero A, Luna L. Quality and sensory profile of ultrasound-treated beef. Italian Journal ofFood Science. 2017;**29**:463-475. DOI: 10.14674/1120-1770/ijfs.v604

[23] Lawrie RA, Ledward DA. Lawries's Meat Science (7th Edn). Cambridge: CRC/Woodhead Publishing; 2006. pp. 98-103

[24] Honikel KO. Reference methods for the assessment of physical characteristics of meat. Meat Science. 1998;**49**:447-457

[25] Pardo JE, Tarjuelo L, Mateos B, Hurtado J, Alvariuz A. Parámetros a evaluar en el control de la calidad de las carnes. Eurocarne. 2006;**152**:19-34

[26] Garcia-Rey C, Fenoll A, Aguilar L, Casal J. Effect of social and climatological factors on antimicrobial use and *Streptococcus pneumoniae* resistance in different provinces in Spain. Journal of Antimicrobial Chemotherapy. 2004;**54**:465-471. http://dx.doi.org/10.1093/jac/dkh375

[27] Mandour HA, Bashari M, Lagnika C, He Q, Sun H. Effect of ultrasound treatment prior to vacuum and modified atmosphere packaging on microbial and physical characteristics of fresh beef. Journal of Food and Nutrition Research. 2014;(6):312-320

[28] Patist A, Bates D. Ultrasonic innovations in the food industry: From the laboratory to commercial production. Innovative Food Science and Emerging Technologies. 2008;**9**: 147-154

[29] Huff-Lonergan E, Lonergan SM. Mechanisms of water-holding capacity of meat: The role of postmortem biochemical and structural changes. Meat Science. 2005;**71**:194-204

[30] Got F, Culioli J, Berge P, Vignon X, Astruc T, Quideau JM, Lethiecq M. Effects of high-intensity high-frequency ultrasound on ageing rate, ultrastructure and some physico-chemical properties of beef. Meat Science. 1999;**51**:35-42

[31] Stadnik J, Dolatowski ZJ, Baranowska HM. Effect of ultrasound treatment on water holding properties and microstructure of beef (m. Semimembranosus) during ageing. LWT Food Science and Technology. 2008;**41**:2151-2158

[32] Dolatowski Z, Stasiak DM, Latoch A. Effect of ultrasound processing of meat before freezing on its texture after thawing. Electronic Journal of Polish Agricultural Universities. 2000;**3**(2)

[33] Stadnik J. Influence of sonication on the oxidative stability of beef. Roczniki Instytutu Przemyslu Miesnego I Tluszczowego. 2008;**47**:63-68

[34] Jayasooriya SD, Torley PJ, D'Arcy BR, Bhandari BR. Effect of high power ultrasound and ageing on the physical properties of bovine semitendinosus and Longissimus muscle. Meat Science. 2007;**75**:628-639

[35] Honikel KO, Rosenbauer H, Fischer K, Muller WD, Przytulla J. Influence of vitamin E and rape seed oil on quality characteristics of pork and pork products. Fleischwirtschaft. 1998;**78**:1205-1207

[36] Lawrie RA. Lawrie's Meat Science. 6th ed. Cambridge: Woodhead Publishing; 1998. pp. 105-118

[37] Melody JL, Lonergan SM, Rowe LJ, Huiatt TW, Mayes MS, Huff-Lonergan E. Early post mortem biochemical factors influence tenderness and water-holding capacity of three porcine muscles. Journal of Animal Science. 2004;**82**:1195-1205

[38] Janz JAM, Morel PCH, Wilkinson BHP, Purchas RW. Preliminary investigation of the effects of low-level dietary inclusion of fragrant essential oils and oleoresins on pig performance and pork quality. Meat Science. 2007;**75**:350-355

[39] Gambuteanu C, Filimon V, Alexe P. Effects of ultrasound on technological properties of meat a review. Annals of Food Science and Technology. 2013;**2**:176-182

[40] Chang HJ, Wang Q, Tang CH, Zhou GH. Effects of ultrasound treatment on connective tissue collagen and meat quality of beef semitendinosus muscle. Journal of Food Quality. 2015;**38**:256-267. DOI: 10.1111/jfq.12141

[41] Gómez-Salazar JA, Ochoa-Montes DA, Ozuna C, Sosa-Morales ME, Cerón-García A. Effect of acid Marination assisted by power ultrasound on the quality of rabbit meat. Journal of Food Quality. 2018:1-6

[42] Smith DP. Effect of ultrasonic marination on broiler breast meat quality and Salmonella contamination. International Journal of Poultry Science. 2011;**10**:757-759

[43] Pohlman FW, Dikeman ME, Kropf DH. Effects of high intensity ultrasound treatment, storage time and cooking method on shear, sensory, instrumental color and cooking properties of packaged and unpackaged beef pectoralis muscle. Meat Science. 1997; **46**:89-100

[44] Kang DC, Gao XQ, Ge QF, Zhou GH, Zhang WG. Effects of ultrasound on the beef structure and water distribution during curing through protein degradation and modification. Ultrasonics Sonochemistry. 2017;**38**:317-325. DOI: 10.1016/j.ultsonch.2017.03.026

[45] Wang A, Kang D, Zhang W, Zhang C, Zou Y, Zhou G. Changes in calpain activity, protein degradation and microstructure of beef m. Semitendinosus by the application of ultrasound. Food Chemistry. 2018;**245**:724-730. DOI: 10.1016/J.FOODCHEM.2017.12.003

[46] O'Sullivan MG, Byrne DV, Martens M. Evaluation of pork colour sensory colour assessment using trained and untrained sensory panellists. Meat Science. 2003;**63**:119-129

[47] MG O'S, Byrne DV, Martens H, Gidskehaug LH, Andersen HJ, Martens M. Evaluation of pork color: Prediction of visual sensory quality of meat from instrumental and computer vision methods of colour analysis. Meat Science. 2003;**65**:909-918

[48] Stadnik J, Dolatowski ZJ. Influence of sonication on Warner-Bratzler shear force, colour and myoglobin of beef (m. Semimembranosus). European Food Research Technology. 2011;**233**:553-559

[49] Sikes AL, Mawson R, Stark J, Warner R. Quality properties of pre- and post-rigor beef muscle after interventions with high frequency ultrasound. Ultrasonics Sonochemistry. 2014;**21**:2138-2143

[50] Koohmaraie M, Seidemann SC, Schollmeyer JE, Dutson TR, Crouse JD. Effect of post-mortem storage on Ca (++)-dependent proteases, their inhibitor and myofibril fragmentation. Meat Science. 1987;**19**:187-196

[51] Lyng JG, Allen P, McKenna BM. The influence of high intensity ultrasounds baths on aspects of beef tenderness. Journal of Muscle Foods. 1997;**8**:237-249

[52] Zhou GH, Xu XL, Liu Y. Preservation technologies for fresh meat—A review. Meat Science. 2010;**86**:119-128

[53] Xiong GY, Zhang LL, Hang W, Wu J. Influence of ultrasound and proteolytic enzyme inhibitors on muscle degradation, tenderness, and cooking loss of hens during aging. Czech Journal of Food Science. 2012;**30**:195

[54] Zhao YY, Wang P, Zou YF, Li K, Kang ZL, Xu XL, Zhou GH. Effect of pre-emulsification of plant lipid treated by pulsed ultrasound on the functional properties of chicken breast myofibrillar protein. Food Research International. 2014;**58**:98-104

[55] Barekat S, Soltanizadeh N. Effects of ultrasound on microstructure and enzyme penetration in beef longissimus lumborum muscle. Food and Bioprocess Technology. 2018;**11**: 680-693. DOI: 10.1007/s11947-017-2043-8

[56] Dolatowski ZJ, Twarda J, Dudek M. Changes in hydration of meat during the ageing process. Annales Universitatis Mariae Curie-Skłodowska, sectio E. 2004;**59**:1595-1606

[57] Chandrapala J. Low intensity ultrasound applications on foods systems. International Food Research Journal. 2015;**22**:888-895

[58] Lyng JG, Allen P, McKenna BM. The effect on aspects of beef tenderness of pre- and post-rigor exposure to a high intensity ultrasound probe. Journal of the Science of Food Agriculture. 1998;**78**:308-314

[59] McDonnell CK, Allen P, Morin C, Lyng JG. The effect of ultrasonic salting on protein and water protein interactions in meat. Food Chemistry. 2014;**147**:245-251

[60] Jørgensen AS, Christensen M, Ertbjerg P. Marination with Kiwifruit Powder Followed by Power Ultrasound Tenderizes Porcine m. Biceps femoris. In: International Conference of Meat Science and Technology, Cape Town. Sudafrica; 2008

[61] Chang HJ, Xu XL, Zhou GH, Li CB, Huang M. Effects of characteristics changes of collagen on meat physicochemical properties of beef semitendinosus muscle during ultrasonic processing. Food and Bioprocess Technology. 2012;**5**:285-297

[62] Leal-Ramos MY, Alarcón-Rojo AD, Mason TJ, Paniwnyk L, Alarjah M. Ultrasound-enhanced mass transfer in halal compared with non-halal chicken. Journal of the Science of Food and Agriculture. 2011;**91**:130-133

[63] Ozuna C, Puig A, García-Pérez JV, Mulet A, Cárcel JA. Influence of high intensity ultrasound application on mass transport, microstructure and textural properties of pork meat (Longissimus dorsi) brined at different NaCl concentrations. Journal of Food Engineering. 2013;**119**:84-93

[64] International Commission on Microbiological Specifications of Foods (ICMSF). Microorganisms in Foods 6: Microbial Ecology of Food Commodities (Microorganisms in Foods). New York, NY: Springer; 2005

[65] Forsythe J. Alimentos seguros: Microbiología. Acribia: España; 2003

[66] Hennekinne A, De Buyser ML, Dragacci S. *Stafilococcus aureus* and its food poisoning toxins: Characterization and outbreak investigation. FEMS Microbiology Reviews. 2012;**36**:815-836

[67] Ercolini D, Russo F, Nasi A. Mesophilic and psychrotrophic bacteria from meat and their spoilage potential in vitro and in beef. Applied and Environmental Microbiology. 2009;**75**(7):1990-2001. DOI: 10.1128/AEM.02762-08

[68] Farrell GM, Upton ME. The effect of low temperature on the growth and survival of *Stafilococcus aureus* and *Salmonella typhimurium* when inoculated into bacon. Journal of Food Technology. 1978;**13**:15-23

[69] Sheridan JJ. The effect of freezing on the survival of pathogens in different meat types and the effect of varying lean fat rations. In: Hinton MH, Rowlings C, editors.Factors Affecting the Microbial Quality of Meat: 3. Cutting and further processing. Bristol: University of Bristol Press; 1996. pp. 97-107

[70] Speck ML, Ray B. Effect of freezing and storage on microorganisms in frozen foods: A review. Journal of Food Protection. 1997;**40**:333-336

[71] Davies A, Board R. The Microbiology of Meat and Poultry. London: Blackie Academic and Professional; 1998

[72] Tortorello ML. Encyclopedia of Food Microbiology. New York: Academic Press; 2014

[73] Piyasena P, Mohareb E, McKellar RC. Inactivation of microbes using ultrasound: A review. International Journal of Food Microbiology. 2003;**87**:207-216

[74] Mason TJ. Power ultrasound in food processing—The way forward. In: Povey MJW, Mason TJ, editors.Ultrasound in Food Processing. Glasgow:Blackie Academic & Professional; 1998. pp 104-124

[75] López-Malo A, Palou E, Jiménez-Fernández M, Maris-Alzamora S, Guerrero S. Multifactorial fungal inactivation combining thermosonication and antimicrobials. Journal of Food Engineering. 2005;**67**:87-93

[76] Torley PJ, Bhandari BR. Handbook and food preservation. Ultrasounds in food processing and preservation. Cap. 29. 2nd Edition. New York: CRC Press; 2007

[77] Luo H, Schmid F, Grbin PR, Jiranek V. Viability of common wine spoilage organisms after exposure to high power ultrasonics. Ultrasonics Sonochemistry. 2012;**19**:415-420

[78] Mukhopadhyay S, Ramaswamy R. Applications of emerging technologies to control Salmonella in foods: A review. Food Research International. 2011;**45**:666-677

[79] Pagan R, Manas P, Alvarez I, Condon S. Resistance of Listeria monocytogenes to ultrasonic waves under pressure at sublethal (manosonication) and lethal (manothermosonication) temperatures. Food Microbiology. 1999;**16**:139-148

[80] Morild RK, Christiansen PS, Anders H, Nonboe U, Aabo S. Inactivation of pathogens on pork by steam-ultrasound treatment. Journal of Food Protection. 2001;**74**:769-775

[81] Smith NB, Cannon JE, Novakofski JE, Mckeith FK, O'Brien WD Jr. Tenderization of *Semitendinosus* muscle using high intensity ultrasound. Ultrasonics Symposium. 1991: 1371-1373

[82] Kordowska-Wiater M, Stasiak DM. Effect of ultrasound on survival of gram-negative bacteria on chicken skin surface. Bulletin of the Veterinary Institute in Pulawy. 2011;**55**:207-210

[83] Musavian HS, Krebs NH, Nonboe U, Corry JE, Purnell G. Combined steam and ultrasound treatment of broilers at slaughter: A promising intervention to significantly reduce numbers of naturally occurring campylobacters on carcasses. International Journal of Food Microbiology. 2014;**176**:23-28

[84] Herceg Z, Markov K, Šalamon BS, Jambrak AR, Vukušić T, Kaliterna J. Effect of high intensity ultrasound treatment on the growth of food spoilage bacteria. Food Technology and Biotechnology. 2013;**51**:352-359

Chapter 4

Role of Dietary Carotenoids in Different Etiologies of Chronic Liver Diseases

Uzma Latief and Riaz Ahmad

Abstract

Carotenoids are tetraterpenoid organic pigments synthesized by a variety of plants and microorganisms. Dietary carotenoids, taken by animals through food, play an essential role in cell differentiation, morphogenesis, vision, prevention of cancer, atherosclerosis, and age-related macular degeneration in humans due to their potential to suppress oxidative stress. As reactive oxygen species and oxidative damage to biomolecules have been found to be involved in the causation and progression of chronic liver diseases (CLDs), including hepatocellular carcinoma (HCC), which is one of the major causes of morbidity and mortality worldwide. Therefore, dietary antioxidants, which inactivate reactive oxygen species and obstruct oxidative damage, are considered as vital prophylactic strategic molecules. Data from various epidemiological studies and clinical trials strongly validate the observation that adequate carotenoid supplementation may significantly reduce the risk of several liver disorders. This chapter, thus, provides a comprehensive account of dietary carotenoids and includes the recent information with respect to their role in prevention of liver diseases.

Keywords: β-carotene, lycopene, lutein, β-cryptoxanthin, oxidative stress, chronic liver diseases

1. Introduction

There are unambiguous evidences that regular consumption of vegetables and fruits decreases the prevalence of chronic liver disease (CLD) [1, 2]. One of the main reasons of the health organizations to increase the consumption of vegetables and fruits is that these are good sources of carotenoids and other biologically active phytochemicals. Carotenoids are naturally occurring tetraterpenoids and represented by approximately 700 different structural

variants, but only 50 have been reported to play an important role in human diet [3]. They are synthesized by plants, fungi, algae, and bacteria [4]. They are classified into two groups, carotenes containing only carbon and hydrogen atoms and xanthophylls containing at least one oxygen atom [5]. These carotenoids are further paired into two classes, provitamin A carotenoids (β-carotene and β-cryptoxanthin) and non-provitamin A carotenoids (lycopene and lutein) [6]. In animals and human beings, carotenoids particularly β-carotene, lycopene, lutein, and β-cryptoxanthin play an important role in protection against photooxidative damage by acting as singlet molecular oxygen and peroxy radical scavenger [7, 8]. There are increasing evidences that an alteration of the cellular redox state involving production of reactive oxygen species (ROS) plays a central role in different steps that initiate and regulate the progression of various liver diseases irrespective of the cause. Liver damage caused by reactive oxygen species is induced by alcohol, alteration of lipid, viruses, carbohydrate metabolism, and xenobiotics [9]. In this context, provitamin A activity of these carotenoids [10, 11] has received considerable interest by researchers and health professionals to prevent chronic liver diseases.

Chronic liver diseases (CLDs) are the major concern throughout the world because of the increasing death rate due to them. CLDs such as alcoholic liver disease (ALD), nonalcoholic fatty liver disease (NAFLD), and viral hepatitis (B and C) progress to fibrosis, cirrhosis, and ultimately hepatocellular carcinoma (HCC) [12, 13]. HCC is the fifth most common type of cancer and third most common cause of cancer mortality throughout the world [14]. Globally, liver cancer is responsible for causing more than 700,000 deaths annually [15]. Oxidative stress resulting from various sources is a major mechanism for hepatic fibrosis and cirrhosis [16].

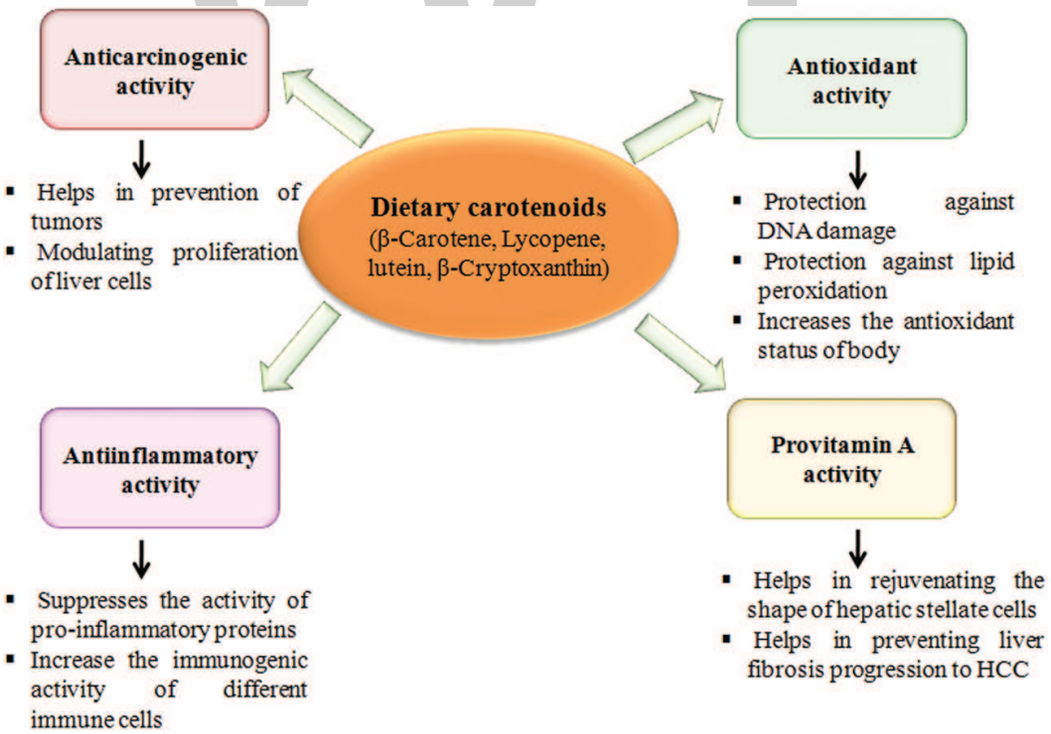

Figure. Functions of various dietary carotenoids against chronic liver diseases.

The major source of ROS in hepatocytes is the mitochondria. Hepatocytes contain many mitochondria and thus have high production of ROS. An imbalance between the production of ROS and antioxidant defense generates various pathophysiological alterations in the hepatic cells, such as activation of hepatic stellate cells (HSCs), initiation of collagen formation, and beginning of proliferative processes [17–21]. HSCs are responsible for storage of more than 90% of the body's vitamin A content as retinyl esters in normal liver. Chronic liver injuries may cause these cells to undergo activation and lose their capacity to store vitamin A while acquiring pro-inflammatory and proliferative properties, responsible for fibrogenesis [22]. Fibrosis if not treated may lead to end stage of liver damage, that is, liver cancer (**Figure**) [15, 23, 24].

In this context, dietary carotenoids being an important part of antioxidant defense system and precursor of vitamin A play an important role in the prevention of chronic liver diseases. Nowadays, due emphasis is given on the search of alternative therapeutics or medicines as these have least side effects and are cost effective. In this context, this chapter focuses on the use of dietary carotenoids in chronic liver disease prevention which have been documented extensively in the recent literature.

2. β-Carotene and chronic liver diseases

β-Carotene is the major carotenoid present in human diet. It is mainly present in plant sources of human diet such as carrots, pumpkin, spinach, sweet potato, cantaloupe, papaya, and mangoes [25]. It is a potent quencher of singlet oxygen, scavenges peroxy radicals, reduces levels of reactive oxygen species, and augments the investigated antioxidant enzyme activities [26, 27]. It is also a precursor of vitamin A and is reportedly converted to vitamin A by the action of β-carotene 15,15′-monooxygenase [10].

Various studies have demonstrated the hepatoprotective potential of β-carotene carried out in animal models, cell lines, and human beings. Patel and Sail reported that β-carotene protects the physiological antioxidants against aflatoxin-B1-induced carcinogenesis in albino rats [28]. β-Carotene supplementation increases the levels of vitamin C, glutathione, and glutathione-related enzymes which function as free radical scavenger and, thus, reduces the toxicity of aflatoxin-B1 in rats. In another study, it was found that various antioxidants including β-carotene modulate the hepatotoxicity induced by aflatoxin-B1 [29]. Both HBV and HCV have been found to elevate inflammatory and oxidative conditions in hepatocytes. Moreover, in chronic liver diseases, hepatic stellate cells, which store almost 70–90% of vitamin A, become activated, lose their retinoid content, and produce extracellular matrix, which is responsible for liver fibrosis [30]. Hepatic fibrosis can lead to cirrhosis and in some cases hepatocellular carcinoma (HCC). Through its provitamin A activity and roles in inhibiting reactive oxygen species, β-carotene has been shown to ameliorate the development and progression of HBV- and HCV-induced HCC. In a human study, it was found that the oxidative stress increased in patients with chronic hepatitis C (CHC). Various antioxidants such as retinol, α- and γ-tocopherol, β-cryptoxanthin, lycopene, and α- and β-carotene were decreased in serum of CHC patients, and levels in liver tissue seem to reflect serum levels [31]. In Chinese patients,

it was observed in Chinese patients that the serum levels of retinoids are low in HBV-induced HCC [32, 33]. In another study, it was shown that a high percentage of patients have vitamin A deficiency in serum in chronic hepatitis C [34, 35]. This condition reflects no response to antiviral therapy, indicating that serum levels of vitamin A could regulate the responsiveness to interferon-based antiviral therapy [35].

The generation of very high levels of oxidative stress during the metabolism of alcohol may exceed the antioxidant defense ability of the body and cause the development of liver dysfunction. It has been reported that β-carotene exerts protective effect on chronic ethanol-fed rats [36]. It was shown that ethanol treatment causes increase in oxidative stress which could stimulate apoptosis in the liver, thus leading to liver injury. However, the lower dose of β-carotene supplementation (0.52 mg/kg BW/day), which acted as an antioxidant, decreased the ROS level by downregulating lipid peroxidation and CYP2E1 expression. Moreover, it prevented ethanol-induced liver damage by impeding hepatic apoptosis via inhibiting caspase-9 and caspase-3 expressions and increasing Bcl-xL expression in the liver. In addition, the higher dose of β-carotene supplementation (6.2 mg/kg BW/day) possibly halted ethanol-induced liver damage through inhibiting TNF-α secretion and lipid peroxidation in ethanol-fed rats. Literature revealed that β-carotene supplementation can prevent liver damage in rats with chronic alcohol consumption [37, 38]. β-carotene supplement is known to attenuate ethanol-induced liver damage, decreased oxidative stress and increased GSH concentrations in erythrocytes and the liver. β-Carotene may act as an antioxidant, scavenging lipophilic radicals produced by ethanol within the membranes [39]. In a human study, it was found that in alcoholic patients with liver damage, the plasma β-carotene level was found to be lowered than in control subjects [40]. ALD is also associated with depleted levels of hepatic vitamin A [41]. As β-carotene is the precursor of vitamin A, thus, the supplementation of this carotenoid tends to regain the hepatic vitamin A content, thus facilitating attenuation of the disease.

In a study, it was found that dietary intake of apricot reduced the risks of hepatic steatosis and damage induced by CCl_4 in Wistar rats [42]. Apricot is believed to have high content of carotenoids, especially β-carotene. Markers of oxidative stress such as malondialdehyde, total GSH levels, catalase, SOD, and GSH peroxidase activities were significantly altered by CCl_4 indicating increased oxidative stress. Hepatic damage and steatosis imposed by high concentration of ROS were ameliorated by β-carotene-rich apricot intake. Consumption of tomato juice which contains lycopene and β-carotene as the major components reduces plasmatic triglycerides, steatosis, and very low-density lipoproteins. Also, it elevates lipid metabolism by inducing the overexpression of genes involved in more efficient fatty acid oxidation in rats [43]. It is also shown that Campari tomato, which contains more β-carotene and lycopene than regular tomato, ameliorated diet-induced obesity, dyslipidemia, and hepatosteatosis via downregulation of gene expression related to lipogenesis in the zebra fish model. It decreased sterol regulatory element-binding transcription factor 1 (SREBF1) mRNA by increasing the forkhead box O1 (foxo1) gene expression. This may be due to high percentage of β-carotene in this strain of tomato which is responsible for downregulating the expression of SREBF1 [44]. In Chinese population greater levels of carotenoids such as β-carotene in serum have been correlated

with low prevalence of NAFLD [45]. These carotenoids mediate the protective effects against NAFLD through antioxidant mechanism, enhancing gap junction communication, reducing inflammation, and modulation of gene expression. In another human study, it was found that NAFLD has reverse relationship with vitamin A nutritional status in individuals with class III obesity. Retinol and β-carotene serum levels were evaluated as biochemical indicators. The researchers observed low serum retinol and β-carotene serum levels in the patients with NAFLD [46].

In one study, β-carotene and vanadium inhibit the diethylnitrosamine (DEN)-induced hepatic carcinogenesis in rats [47]. It was observed that treatment of β-carotene and vanadium reduced the number and size of the hyperplastic nodules significantly, while the combination treatment proved as an additive effect, decreasing number and size of the hyperplastic nodules from 89 to 22%. Further, it significantly reduced the level of cytosolic glutathione and glutathione-S-transferase (GST) activity and stabilized the aerobic metabolism and hepatic architecture of the cells. In another study involving cell lines, it was found that acyclic retinoid (synthetic analog of retinoids) retards overexpression of Ras/Erk signaling system, thereby declining the progression of HCC [48]. In a human study, it was found that greater intake of retinol, total vitamin A, and carotenes decreases the risk of primary liver cancer at an intake of 1000 μg retinol equivalent (RE)/day or greater from food sources [49]. In another human study in the presence of hepatitis B virus, levels of dietary and serum vitamin A and β-carotene were significantly lower in HCC patients than in the control subjects [32].

3. Lycopene and chronic liver diseases

Lycopene, like other carotenoids, is a natural pigment mainly present in tomato and products of tomato. It is also present in watermelon, apricot, pink guava, pink grapefruit, and papaya [50]. It does not show provitamin A activity since it lacks the β-ionone ring structure which is characteristic in other carotenes that are precursors of vitamin A [50, 51]. Various studies showed that lycopene possesses antioxidant, anticancer, anti-cardiovascular disease, and detoxification abilities in many epidemiological and animal experiments with few side effects [52, 53, 54].

As a dietary phytochemicals, lycopene has been demonstrated to mitigate AFB1-induced adverse effects in vitro and in vivo. The carcinogenicity of aflatoxin B1 (AFB1) in HepG2 cells was prevented by lycopene through decreasing DNA damage and AFB1-N7-guanine (AFB1-N7-Gua) adduct formation [55]. In another study, it was found that lycopene, because of its high antioxidant activity and free radical scavenging capacity, has been shown to be effective against oxidative stress due to aflatoxin. Lycopene blocks phase 1 metabolic enzymes of AFB such as 3A4, 2A6, and 1A2 [56]. In another study, lycopene relieves AFB1-induced liver injury through enhancing hepatic antioxidation and detoxification potential with Nrf2 activation [57]. Lycopene, a nutritional antioxidant, has also been shown for its hepatoprotective potential in D-galactosamine/lipopolysaccharide (D-GalN/LPS)-induced

hepatitis in rats [58, 59]. It is able to affect the lipoprotein metabolism by restoring the altered levels of lipid-metabolizing enzymes and stabilizing the arrangement of lipoprotein levels during experimentally induced hepatitis. Another study demonstrated that the regular use of our carotenoid-based functional food minimizes the severity of ribavirin-induced anemia in patients with CHC and improves tolerance to the full dose of antiviral therapy [60]. It was found that a mixture of various carotenoids particularly lycopene seems to be promising for the prevention of liver cancer in hepatitis virus-infected patients with cirrhosis [61, 62].

It is found that tomato powder which is rich in lycopene acts as a novel candidate for prevention against alcohol-related hepatic injury in rodents [63]. It provides protection against alcohol-induced liver injury by suppressing CYP450 2E1 induction. It has been reported that lycopene prevents nonalcoholic steatohepatitis induced by high-fat diet [64, 65, 66]. It can reduce high-fat diet-induced steatohepatitis by reducing oxidative stress to the cells. It has been also shown that incorporation of lycopene in balanced diet prevents NAFLD [67, 68]. Lycopene may be a useful functional compound for treating NAFLD by regulating hepatic lipid metabolism [69]. Lycopene inhibits the downregulation of miR-21, which led to the downregulation of fatty acid-binding protein 7 (FABP7) at both the transcriptional and translational levels, thus inhibiting hepatic steatosis induced by high-fat diet.

Lycopene also showed beneficial effects against hepatocellular carcinoma. It provides protection against HCC by modulation of cellular proliferation, glycolysis, and ultrastructure of hepatic cells [70]. Lycopene supplementation also prevents high-fat diet-induced HCC incidence in mice. It suppressed oncogenic signals, including methionine mRNA, β-catenin protein, and mammalian target of rapamycin (mTOR) complex 1 activation. These results provide novel experimental evidence that dietary lycopene and its metabolites can be used to prevent liver cancer and reduce cancer risk in patients with NAFLD [71].

4. Lutein and chronic liver diseases

Lutein is a non-provitamin A carotenoid that belongs to the oxycarotenoid family. It is present in dark green leafy vegetables, such as kale and spinach and eggs [72, 73]. It is commercially prepared from the marigold flower (*Tagetes erecta* L.) in which it occurs at 1.5–1.8%. It directly quenches free radicals, especially singlet oxygen species [74].

Lutein possesses an antiviral activity against hepatitis B. It exerts its antivirus effects through inhibition of hepatitis B virus transcription [75]. Because of its strong antioxidant potential, lutein has been shown to provide protection against ethanol-induced hepatic damage [76]. It increases levels of antioxidant enzymes, like superoxide dismutase, catalase, glutathione peroxidase, and glutathione, and decreases levels of hydroxyproline. Another study demonstrated that lutein attenuates alcohol-induced liver damage in rats by regulating inflammation and oxidative stress [77]. Its supplementation downregulated inflammatory proteins and cytokines with collateral upregulation of Nrf2 levels and antioxidant enzymatic activities.

Lutein decreases inflammation and oxidative stress in the liver and eyes of guinea pigs fed with hypercholesterolemic diet [78]. This carotenoid could prevent degenerative conditions of the liver by decreasing the free cholesterol pool and attenuating lipid peroxidation and pro-inflammatory cytokine production. Further, attenuated inflammatory state in the liver could be explained by decreased NF-κB DNA-binding activity. Another study demonstrated that lutein possesses ameliorative effect against NAFLD [79]. It suggests that lutein supplementation could protect against hepatic lipid accumulation and insulin resistance induced by high-fat diet, possibly via the activation of the expression of sirtuin 1 (SIRT1) and, subsequently, peroxisome proliferator activated receptor (PPAR)-α and other key factors in insulin signaling [80]. Sirtuin 1 is reported to have therapeutic potential in NAFLD and play a key role in insulin sensitivity. SIRT1 regulates the expression of PPAR-α, a key factor in the regulation of lipid metabolism [81, 82]. Lutein has also been found to have anticarcinogenetic effects against NDEA-induced HCC in rats [83]. Inhibition of carcinogenesis by this carotenoid could be because of the combined effect of its antioxidant activity along with the inhibition of cytochrome P450 enzymes, inducing detoxifying enzymes such as glutathione-S-transferase and UDP glucuronyl transferase.

5. β-Cryptoxanthin and chronic liver diseases

β-Cryptoxanthin is an oxygenated carotenoid usually present in squash, pepper, papaya, sweet pickles, carrots, and orange juice [84]. It is a xanthophyll carotenoid specifically found in the Satsuma mandarin (*Citrus unshiu* Marc.). Similar to other carotenoids, β-cryptoxanthin has an antioxidant activity [85, 86]. β-Cryptoxanthin is readily absorbed and relatively abundant in human plasma, together with β-carotene, lycopene, lutein, and zeaxanthin [87].

It has been shown that β-cryptoxanthin ameliorates diet-induced nonalcoholic steatohepatitis by repressing inflammatory gene expression in mice [88]. β-Cryptoxanthin suppressed the expression of lipopolysaccharide (LPS)-inducible and TNFα-inducible genes in NASH. Elevated levels of the oxidative stress marker thiobarbituric acid-reactive substances (TBARS) were lowered by β-cryptoxanthin in NASH. Thus, it represses inflammation and the resulting fibrosis probably by primarily repressing the increase and activation of macrophages and other immune cells. Reducing reactive oxygen species is likely to be a major mechanism of inflammation and injury suppression in the livers of mice with NASH. Another study revealed that β-cryptoxanthin reversed steatosis, inflammation, and fibrosis progression in preexisting NASH in mice [89]. Thus, β-cryptoxanthin prevents and reverses insulin resistance and steatohepatitis through decreasing activation of macrophages or Kupffer cells in a lipotoxic model of NASH. It was found that plasma levels of carotenoids such as β-carotene, lycopene, lutein, and β-cryptoxanthin were decreased in patients with NASH [90]. This study suggests that antioxidant supplementation may be a rational option for the treatment of NASH. In patients of NAFLD, it was found that β-cryptoxanthin treatment inhibits its progression [91]. β-Cryptoxanthin supplementation is very effective in raising antioxidant and anti-inflammation activities in patients of NAFLD (**Table**).

S. No.	Carotenoid investigated	Object of study	Results	References
1	β-Carotene	Rat liver	Liver antioxidant enzymes such as glutathione peroxidase, glutathione-S-transferase, catalase, and vitamin C were elevated	[28]
2	β-Carotene, vitamin E, selenium, silymarin, and coenzyme Q_{10}	Rat liver	β-Carotene intake restored the levels of hepatic glutathione, RNA, and serum protein thiols relative to control animals	[29]
3	β-Carotene	Rat liver	Oxidative stress is decreased by CYP2E1 expression and lipid peroxidation. β-Carotene also inhibited hepatic apoptosis via inhibiting caspase-3 and caspase-9 and increasing Bcl-xL expression in the liver	[36]
4	Retinol, α- and γ-tocopherol, lutein, β-cryptoxanthin, lycopene, and α- and β-carotene	Human liver and serum	Increased oxidative stress is present in patients with chronic hepatitis C. Antioxidants were severely depleted in serum and liver tissue	[31]
5	β-Carotene	Rat liver	β-Carotene prevented ethanol-induced lipid peroxidation in hepatic tissues	[38]
6	β-Carotene	Rat liver	Malondialdehyde, total glutathione levels and catalase, superoxide dismutase, and glutathione peroxidase activities were restored	[42]
7	α- and β-Carotene, lutein, and zeaxanthin	Human serum	Serum carotenoid levels were inversely associated with the risk of NAFLD	[45]
8	Dietary carotenes and vitamin A	Human liver	Dietary consumption of retinol, carotenes, and total vitamin A decreases the risk of primary liver cancer risk	[49]
9	β-Carotene, β-apo-8′-carotenal, canthaxanthin, astaxanthin, and lycopene	Rat liver	Carotenoids exert protective effect against aflatoxin B1-induced liver preneoplastic foci and DNA damage	[55]
10	Lycopene	Rat liver	Enhanced hepatic antioxidant and detoxification potential	[57]
11	Lycopene	Rat liver	Liver function test enzymes, cholesterol, triglycerides, free fatty acids, and phospholipids in serum and liver were restored	[58]
12	Lycopene	Rat liver	It restored the increase in very-low-density lipoproteins, decrease in high-density lipoproteins, and lipid-metabolizing enzymes	[59]
13	Lycopene	Rat and mouse liver	Hepatic CYP2E1 protein levels, peroxisome proliferator-activated receptor-α, inflammatory gene expression, and reticulum stress markers were restored	[63]

S. No.	Carotenoid investigated	Object of study	Results	References
14	Lycopene	Rat liver	Supplementation with lycopene lowered serum malondialdehyde and tumor necrosis factor (TNF-α) levels and elevated liver GSH level	[64]
15	Lycopene	Rat liver	Lycopene treatment reverted changes in liver weight, serum low-density lipoproteins, total hepatic cholesterol, and activity of hepatic SOD, catalase, and glutathione peroxidase	[68]
16	Lycopene	Rat liver	Lycopene supplementation downregulated TNF-α and CYP2E1 expression and decreased infiltration of liver fats	[67]
17	Lycopene	Rat liver	Lycopene provides protection against NAFLD by alleviating amino acid depletion, recovery of the redox balance in liver, and incrementing L-carnitine levels	[65]
18	Lycopene	Mouse liver	It decreases the expression of cell proliferation-associated genes (PCNA, cyclin D1, and p21) and glycolytic enzymes	[70]
19	Lycopene	Mouse liver	Lycopene and its metabolites reduce cancer in NAFLD	[71]
20	Lutein	Human hepatoblastoma cells	Lutein inhibited the activity of HBV full-length promoter	[75]
21	Lutein	Rat liver	SOD, catalase, glutathione peroxidase, glutathione, and hepatic hydroxyproline content were restored by lutein	[76]
22	Lutein	Rat liver	Inflammatory proteins such as NF-κB, COX-2, and iNOS were downregulated along with the upregulation of Nrf2 levels and activities of antioxidant enzymes	[77]
23	Lutein	Rat liver	Lutein administration decreased serum and hepatic cholesterol and triglyceride. It also increased the expression of key factors involved in hepatic insulin signaling	[79]
24	Lutein	Rat liver	Lutein treatment inhibits cytochrome P450 enzymes and increases detoxifying enzymes such as glutathione-S-transferase and UDP glucuronyl transferase	[83]
25	β-Cryptoxanthin	Mouse liver	β-Cryptoxanthin reduced the levels of TBARS and suppressed the expression of lipopolysaccharide and TNF-α inducible gene. It further suppressed the activation of macrophages, T helper, and cytotoxic cells	[88]

S. No.	Carotenoid investigated	Object of study	Results	References
26	β-Cryptoxanthin	Mouse liver	β-Cryptoxanthin reduced total hepatic macrophage content	[89]
27	β-Cryptoxanthin	Human liver	β-Cryptoxanthin induced antioxidant and anti-inflammatory activities in NAFLD patients	[91]
28	Fucoxanthin	Human liver	It reduced liver and body fat content and improved liver function tests	[93]

Table. Effects of carotenoids on chronic liver diseases in cell line, human, and animal models.

6. Other carotenoids and chronic liver diseases

Other carotenoids such as α-carotene, fucoxanthin, and zeaxanthin also show promising effects against chronic liver injury. It has been found that α-carotene has an inhibitory effect on spontaneous liver carcinogenesis in male mice [92]. α-Carotene significantly decreases the mean number of hepatomas per mouse. In another study, it was found that fucoxanthin promoted weight loss, reduced body and liver fat content, and improved liver function tests in obese nondiabetic women [93]. Zeaxanthin also shows protective effects against nonalcoholic steatohepatitis [94]. It significantly prevented NASH progression by decreasing oxidative stress and liver fibrosis. It has been found also that zeaxanthin showed therapeutic effects against alcoholic liver diseases [95]. Zeaxanthin show protective effects through the lower expression level of cytochrome P450 2E1 (CYP2E1), diminished activity of nuclear factor kappa B (NF-κB) through the restoration of its inhibitor kappa B alpha (IκBα), and the modulation of MAPK pathways including p38 MAPK, JNK, and ERK.

7. Conclusions and future directions

Various epidemiological studies investigate the effects of dietary carotenoids on various markers of oxidative stress and inflammation indicating their preventive role in chronic liver disease prevention. Oxidative stress is clearly associated with the etiology of chronic liver diseases. Thus, the use of carotenoid-rich fruits and vegetables should be the part of diet (**Tables**). Although several pathways related to inhibition of oxidative stress by carotenoids have been uncovered, many aspects remain poorly understood and warrant further research. As many carotenoids have been found to have provitamin A activity, the mechanism related to their regaining of vitamin A content by hepatic stellate cells and restoring their normal functions should be properly understood (**Figure**).

There is further need of long-term controlled trials in normal and diseased groups to study the dose response of each dietary carotenoid. Future research areas may include their bioavailability, metabolism, safety, and mechanism of action. Studies on the type of carotenoid and its metabolites which may act as a suitable regulator to alter pathways related to oxidative

Dietary sources	β-Carotene	Lycopene	Lutein	β-Cryptoxanthin
Apricot (dried)	17.6[$]	0.9[$]	—	—
Carrot (raw)	7.9[$]	—	—	—
Spinach (raw)	4.1[$]	—	11.9[$]	—
Kale	4.7[$]	—	15.8[$]	—
Tomato juice	—	8.6[$]	—	—
Avocado (raw)	0.053[#]	—	—	0.036[#]
Grape fruit, pink (raw)	—	3.4[$]	—	—
Guava (raw)	—	5.4[$]	—	—
Corn, sweet (cooked)	—	—	1.8[$]	—
Papaya (raw)	0.276[#]	—	—	0.761[#]
Orange (raw)	0.051[#]	—	0.187[#]	0.122[#]
Sweet potato (raw)	8.8[$]	—	—	—
Lettuce (raw)	1.27[#]	—	2.6[#]	—
Watermelon (raw)	0.295[#]	4.8[#]	0.017[#]	0.105[#]
Cabbage (raw)	0.065[#]	—	0.310[#]	—
Broccoli (raw)	2.4[#]	—	0.78[#]	—
Brussels sprouts (raw)	0.45[#]	—	1.5[#]	—
Peas, green (raw)	0.48[#]	—	—	—
Tangerine (raw)	0.071[#]	—	0.243[#]	0.485[#]
Pepper, sweet red (raw)	2.379[#]	—	—	2.205[#]

[#]Derived from Arscott [96].
[$]Derived from Johnson [97].

Table. Dietary sources of major carotenoids (mg/100 g).

S. no.	Carotenoid	Recommended daily dose (mg)	References
1	β-Carotene	7	[98]
2	Lycopene	35	[99]
3	Lutein	1–4	[100]
4	β-Cryptoxanthin	3	[91]

Table. Ingestion levels of dietary carotenoids to prevent liver diseases.

stress, inflammation, and carcinogenicity shall also be among the priority areas. However, studying the molecular targets of these dietary carotenoids cannot be ignored and be given full consideration.

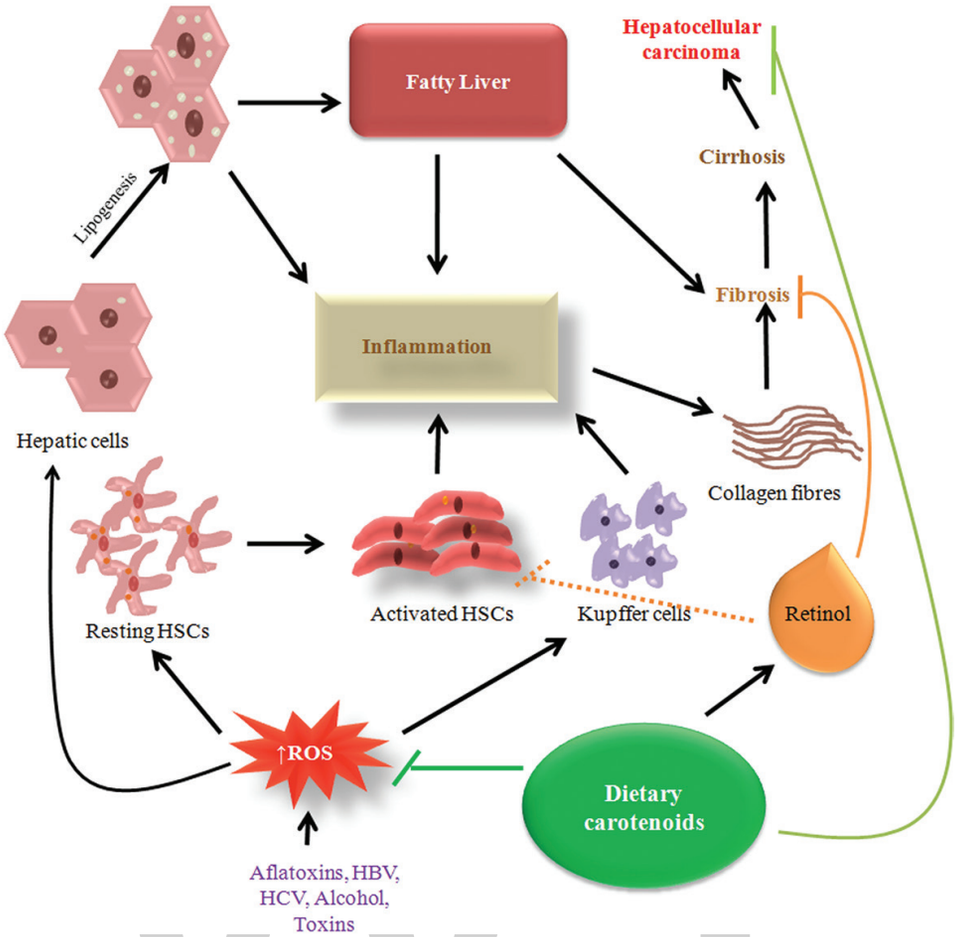

Figure. Hypothesis of progression of chronic liver diseases and their prevention by dietary carotenoids.

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Conflict of interest

The authors declare no potential conflict of interest and are responsible for the writing and content of the chapter.

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References

[1] Nishino H, Tokuda H, Murakoshi M, Satomi Y, Masuda M, Onozuka M, et al. Cancer prevention by natural carotenoids. BioFactors. 2000;**13**:89-94. DOI: 10.1002/biof.5520130115

[2] Coyne T, Ibiebele TI, Baade PD, Dobson A, McClintock C, Dunn S, et al. Diabetes mellitus and serum carotenoids: Findings of a population-based study in Queensland, Australia. The American Journal of Clinical Nutrition. 2005;**82**:685-693. DOI: 10.1093/ajcn/82.3.685

[3] Mueller L, Boehm V. Antioxidant activity of β-carotene compounds in different in vitro assays. Molecules. 2011;**16**:1055-1069. DOI: 10.3390/molecules16021055

[4] Bohn T. Bioavailability of non-provitamin A carotenoids. Current Nutrition & Food Science. 2008;**4**:240-258. DOI: 10.2174/157340108786263685

[5] Britton G. Structure and properties of carotenoids in relation to function. The FASEB Journal. 1995;**9**:1551-1558. DOI: 10.1096/fasebj.9.15.8529834

[6] Waris G, Ahsan H. Reactive oxygen species: Role in the development of cancer and various chronic conditions. Journal of Carcinogenesis. 2006;**5**:14. DOI: 10.1186/1477-3163-5-14

[7] Tapiero H, Townsend DM, Tew KD. The role of carotenoids in the prevention of human pathologies. Biomedicine & Pharmacotherapy. 2004;**58**:100-110. DOI: 10.1016/j.biopha.2003.12.006

[8] Stahl W, Sies H. Antioxidant activity of carotenoids. Molecular Aspects of Medicine. 2003;**24**:345-351. DOI: 10.1016/S0098-2997(03)00030-X

[9] Loguercio C, De Girolamo V, de Sio I, Tuccillo C, Ascione A, Baldi F, et al. Non-alcoholic fatty liver disease in an area of southern Italy: Main clinical, histological, and pathophysiological aspects. Journal of Hepatology. 2001;**35**:568-574. DOI: 10.1016/S0168-8278(01)00192-1

[10] Krinsky NI, Johnson EJ. Carotenoid actions and their relation to health and disease. Molecular Aspects of Medicine. 2005;**26**:459-516. DOI: 10.1016/j.mam.2005.10.001

[11] Burri BJ. Beta-cryptoxanthin as a source of vitamin A. Journal of the Science of Food and Agriculture. 2015;**95**:1786-1794. DOI: 10.1002/jsfa.6942

[12] Sanyal AJ, Yoon SK, Lencioni R. The etiology of hepatocellular carcinoma and consequences for treatment. The Oncologist. 2010;**15**:14-22. DOI: 10.1634/theoncologist.2010-S4-14

[13] Gao B, Bataller R. Alcoholic liver disease: Pathogenesis and new therapeutic targets. Gastroenterology. 2011;**141**:1572-1585. DOI: 10.1053/j.gastro.2011.09.002

[14] HB E-S, Rudolph KL. Hepatocellular carcinoma: Epidemiology and molecular carcinogenesis. Gastroenterology. 2007;**132**:2557-2576. DOI: 10.1053/j.gastro.2007.04.061

[15] Affo S, Yu LX, Schwabe RF. The role of cancer-associated fibroblasts and fibrosis in liver cancer. Annual Review of Pathology: Mechanisms of Disease. 2017;**12**:153-186. DOI: 10.1146/annurev-pathol-052016-100322

[16] Ha HL, Shin HJ, Feitelson MA, Yu DY. Oxidative stress and antioxidants in hepatic pathogenesis. World Journal of Gastroenterology: WJG. 2010;**16**:6035-6043. DOI: 10.3748/wjg.v16.i48.6035

[17] Friedman SL. Liver fibrosis–from bench to bedside. Journal of Hepatology. 2003;**38**:38-53. DOI: 10.1016/S0168-8278(02)00429-4

[18] Ahmad R, Ahmed S, Khan NU, Hasnain A. *Operculina turpethum* attenuates N-nitrosodimethylamine induced toxic liver injury and clastogenicity in rats. Chemico-Biological Interactions. 2009;**181**:145-153. DOI: 10.1016/j.cbi.2009.06.021

[19] Ahmad A, Ahmad R. Understanding the mechanism of hepatic fibrosis and potential therapeutic approaches. Saudi Journal of Gastroenterology: Official Journal of the Saudi Gastroenterology Association. 2012;**18**:155-167. DOI: 10.4103/1319-3767.96445

[20] Latief U, Ahmad R. Herbal remedies for liver fibrosis: A review on the mode of action of fifty herbs. Journal of Traditional and Complementary Medicine. 2017;**8**:352-360 DOI: 10.1016/j.jtcme.2017.07.002

[21] Husain H, Ahmad R, Khan A, Asiri AM. Proteomic-genomic adjustments and their confluence for elucidation of pathways and networks during liver fibrosis. International Journal of Biological Macromolecules. 2018;**111**:379-392. DOI: 10.1016/j.ijbiomac.2017.12.168

[22] Senoo H, Yoshikawa K, Morii M, Miura M, Imai K, Mezaki Y. Hepatic stellate cell (vitamin A-storing cell) and its relative–past, present and future. Cell Biology International. 2010;**34**:1247-1272. DOI: 10.1042/CBI20100321

[23] Sakurai T, Kudo M. Molecular link between liver fibrosis and hepatocellular carcinoma. Liver Cancer. 2013;**2**:365-366. DOI: 10.1159/000343851

[24] Mukherjee D, Ahmad R. Dose-dependent effect of N′-Nitrosodiethylamine on hepatic architecture, RBC rheology and polypeptide repertoire in Wistar rats. Interdisciplinary Toxicology. 2015;**8**:1-7. DOI: 10.1515/intox-2015-0001

[25] Shete V, Quadro L. Mammalian metabolism of β-carotene: Gaps in knowledge. Nutrients. 2013;**5**:4849-4868. DOI: 10.3390/nu5124849

[26] Wang XD, Russell RM. Procarcinogenic and anticarcinogenic effects of β-carotene. Nutrition Reviews. 1999;**57**:263-272. DOI: 10.1111/j.1753-4887.1999.tb01809.x

[27] Vardi N, Parlakpinar H, Cetin A, Erdogan A, Cetin Ozturk I. Protective effect of β-carotene on methotrexate–induced oxidative liver damage. Toxicologic Pathology. 2010;**38**:592-597. DOI: 10.1177/0192623310367806

[28] Patel V, Sail S. β-Carotene protects the physiological antioxidants against aflatoxin-B1 induced carcinogenesis in albino rats. Pakistan Journal of Biological Sciences. 2006;**9**:1104-1111

[29] Kheir Eldin AA, Motawi TM, Sadik NA. Effect of some natural antioxidants on aflatoxin B1-induced hepatic toxicity. EXCLI Journal. 2008;**7**:119-131

[30] Lee YS, Jeong WI. Retinoic acids and hepatic stellate cells in liver disease. Journal of Gastroenterology and Hepatology. 2012;**27**:75-79. DOI: 10.1111/j.1440-1746.2011.07007.x

[31] Yadav D, Hertan HI, Schweitzer P, Norkus EP, Pitchumoni CS. Serum and liver micronutrient antioxidants and serum oxidative stress in patients with chronic hepatitis C. The American Journal of Gastroenterology. 2002;**97**:2634-2639. DOI: 10.1111/j.1572-0241.2002.06041.x

[32] Pan WH, Wang CY, Huang SM, Yeh SY, Lin WG, Lin DI, et al. Vitamin A, vitamin E or beta-carotene status and hepatitis B-related hepatocellular carcinoma. Annals of Epidemiology. 1993;**3**:217-224. DOI: 10.1016/1047-2797(93)90022-V

[33] Yuan JM, Gao YT, Ong CN, Ross RK, Yu MC. Prediagnostic level of serum retinol in relation to reduced risk of hepatocellular carcinoma. Journal of the National Cancer Institute. 2006;**98**:482-490. DOI: 10.1093/jnci/djj104

[34] Peres WA, Chaves GV, Gonçalves JC, Ramalho A, Coelho HS. Vitamin A deficiency in patients with hepatitis C virus-related chronic liver disease. The British Journal of Nutrition. 2011;**106**:1724-1731. DOI: 10.1017/S0007114511002145

[35] Bitetto D, Bortolotti N, Falleti E, Vescovo S, Fabris C, Fattovich G, et al. Vitamin A deficiency is associated with hepatitis C virus chronic infection and with unresponsiveness to interferon-based antiviral therapy. Hepatology. 2013;**57**:925-933. DOI: 10.1002/hep.26186

[36] Peng HC, Chen YL, Yang SY, Ho PY, Yang SS, Hu JT, et al. The antiapoptotic effects of different doses of β-carotene in chronic ethanol-fed rats. Hepatobiliary Surgery and Nutrition. 2013;**2**:132-141. DOI: 10.3978/j.issn.2304-3881.2013.06.08

[37] Lin WT, Huang CC, Lin TJ, Chen JR, Shieh MJ, Peng HC, et al. Effects of β-carotene on antioxidant status in rats with chronic alcohol consumption. Cell Biochemistry and Function. 2009;**27**:344-350. DOI: 10.1002/cbf.1579

[38] Werman MJ, Ben-Amotz A, Mokady S. Availability and antiperoxidative effects of β-carotene from Dunaliella bardawil in alcohol-drinking rats. The Journal of Nutritional Biochemistry. 1999;**10**:449-454. DOI: 10.1016/S0955-2863(99)00026-1

[39] Tsuchihashi H, Kigoshi M, Iwatsuki M, Niki E. Action of β-carotene as an antioxidant against lipid peroxidation. Archives of Biochemistry and Biophysics. 1995;**32**:137-147. DOI: 10.1006/abbi.1995.0019

[40] Stryker WS, Kaplan LA, Stein EA, Stampfer MJ, Sober A, Willett WC. The relation of diet, cigarette smoking, and alcohol consumption to plasma beta-carotene and alpha-tocopherol levels. American Journal of Epidemiology. 1988;**127**:283-296. DOI: 10.1093/oxfordjournals.aje.a114804

[41] Leo MA, Sato M, Lieber CS. Effect of hepatic vitamin A depletion on the liver in humans and rats. Gastroenterology. 1983;**84**:562-572

[42] Ozturk F, Gul M, Ates B, Ozturk IC, Cetin A, Vardi N, et al. Protective effect of apricot (*Prunus armeniaca* L.) on hepatic steatosis and damage induced by carbon tetrachloride

in Wistar rats. The British Journal of Nutrition. 2009;**102**:1767-1775. DOI: 10.1017/S0007114509991322

[43] Martín-Pozuelo G, Navarro-González I, González-Barrio R, Santaella M, García-Alonso J, Hidalgo N, et al. The effect of tomato juice supplementation on biomarkers and gene expression related to lipid metabolism in rats with induced hepatic steatosis. European Journal of Nutrition. 2015;**54**:933-944. DOI: 10.1007/s00394-014-0770-4

[44] Tainaka T, Shimada Y, Kuroyanagi J, Zang L, Oka T, Nishimura Y, et al. Transcriptome analysis of anti-fatty liver action by Campari tomato using a zebrafish diet-induced obesity model. Nutrition and Metabolism. 2011;**8**:88. DOI: 10.1186/1743-7075-8-88

[45] Cao Y, Wang C, Liu J, Liu ZM, Ling WH, Chen YM. Greater serum carotenoid levels associated with lower prevalence of nonalcoholic fatty liver disease in Chinese adults. Scientific Reports. 2015;**5**:12951. DOI: 10.1038/srep12951

[46] Chaves GV, Pereira SE, Saboya CJ, Ramalho A. Non-alcoholic fatty liver disease and its relationship with the nutritional status of vitamin A in individuals with class III obesity. Obesity Surgery. 2008;**18**:378-385. DOI: 10.1007/s11695-007-9361-2

[47] Chattopadhyay MB, Kanna PS, Ray RS, Roy S, Chatterjee M. Combined supplementation of vanadium and beta-carotene suppresses placental glutathione S-transferase-positive foci and enhances antioxidant functions during the inhibition of diethylnitrosamine-induced rat liver carcinogenesis. Journal of Gastroenterology and Hepatology. 2004;**19**:683-693. DOI: 10.1111/j.1440-1746.2004.03378.x

[48] Matsushima-Nishiwaki R, Okuno M, Takano Y, Kojima S, Friedman SL, Moriwaki H. Molecular mechanism for growth suppression of human hepatocellular carcinoma cells by acyclic retinoid. Carcinogenesis. 2003;**24**:1353-1359. DOI: 10.1093/carcin/bgg090

[49] Lan QY, Zhang YJ, Liao GC, Zhou RF, Zhou ZG, Chen YM, et al. The association between dietary vitamin A and carotenes and the risk of primary liver cancer: A case–control study. Nutrients. 2016;**8**:624. DOI: 10.3390/nu8100624

[50] Gerster H. The potential role of lycopene for human health. Journal of the American College of Nutrition. 1997;**16**:109-126. DOI: 10.1080/07315724.1997.10718661

[51] Rao AV, Rao LG. Carotenoids and human health. Pharmacological Research. 2007;**55**:207-216. DOI: 10.1016/j.phrs.2007.01.012

[52] Kavanaugh CJ, Trumbo PR, Ellwood KC. The US Food and Drug Administration's evidence-based review for qualified health claims: Tomatoes, lycopene, and cancer. Journal of the National Cancer Institute. 2007;**99**:1074-1085. DOI: 10.1093/jnci/djm037

[53] Islamian JP, Mehrali H. Lycopene as a carotenoid provides radioprotectant and antioxidant effects by quenching radiation-induced free radical singlet oxygen: An overview. Cell Journal (Yakhteh). 2015;**16**:386-391. DOI: 10.22074/cellj.2015.485

[54] Müller L, Caris-Veyrat C, Lowe G, Böhm V. Lycopene and its antioxidant role in the prevention of cardiovascular diseases—A critical review. Critical Reviews in Food Science and Nutrition. 2016;**56**:1868-1879. DOI: 10.1080/10408398.2013.801827

[55] Gradelet S, Le Bon AM, Berges R, Suschetet M, Astorg P. Dietary carotenoids inhibit aflatoxin B1-induced liver preneoplastic foci and DNA damage in the rat: Role of the modulation of aflatoxin B1 metabolism. Carcinogenesis. 1998;**19**:403-411. DOI: 10.1093/carcin/19.3.403

[56] Yilmaz S, Kaya E, Kisacam MA. The Effect on Oxidative Stress of Aflatoxin and Protective Effect of Lycopene on Aflatoxin Damage. In: Aflatoxin-Control, Analysis, Detection and Health Risks. 2017. Pp. 67-90. DOI: 10.5772/intechopen.69321

[57] Xu F, Yu K, Yu H, Wang P, Song M, Xiu C, et al. Lycopene relieves AFB 1-induced liver injury through enhancing hepatic antioxidation and detoxification potential with Nrf2 activation. Journal of Functional Foods. 2017;**39**:215-224. DOI: 10.1016/j.jff.2017.10.027

[58] Shivashangari KS, Ravikumar V, Vinodhkumar R, Sheriff SA, Devaki T. Hepatoprotective potential of lycopene on D-galactosamine/lipopolysaccharide induced hepatitis in rats. Pharmacologyonline. 2006;**2**:151-170

[59] Sheriff SA, Devaki T. Lycopene stabilizes lipoprotein levels during D-galactosamine/lipopolysaccharide induced hepatitis in experimental rats. Asian Pacific Journal of Tropical Biomedicine. 2012;**2**:975-980. DOI: 10.1016/S2221-1691(13)60009-X

[60] Morisco F, Vitaglione P, Carbone A, Stingo S, Scarpati S, Ascione A, et al. Tomato-based functional food as interferon adjuvant in HCV eradication therapy. Journal of Clinical Gastroenterology. 2004;**38**:S118-S120. DOI: 10.1097/01.mcg.0000128935.48082.f9

[61] Nishino H. Prevention of hepatocellular carcinoma in chronic viral hepatitis patients with cirrhosis by carotenoid mixture. In: Cancer Prevention. Berlin, Heidelberg: Springer; 2007. pp. 67-71. DOI: 10.1007/978-3-540-37696-5_6

[62] Seren S, Mutchnick M, Hutchinson D, Harmanci O, Bayraktar Y, Mutchnick S, et al. Potential role of lycopene in the treatment of hepatitis C and prevention of hepatocellular carcinoma. Nutrition and Cancer. 2008;**60**:729-735. DOI: 10.1080/01635580802419772

[63] Stice CP, Liu C, Aizawa K, Greenberg AS, Ausman LM, Wang XD. Dietary tomato powder inhibits alcohol-induced hepatic injury by suppressing cytochrome p450 2E1 induction in rodent models. Archives of Biochemistry and Biophysics. 2015;**572**:81-88. DOI: 10.1016/j.abb.2015.01.004

[64] Bahcecioglu IH, Kuzu N, Metin K, Ozercan IH, Ustündag B, Sahin K, et al. Lycopene prevents development of steatohepatitis in experimental nonalcoholic steatohepatitis model induced by high-fat diet. Veterinary Medicine International. 2010; Article ID. 262179, 1-8. DOI: 10.4061/2010/262179

[65] Bernal C, Martín-Pozuelo G, Lozano AB, Sevilla Á, García-Alonso J, Canovas M, et al. Lipid biomarkers and metabolic effects of lycopene from tomato juice on liver of rats with induced hepatic steatosis. The Journal of Nutritional Biochemistry. 2013;**24**:1870-1881. DOI: 10.1016/j.jnutbio.2013.05.003

[66] Ip BC, Wang XD. Non-alcoholic steatohepatitis and hepatocellular carcinoma: Implications for lycopene intervention. Nutrients. 2013;**6**:124-162. DOI: 10.3390/nu6010124

[67] Jiang W, Guo MH, Hai X. Hepatoprotective and antioxidant effects of lycopene on non-alcoholic fatty liver disease in rat. World Journal of Gastroenterology. 2016;**22**:10180-10188. DOI: 10.3748/wjg.v22.i46.10180

[68] Piña-Zentella RM, Rosado JL, Gallegos-Corona MA, Madrigal-Pérez LA, García OP, Ramos-Gomez M. Lycopene improves diet-mediated recuperation in rat model of non-alcoholic fatty liver disease. Journal of Medicinal Food. 2016;**19**:607-614. DOI: 10.1089/jmf.2015.0123

[69] Ahn J, Lee H, Jung CH, Ha T. Lycopene inhibits hepatic steatosis via microRNA-21-induced downregulation of fatty acid-binding protein 7 in mice fed a high-fat diet. Molecular Nutrition & Food Research. 2012;**56**:1665-1674. DOI: 10.1002/mnfr.201200182

[70] Gupta P, Bhatia N, Bansal MP, Koul A. Lycopene modulates cellular proliferation, glycolysis and hepatic ultrastructure during hepatocellular carcinoma. World Journal of Hepatology. 2016;**8**:1222. DOI: 10.4254/wjh.v8.i29.1222-1233

[71] Ip BC, Liu C, Ausman LM, Von Lintig J, Wang XD. Lycopene attenuated hepatic tumorigenesis via differential mechanisms depending on carotenoid cleavage enzyme in mice. Cancer Prevention Research. 2014;**7**:1219-1227. DOI: 10.1158/1940-6207

[72] Mangels AR, Holden JM, Beecher GR, Forman MR, Lanza E. Carotenoid content of fruits and vegetables: An evaluation of analytic data. Journal of the American Dietetic Association. 1993;**93**:284-296. DOI: 10.1016/0002-8223(93)91553-3

[73] Perry A, Rasmussen H, Johnson EJ. Xanthophyll (lutein, zeaxanthin) content in fruits, vegetables and corn and egg products. Journal of Food Composition and Analysis. 2009;**22**:9-15. DOI: 10.1016/j.jfca.2008.07.006

[74] Landrum JT, Bone RA. Lutein, zeaxanthin, and the macular pigment. Archives of Biochemistry and Biophysics. 2001;**385**:28-40. DOI: 10.1006/abbi.2000.2171

[75] Pang R, Tao JY, Zhang SL, Zhao L, Yue X, Wang YF, et al. In vitro antiviral activity of lutein against hepatitis B virus. Phytotherapy Research. 2010;**24**:1627-1630. DOI: 10.1002/ptr.3155

[76] Sindhu ER, Firdous AP, Preethi KC, Kuttan R. Carotenoid lutein protects rats from paracetamol-, carbon tetrachloride-and ethanol-induced hepatic damage. The Journal of Pharmacy and Pharmacology. 2010;**62**:1054-1060. DOI: 10.1111/j.2042-7158.2010.01123.x

[77] Du SY, Zhang YL, Bai RX, Ai ZL, Xie BS, Yang HY. Lutein prevents alcohol-induced liver disease in rats by modulating oxidative stress and inflammation. International Journal of Clinical and Experimental Medicineis. 2015;**8**:8785-8793

[78] Kim JE, Clark RM, Park Y, Lee J, Fernandez ML. Lutein decreases oxidative stress and inflammation in liver and eyes of Guinea pigs fed a hypercholesterolemic diet. Nutrition Research and Practice. 2012;**6**:113-119. DOI: 10.4162/nrp.2012.6.2.113

[79] Qiu X, Gao DH, Xiang X, Xiong YF, Zhu TS, Liu LG, et al. Ameliorative effects of lutein on non-alcoholic fatty liver disease in rats. World Journal of Gastroenterology. 2015;**21**:8061-8072. DOI: 10.3748/wjg.v21.i26.8061

[80] Colak Y, Yesil A, Mutlu HH, Caklili OT, Ulasoglu C, Senates E, et al. A potential treatment of non-alcoholic fatty liver disease with SIRT1 activators. Journal of Gastrointestinal and Liver Diseases. 2014;**23**:311-319. DOI: 10.1543/jgld.2014.1121.233.yck

[81] Purushotham A, Schug TT, Xu Q, Surapureddi S, Guo X, Li X. Hepatocyte-specific deletion of SIRT1 alters fatty acid metabolism and results in hepatic steatosis and inflammation. Cell Metabolism. 2009;**9**:327-338. DOI: 10.1016/j.cmet.2009.02.006

[82] Ding X, Guo L, Zhang Y, Fan S, Gu M, Lu Y, et al. Extracts of pomelo peels prevent high-fat diet-induced metabolic disorders in c57bl/6 mice through activating the PPARα and GLUT4 pathway. PLoS One. 2013;**8**:e77915. DOI: 10.1371/journal.pone.0077915

[83] Sindhu ER, Firdous AP, Ramnath V, Kuttan R. Effect of carotenoid lutein on N-nitrosodiethylamine-induced hepatocellular carcinoma and its mechanism of action. European Journal of Cancer Prevention. 2013;**22**:320-327. DOI: 10.1097/CEJ.0b013e32835b69ff

[84] Maiani G, Periago Castón MJ, Catasta G, Toti E, Cambrodón IG, Bysted A, et al. Carotenoids: Actual knowledge on food sources, intakes, stability and bioavailability and their protective role in humans. Molecular Nutrition & Food Research. 2009;**53**:S194-S218. DOI: 10.1002/mnfr.200800053

[85] Lorenzo Y, Azqueta A, Luna L, Bonilla F, Domínguez G, Collins AR. The carotenoid β-cryptoxanthin stimulates the repair of DNA oxidation damage in addition to acting as an antioxidant in human cells. Carcinogenesis. 2008;**30**:308-314. DOI: 10.1093/carcin/bgn270

[86] Unno K, Sugiura M, Ogawa K, Takabayashi F, Toda M, Sakuma M, et al. Beta-cryptoxanthin, plentiful in Japanese mandarin orange, prevents age-related cognitive dysfunction and oxidative damage in senescence-accelerated mouse brain. Biological and Pharmaceutical Bulletin. 2011;**34**:311-317. DOI: 10.1248/bpb.34.311

[87] Sugiura M, Nakamura M, Ikoma Y, Yano M, Ogawa K, Matsumoto H, et al. High serum carotenoids are inversely associated with serum gamma-glutamyltransferase in alcohol drinkers within normal liver function. Journal of Epidemiology. 2005;**15**:180-186. DOI: 10.2188/jea.15.180

[88] Kobori M, Ni Y, Takahashi Y, Watanabe N, Sugiura M, Ogawa K, et al. β-Cryptoxanthin alleviates diet-induced nonalcoholic steatohepatitis by suppressing inflammatory gene expression in mice. PLoS One. 2014;**9**:e98294. DOI: 10.1371/journal.pone.0098294

[89] Ni Y, Nagashimada M, Zhan L, Nagata N, Kobori M, Sugiura M, et al. Prevention and reversal of lipotoxicity-induced hepatic insulin resistance and steatohepatitis in mice by an antioxidant carotenoid, β-cryptoxanthin. Endocrinology. 2015;**156**:987-999. DOI: 10.1210/en.2014-1776

[90] Erhardt A, Stahl W, Sies H, Lirussi F, Donner A, Häussinger D. Plasma levels of vitamin E and carotenoids are decreased in patients with nonalcoholic steatohepatitis (NASH). European Journal of Medical Research. 2011;**16**:76. DOI: 10.1186/2047-783X-16-2-76

[91] Matsuura B, Miyake T, Yamamoto S, Furukawa S, Hiasa Y. Usefulness of beta-cryptoxanthin for nonalcoholic fatty liver diseases. Journal of Food and Nutritional Disorders. 2016;**5**:3. DOI: 10.4172/2324-9323.1000196

[92] Murakoshi M, Nishino H, Satomi Y, Takayasu J, Hasegawa T, Tokuda H, et al. Potent preventive action of α-carotene against carcinogenesis: Spontaneous liver carcinogenesis and promoting stage of lung and skin carcinogenesis in mice are suppressed more effectively by α-carotene than by β-carotene. Cancer Research. 1992;**52**:6583-6587

[93] Abidov M, Ramazanov Z, Seifulla R, Grachev S. The effects of Xanthigen™ in the weight management of obese premenopausal women with non-alcoholic fatty liver disease and normal liver fat. Diabetes, Obesity & Metabolism. 2010;**12**:72-81. DOI: 10.1111/j.1463-1326.2009.01132.x

[94] Chamberlain SM, Hall JD, Patel J, Lee JR, Marcus DM, Sridhar S, et al. Protective effects of the carotenoid zeaxanthin in experimental nonalcoholic steatohepatitis. Digestive Diseases and Sciences. 2009;**54**:1460-1464. DOI: 10.1007/s10620-009-0824-2

[95] Xiao J, Wang J, Xing F, Han T, Jiao R, Liong EC, et al. Zeaxanthin dipalmitate therapeutically improves hepatic functions in an alcoholic fatty liver disease model through modulating MAPK pathway. PLoS One. 2014;**9**:e95214. DOI: 10.1371/journal.pone.0095214

[96] Arscott SA. Food sources of carotenoids. In: Carotenoids and Human Health. Totowa, NJ: Humana Press; 2013. pp. 3-19. DOI: 10.1007/978-1-62703-203-2_1

[97] Johnson EJ. The role of carotenoids in human health. Nutrition in Clinical Care. 2002;**5**:56-65. DOI: 10.1046/j.1523-5408.2002.00004.x

[98] Grune T, Lietz G, Palou A, Ross AC, Stahl W, Tang G, et al. β-Carotene is an important vitamin A source for humans-3. The Journal of Nutrition. 2010;**140**:2268S-2285S. DOI: 10.3945/jn.109.119024

[99] Rao AV, Agarwal S. Role of antioxidant lycopene in cancer and heart disease. Journal of the American College of Nutrition. 2000;**19**:563-569. DOI: 10.1080/07315724.2000.10718953

[100] Mares-Perlman JA, Millen AE, Ficek TL, Hankinson SE. The body of evidence to support a protective role for lutein and zeaxanthin in delaying chronic disease. Overview. The Journal of Nutrition. 2002;**132**:518S-524S. DOI: 10.1093/jn/132.3.518S

Chapter 5

Food Authenticity: Provenancing. A Case Study of Fish

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Abstract

Authentication of food products is of ongoing interest to consumers in developed countries. Recently, a general interest in the sustainability of food productions, from both societal and environmental perspectives, developed and added a new dimension. Fish and fish products are common targets for food adulteration. The most important issue is fish management, e.g., the environmental impact of overfishing. Analytical means would be helpful for verification. The aim of the present study was to evaluate various marker groups for the distinction of European plaice from the North Sea from European plaice from other geographical origins: volatile organic compounds (VOCs), fatty acids (FA), and isotope ratios. VOCs were analyzed using proton transfer reaction mass spectrometry (PTR-MS); the FA composition was analyzed using gas chromatography with a flame ionization detector, and carbon, hydrogen, nitrogen; and sulfur isotope ratios were analyzed using isotope ratio mass spectrometry. In a principal component analysis, FA profiling appeared the best option to distinguish European plaice from the North Sea from those originating from other seas.

Keywords: European plaice, fatty acid composition, geographical origin, isotope ratio, PCA, *Pleuronectes platessa*, PTR-MS

1. Introduction

In food science and technology, considerable attention to the preservation and quality of foods has been paid throughout history. Food authenticity is a typical food quality issue and is not new either. Some food authenticity infringements were reported over 2000 years ago, and the first related food standards appeared 150 years ago. The classical form of food authenticity violations is the removal or addition of undeclared ingredients or constituents. However,

more recently, a general interest in the sustainability of food productions, from both societal and environmental perspectives, developed and added a new dimension. With advancing technology and increased awareness of social corporate responsibility, new questions have emerged in the food authenticity arena. How are production in terms of raw materials and processing mirrored in unique product characteristics? Do special production (farming management systems, geographical origin, etc.) result in special traits? How to discern and measure these relevant characteristics? And, can these characteristics be used for discriminatory purposes and/or substantiate the produce quality?

Adulteration has been defined as "the fraudulent addition of non-authentic substances or removal or replacement of authentic substances without the purchaser's knowledge for economic gain of the seller" by the United States Pharmacopeial Convention (USP). Fish and fish products are common targets for food adulteration [1]. There are different issues in regard to the traceability of fish: (1) the species of the fish, (2) geographical origin, and (3) the production method [2]. During production, fish types can be completely interchanged or mixed with cheaper or less sustainable fish types. According to European law (Regulation 1379/2013), information concerning the commercial designation, the catch area, and the production method shall be available at each stage of marketing of the species concerned [3]. Next to the fact that geographical origin of food products is laid down in legislation, consumers find it important because of economic, safety, and sustainability reasons. Different types of food adulteration can be distinguished: most types influence the product composition by, e.g., substitution or dilution, while other issues involve cultivar and variety of products of plant origin, species of products of animal origin, geographical origin of foods, production system, and processing. Geographical origin has become a more important issue in recent years for economic, safety, and sustainability reasons [4]. Counterfeiting occurs, for example, with the European Union's protected designation of origin, like certain cheeses or olive oils.

Authenticity and traceability research aims to combine different complementary analytical strategies to determine the authenticity of materials and commodities. Various techniques have been studied based on organic constituents, mineral contents or composition, light- or heavy-element isotope ratios, or combinations thereof. If the components have sufficient discriminatory power, the set of their concentrations will form a characteristic pattern or "fingerprint" relating to the geographical origin of the sample. Chemometrics provides the ability to detect these patterns, and is essentially helpful when the number of components necessary to differentiate samples from different geographical origins increases [5]. The three major groups of analytical techniques are: mass spectrometry techniques, spectroscopic techniques, and separation techniques.

Markers that have been successfully used for the verification of the geographical origin of food and feed products are volatile organic compounds (VOCs), the fatty acid (FA) profile, and isotope ratios. These markers can be analyzed using mass spectrometry (like isotope ratio mass spectrometry—IRMS; and proton transfer reaction mass spectrometry—PTR-MS), spectroscopic techniques (like NMR and infrared spectroscopy), and separation techniques (such as liquid chromatography—LC; and gas chromatography—GC) [6].

1.1. VOC profile in the verification of geographical origin

VOCs have shown to satisfactorily predict the country of origin of olive oil, truffles, Grana Padano cheese, as reviewed by Luykx et al. [5] and crude palm oil, butter, cumin cheese, dry cured hams, and coffees, as listed by Pustjens et al. [6]. More recently, VOCs have also shown to successfully verify geographical origin of Chinese cabbage [7], plant-related liquors [8], saffron [9], rosemary [10], teas [11], and capers [12]. For plant products, the difference in VOC profile was ascribed to differences in climate and soil type [13]. This can be translated to fish, since the sea bed and climate will vary for fish from different geographical origins. The VOC profile of fish has been linked to lipid type and source and would therefore be determined by the diet they consumed [14].

1.2. FA profile in the verification of geographical origin

FAs have shown to satisfactorily predict the country of origin of milk and olive oils, as reviewed by Luykx et al. [5] and chocolate and wheat, as listed by Pustjens et al. [6]. More recently, FAs have also shown to successfully verify geographical origin of berries [15], cockles [16], and almonds [17]. FAs in the fish feed are incorporated into its tissues; therefore, the FA profile of the fish tissue reflects the diet over a longer period of time [18]. Especially the content of polyunsaturated fatty acids (PUFAs) seems to be dependent on the geographical origin, i.e., the latitude, and thus broadly related to temperature [19]. They found that at a higher latitude, marine organisms contain more n-3 long-chain PUFAs.

1.3. IR in the verification of geographical origin

All food products have their own isotopic composition, which is determined by the animal's diet (carbon and nitrogen), climate (hydrogen and oxygen), and soil composition (sulfur) [6]. IRMS analysis combined with or without other techniques and/or chemometric models has been successfully applied to determine the geographical origin of dairy and animal products, vegetables, natural flavors, honey, wines, coffee, and fruit, as reviewed by Luykx et al. [5] and cereals, fish and crustaceans, and olive oil and wine, as listed by Pustjens et al. [6]. IRMS analysis of carbon, hydrogen, nitrogen, and oxygen has shown to be able to verify geographical origin of different fish species [20, 21].

1.4. Case study: geographical origin of European plaice

European plaice (*Pleuronectes platessa*) is the principal commercial flatfish in Europe [22]. European plaice live at the bottom of the sea not far from the shore, predominantly in the North Sea, but extend to the Baltic Sea, the Barents Sea, and the sea around Ireland and Iceland [23]. Juvenile European plaice feed on a variety of microbenthic species [24], whereas larger European plaice mainly feed on Polychaeta and mollusks [25]. The habitat of European plaice is dependent on: (a) their life stages: they gradually move from shallow coastal nurseries into deeper water [26, 27] and (b) climate change: European plaice

have shifted toward deeper and more northern areas [28]. This information can be used to find specific groups of markers potentially useful for the verification of their geographical origin.

The aim of this study was to examine how well VOCs, FAs, and isotope ratios are able to distinguish European plaice from the North Sea from European plaice from other provenance.

2. Materials and methods

2.1. Samples

A reference set of 49 European plaice were collected by local fishermen from five different locations in the Northeast Atlantic ocean—FAO area 27: the North Sea, subarea IV a, b, c (n = 31); other research institutes from the Baltic Sea, subarea IIId (n = 3); the Barents Sea, subarea I (n = 5); the sea around Ireland, subarea Va (n = 5); and the sea around Iceland, subarea VIIj2 (n = 5). Locations are labeled as NS, BalS, BarS, SIr, and SIc, respectively, in **Figure**. European plaice from the North Sea were both collected in 2013 and 2014; all other samples were collected in 2014. Samples were transported on dry ice to our institute, filleted and stored at −18°C until further analyses. In 2014, a set of market samples were also collected: 11 non-breaded and non-marinated frozen European plaice fillets from Italian (n = 6), German (n = 4), and Dutch (n = 1) supermarkets. According to the package, they originated from the Northeast Atlantic Ocean (FAO zone 27). For some fish, the North Sea, which is part of this FAO catch area, was mentioned specifically.

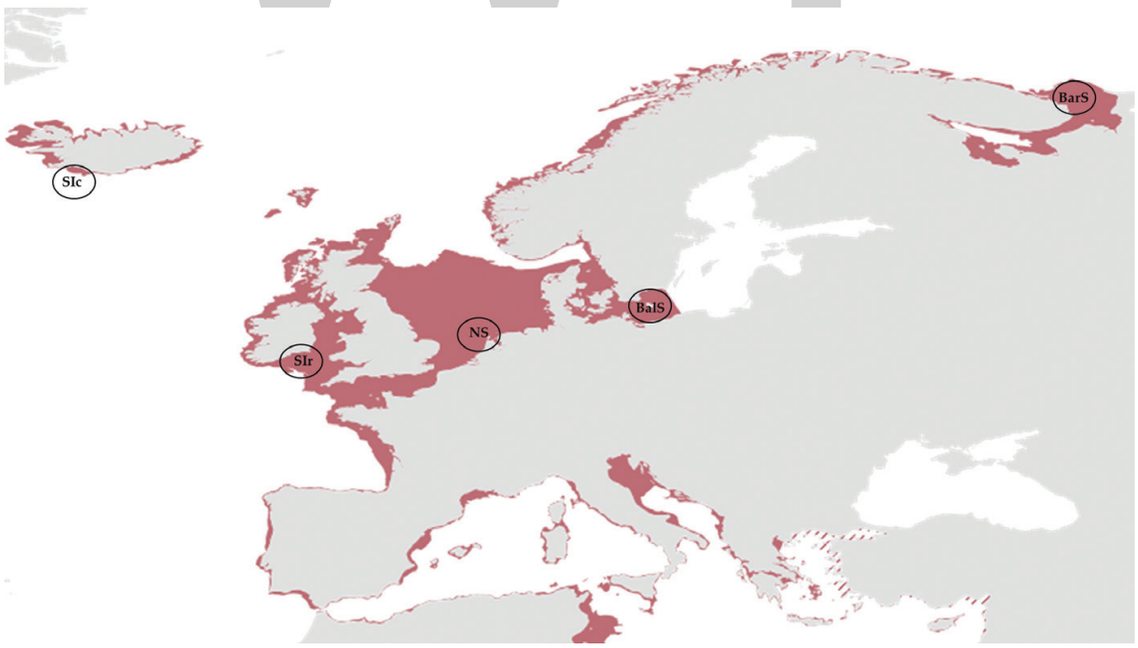

Figure. Habitat of European plaice, with the sample locations marked as NS—North Sea, BalS—Baltic Sea, BarS—Barents Sea, SIr—sea around Ireland, and SIc—sea around Iceland. Original figure from FAO Fish Finder (23).

2.2. Chemicals

All chemicals used were of analytical grade purchased from Merck (Darmstadt, Germany), Sigma-Aldrich (St. Louis, MO, USA), or VWR International (Radnor, PA, USA), unless stated otherwise.

2.3. Analytical methods

2.3.1. PTR-MS

The samples were thawed to room temperature in their package and 1 gram of finely sliced material was placed in a 250-mL glass bottle. The headspace above the samples was equilibrated in a waterbath at 25°C for 30 minutes and kept at 25°C for the duration of the measurement. Two independent replicates of each sample were analyzed. The headspace was drawn from the sample flask at a rate of 60 ml/min, which was led through a heated transfer line into a high-sensitivity PTR-MS system (Ionicon GmbH, Innsbruck, Austria). A constant drift voltage of 600 V and a pressure of 2.20 ± 0.02 mbar were maintained in the reaction chamber. Data were collected for the mass range m/z 20–160 using a dwell time of 0.2 s/mass, resulting in a duration of 28 s/cycle. The instrument was operated at a standard E/N (ratio of electric field strength across the drift tube, E, to buffer gas density, N) of 138 Td (1Td = 10^{-17} cm^2 V $molecule^{-1}$). Inlet and drift chamber temperatures were 60°C. Masses are analyzed after H_3O^+ ionization in a quadrupole mass spectrometer and detected as ion count/s, resulting in a fingerprint of the volatiles. Each sample was analyzed for five full mass cycles. The headspace concentrations of the compounds during the second, third, and fourth cycles were calculated as described by Hansel et al. [29]. Blank measurements (empty bottle) were performed for five cycles, with the average of the third, fourth, and fifth mass cycles subtracted from the samples' spectra as background correction. These corrected headspace concentrations (ppbv) were subsequently used for further statistical analysis.

2.3.2. GC flame ionization detection (GC-FID)

The samples were freeze-dried. Fat was extracted from the samples (2 g) using chloroform:methanol (2:1, v/v) as described by Tres et al. [30]. Fatty acids were methyl esterified according to Guardiola et al. [31]. The obtained methyl esters were analyzed in duplicate using an Agilent 7890A GC system, fitted with a flame ionization detector, using a CP7419 50 m × 0.25 mm FAME column (Agilent, Santa Clara, CA, USA). Temperature was kept at 100°C for 1 minute and then increased to 230°C with 5°C/min, after which the temperature was kept at 230°C for 9 min. Helium was used as a carrier gas using a flow of 0.9 ml per min and a split ratio of 1:50. Fatty acids were identified by comparing retention times with those of standard mixtures (Supelco 37 component FAME mix (Supelco, St. Louis, MO, USA)). Results were expressed as normalized peak areas (% of total fatty acids).

2.3.3. IR-MS

The dried defatted samples (1 g) were placed in a paper filter and washed three times with 10 ml of distilled water for removal of sodium sulfate, then three times with 10 ml of acetone to accelerate drying, and then placed into glass containers and dried in vacuum overnight.

For analysis of δ^2H, 250–300 µg of dried sample material was weighed into a silver capsule. δ^2H was determined using a high-temperature pyrolysis system working at 1450°C on the basis of ceramic tubes with glassy carbon reactor filling coupled with a Thermo Finnigan Delta-Plus XL IRMS system (Thermo Fisher Inc.), designed for continuous flow isotope ratio mass spectrometry (CF-IRMS) of hydrogen isotopes in helium carrier gas.

For the other isotopes (for $\delta^{13}C$, $\delta^{15}N$, and $\delta^{34}S$), 3–4 mg of sample material was weighed into tin capsules. The measurements of $\delta^{13}C$, $\delta^{15}N$, and $\delta^{34}S$ were carried out on an elemental analyzer (Vario EL, Elementar, Hanau, Germany) coupled with an isotope-ratio mass spectrometer, IRMS (IsoPrime, GV Instruments, Manchester, UK). All isotopes were analyzed in duplicate.

The values of the isotope ratios are expressed in δ (‰) and correspond to international standards (V-SMOW for δ^2H, V-PDB for $\delta^{13}C$, air for $\delta^{15}N$, and V-CDT for $\delta^{34}S$) according to the relation:

$$\delta\ (‰) = 1000\ x\ \frac{R_{sample} - R_{standard}}{R_{standard}} \quad (1)$$

where R represents the ratio of the higher mass to the lower mass isotopes measured in the sample (R_{sample}) and in the standard ($R_{standard}$).

2.4. Data analysis

Normality of the distribution of VOC profiles, FA profiles, and isotope ratios within the groups of geographical origin was checked using the Shapiro-Wilk test. Distributions appeared to be nonnormal (Shapiro-Wilk $P < 0.05$). Therefore, Kruskal-Wallis test for group comparisons was performed among the provenance groups using SPSS version 23.0.0.2 (IBM Corp., Armonk, NY, USA). Principal component analysis (PCA) was performed using the Unscrambler (Version X 10.3). Raw data were auto-scaled prior to PCA.

3. Results and discussion

3.1. Distinction of the provenance of European plaice

3.1.1. VOC profiles analyzed by PTR-MS

The reference set of 49 European plaice samples was subjected to PTR-MS analyses. Most abundant masses of protonated VOCs of European plaice collected from the North Sea are m/z 33, 45, and 59. Subsequently, significant differences in mass intensities between European plaice originating from the North Sea versus other provenance were examined (Kruskal-Wallis $P < 0.05$). Results are presented in **Table 1**.

In order to explore the data, PCA was carried out. PCA (**Figure A**) revealed grouping of samples, with two-third of the samples from the North Sea showing high positive scores in

the first dimension. Samples from the other seas demonstrated high negative scores in this dimension. Remarkable is the fact that there is more variation in European plaice from the North Sea than in European plaice from the other seas. This might be caused by the fact that the European plaice from the North Sea have been sampled at six different locations within the North Sea, while the other seas have been sampled only on one location. For this reference set, distinction of the North Sea European plaice from other European plaice by their VOCs is a promising approach.

However, the VOC profiles are affected not only by the fish diet [14] but also by the freshness of the fish [32, 33]. This factor may interfere in the analysis. VOCs formed during storage are alcohols, aldehydes, esters, organic acids, and sulfur compounds, which can be either of microbial or nonmicrobial origin [34]. Their non-protonated masses range from m/z 46 for ethanol to m/z 176 for 3-hydroxy-2-butanone. This is a complicating factor and can influence the robustness of the method.

Protonated VOC (m/z)	NS (n = 31)	BalS (n = 3)	BarS (n = 5)	SIr (n = 5)	SIc (n = 5)	P-value NS versus other seas
33	398 ± 623	43 ± 14	60 ± 15	30 ± 17	26 ± 3	0.000
38	3 ± 1	3 ± 0	4 ± 0	4 ± 0	4 ± 0	0.000
39	10 ± 1	10 ± 1	12 ± 1	11 ± 2	10 ± 1	0.063
41	14 ± 7	12 ± 4	11 ± 2	12 ± 3	7 ± 4	0.044
42	39 ± 43	2 ± 1	1 ± 0	0 ± 0	0 ± 0	0.000
43	23 ± 12	13 ± 4	10 ± 1	7 ± 1	16 ± 5	0.001
45	301 ± 240	204 ± 161	69 ± 34	101 ± 29	579 ± 239	0.044
46	7 ± 5	4 ± 4	2 ± 1	2 ± 1	13 ± 6	0.051
47	6 ± 8	14 ± 12	3 ± 1	2 ± 1	55 ± 25	0.793
55	6 ± 3	4 ± 1	6 ± 1	5 ± 1	5 ± 1	0.983
57	10 ± 10	4 ± 1	3 ± 1	25 ± 13	2 ± 1	0.049
59	168 ± 112	79 ± 8	53 ± 17	45 ± 13	66 ± 52	0.000
60	6 ± 4	3 ± 0	2 ± 1	2 ± 0	2 ± 2	0.000
63	1 ± 1	2 ± 2	1 ± 0	1 ± 0	2 ± 0	0.001
69	7 ± 2	3 ± 2	4 ± 1	3 ± 1	4 ± 2	0.000
71	4 ± 2	1 ± 0	0 ± 0	1 ± 0	3 ± 1	0.000
73	7 ± 2	8 ± 1	9 ± 4	6 ± 1	6 ± 3	0.369
87	3 ± 2	1 ± 1	2 ± 0	1 ± 0	1 ± 0	0.000

Table. Average protonated VOC composition (ppbv) of European plaice sampled from the North Sea (NS), the Baltic Sea (BalS), the Barents Sea (BarS), the sea around Ireland (SIr), and the sea around Iceland (SIc); mean intensity, standard deviation, and significant differences between European plaice from the North Sea and European plaice caught in other seas (Kruskal-Wallis).

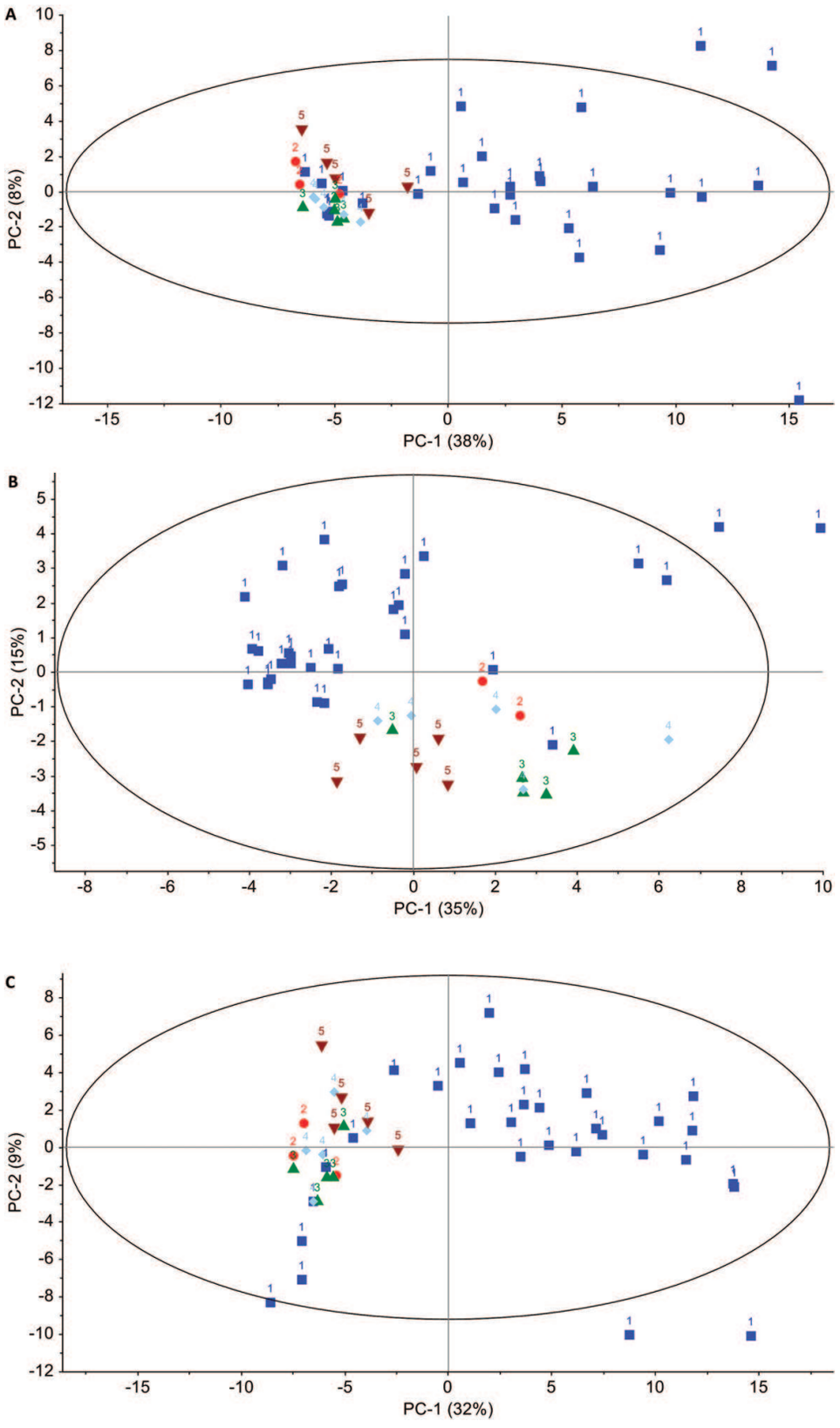

Figure. Principal component analysis plot of (A) PTR-MS data, (B) fatty acid profile, and (C) all data combined, on European plaice from the North Sea (1—box), the Baltic Sea (2—dot), the Barents Sea (3—triangle), the sea around Ireland (4—diamond), and the sea around Iceland (5—inverted triangle).

FA	NS (n = 31)	BalS (n = 3)	BarS (n = 5)	SIr (n = 5)	SIc (n = 5)	P-value NS versus other seas
C12:0	0.03 ± 0.04	0.02 ± 0.01	0.02 ± 0.01	0.06 ± 0.03	0.01 ± 0.01	0.436
isoC14:0	0.03 ± 0.05	0.00 ± 0.00	0.02 ± 0.01	0.01 ± 0.02	0.00 ± 0.01	0.146
C14:0	2.02 ± 0.72	2.01 ± 0.28	2.54 ± 0.24	3.10 ± 0.91	2.69 ± 0.83	0.002
C14:1n5	0.15 ± 0.13	0.06 ± 0.01	0.06 ± 0.04	0.04 ± 0.04	0.03 ± 0.02	0.000
isoC15:0	0.06 ± 0.04	0.00 ± 0.00	0.03 ± 0.02	0.01 ± 0.02	0.01 ± 0.01	0.000
C15:0	0.74 ± 0.16	0.95 ± 0.05	0.70 ± 0.04	0.76 ± 0.11	0.50 ± 0.10	0.332
isoC16:0	0.26 ± 0.19	0.38 ± 0.00	0.35 ± 0.07	0.28 ± 0.09	0.26 ± 0.06	0.004
C15:1n5	0.02 ± 0.04	0.01 ± 0.01	0.03 ± 0.02	0.00 ± 0.00	0.05 ± 0.03	0.279
C16:0	17.22 ± 2.06	17.57 ± 0.23	15.89 ± 1.83	18.89 ± 2.84	20.02 ± 0.68	0.134
C16:1n9	0.43 ± 0.17	0.65 ± 0.06	0.53 ± 0.06	0.70 ± 0.16	0.42 ± 0.04	0.006
C16:1n7	4.28 ± 1.97	7.64 ± 2.21	16.74 ± 3.10	4.88 ± 1.24	6.45 ± 1.53	0.000
isoC17:0	0.43 ± 0.16	0.36 ± 0.05	0.39 ± 0.08	0.33 ± 0.11	0.28 ± 0.06	0.033
anteisoC17:0	0.40 ± 0.29	0.52 ± 0.05	0.49 ± 0.12	0.34 ± 0.14	0.35 ± 0.13	0.140
C17:1n7	0.31 ± 0.17	0.65 ± 0.03	0.28 ± 0.05	0.34[a] ± 0.15	0.25 ± 0.08	0.371
C18:0	4.53 ± 0.64	4.07 ± 0.04	3.16 ± 0.43	4.15 ± 0.42	3.94 ± 0.73	0.001
Trans C18:1 + C18:T2	0.38 ± 0.33	0.49 ± 0.10	0.40 ± 0.15	0.91 ± 0.40	0.65 ± 0.23	0.001
C18:1n9	6.38 ± 2.41	8.60 ± 1.06	8.81 ± 1.41	8.46 ± 1.62	8.21 ± 1.23	0.000
C18:1n7	3.31 ± 0.93	4.50 ± 0.47	4.17 ± 0.62	3.03 ± 0.66	4.23 ± 0.53	0.018
C18:2n6t	0.09 ± 0.07	0.14 ± 0.01	0.08 ± 0.02	0.09 ± 0.06	0.13 ± 0.06	0.253
c9t12C18:2	0.01 ± 0.03	0.02 ± 0.03	0.01 ± 0.02	0.02 ± 0.03	0.03 ± 0.05	0.189
t9c12C18:2	0.21 ± 0.11	0.14 ± 0.00	0.09 ± 0.02	0.23 ± 0.09	0.10 ± 0.02	0.001
C18:2n6c	0.84 ± 0.95	0.85 ± 0.08	1.21 ± 0.29	1.20 ± 0.57	0.41 ± 0.12	0.095
C18:3n6	0.07 ± 0.05	0.13 ± 0.03	0.16 ± 0.03	0.09 ± 0.02	0.06 ± 0.01	0.019
C18:3n3 + C20:0	0.38 ± 0.18	0.41 ± 0.02	0.57 ± 0.18	0.63 ± 0.19	0.29 ± 0.08	0.057
C20:1n9	1.16 ± 0.52	1.99 ± 0.07	2.45 ± 0.71	3.49 ± 1.36	1.98 ± 0.57	0.000
C21:0	0.07 ± 0.06	0.12 ± 0.02	0.08 ± 0.01	0.04 ± 0.02	0.09 ± 0.03	0.482
C20:2n6	0.35 ± 0.19	0.53 ± 0.01	0.40 ± 0.09	0.69 ± 0.33	0.30 ± 0.11	0.054
C20:3n6	0.14 ± 0.05	0.17 ± 0.04	0.09 ± 0.06	0.13 ± 0.05	0.05 ± 0.01	0.044
C20:3n3 + C20:4n6 + C22:0	6.00 ± 2.20	3.44 ± 0.26	3.28 ± 1.20	4.24 ± 0.75	2.44 ± 0.91	0.000
C22:1n9	0.19 ± 0.26	0.41 ± 0.04	0.41 ± 0.07	0.64 ± 0.17	0.48 ± 0.15	0.000
C20:5n3	17.70 ± 3.27	13.72 ± 1.45	13.19 ± 3.05	15.63 ± 3.33	19.44 ± 2.70	0.075
C22:2n6	0.07 ± 0.03	0.06 ± 0.00	0.10 ± 0.04	0.04 ± 0.05	0.05 ± 0.02	0.991

FA	NS (n = 31)	BalS (n = 3)	BarS (n = 5)	SIr (n = 5)	SIc (n = 5)	P-value NS versus other seas
C24:0	0.15 ± 0.08	0.19 ± 0.05	0.23 ± 0.12	0.15 ± 0.11	0.07 ± 0.05	0.889
C24:1n9	0.23 ± 0.29	0.47 ± 0.07	0.25 ± 0.08	0.52 ± 0.15	0.41 ± 0.12	0.000
C22:6n3	15.78 ± 4.22	13.31 ± 2.72	6.35 ± 1.30	10.60 ± 4.34	11.45 ± 3.24	0.000
SFA*	26.51 ± 1.62	25.77 ± 0.08	23.50 ± 1.83	28.15 ± 2.00	28.05 ± 0.77	0.788
MUFA	16.31 ± 4.99	24.42 ± 3.93	32.07 ± 4.75	20.10 ± 3.57	21.52 ± 2.78	0.000
PUFA	41.52 ± 6.35	32.75 ± 4.23	25.31 ± 4.74	33.14 ± 6.83	34.53 ± 2.89	0.000
OFA	15.66 ± 3.89	17.06 ± 0.38	19.12 ± 1.90	18.60 ± 5.64	15.90 ± 1.24	0.016
Omega 3	39.05 ± 7.85	27.32 ± 4.17	20.01 ± 3.83	26.58 ± 6.68	31.05 ± 2.66	0.000
Omega 6	1.38 ± 1.04	2.05 ± 0.20	2.12 ± 0.36	2.48 ± 0.74	1.14 ± 0.21	0.002
Omega 3/omega 6 ratio	38.09 ± 18.72	13.51 ± 3.33	9.90 ± 3.66	11.75 ± 5.44	28.21 ± 6.26	0.000

*SFA = saturated fatty acids, MUFA = monounsaturated fatty acids, PUFA = polyunsaturated fatty acids, OFA = other fatty acids.

Table. Average fatty acid composition of European plaice sampled from the North Sea (NS), the Baltic Sea (BalS), the Barents Sea (BarS), the sea around Ireland (SIr), and the sea around Iceland (SIc); mean concentration, standard deviation, and significant differences between European plaice from the North Sea and European plaice caught in other seas (Kruskal-Wallis).

3.1.2. FA profiles by GC-FID

Subsequently, the reference set of European plaice was subjected to GC-FID analysis. European plaice contains around 0.8% fat [35]. This was extracted and its FA composition was analyzed. The most abundant FAs in European plaice were palmitic acid (C16:0), eicosapentaenoic acid (EPA, C20:5n3), and docosahexaenoic acid (DHA, C22:6n3) (**Table**). Karl et al. [35] found a similar FA profile for European plaice from the North Sea.

When comparing the origins for each FA, 19 out of 35 FAs revealed significant differences between European plaice from the North Sea and European plaice from other seas (Kruskal-Wallis $P < 0.05$; **Table**). European plaice from the Barents Sea seems to have a rather distinct FA profile compared to the other seas. When considering groups of FAs instead of individual FAs, contents of MUFA (monounsaturated FA), PUFA (polyunsaturated FA), OFA (other FA), omega-3 FAs, and omega-6 FAs were significantly different in North Sea European plaice from European plaice originating from other seas (Kruskal-Wallis $P < 0.05$). Colombo et al. [19] suggested that fish originating from a higher latitude would contain more PUFA. However, this was not confirmed in our study. European plaice from the sea with the highest latitude, the Barents Sea, showed to have the lowest PUFA content, namely 25%. The sea around Ireland, which has the lowest latitude, showed a rather average PUFA content, namely 33%.

PCA reveals clustering of samples (**Figure B**). Generally, the samples from the North Sea and those from the other seas are separated in the second dimension. Therefore, the FA profile seems to be a suitable option to distinguish North Sea European plaice from those

originating from other seas. This was expected since the FA composition of the fish tissue is reflected by their diets and these will vary for the different geographical locations [18].

3.1.3. Isotope ratios by IR-MS

Finally, all reference samples were subjected to IR-MS for analysis of carbon, hydrogen, nitrogen, and sulfur isotope ratios. Results are visualized in a scatter plot (**Figure**). Hydrogen isotope ratio (δ^2H) is severely depleted compared to sea water, ranging from −43.7 to −112.5‰ (versus Vienna Standard Mean Ocean Water). This can be explained by the fact that organic tissue is synthesized from metabolized products and their isotopic composition is referenced to local food chains rather than dissolved water isotopes [36]. Sulfur isotope ratio ($\delta^{34}S$) is enriched in European plaice, ranging from +4.6 to +20.1‰. This is typical for marine fish, whereas freshwater fish typically shows depleted $\delta^{34}S$ [37]. Carbon isotope ratio ($\delta^{13}C$) is depleted, ranging from −14.6 to −21.1‰. Nitrogen isotope ratio ($\delta^{15}N$) is enriched, ranging from +8.9 to +13.0‰. Both $\delta^{13}C$ and $\delta^{15}N$ ratios are in accordance with previous research on flatfish from the southern North Sea and claimed to reflect the European plaices' diet [38].

In both scatter plots, grouping of the samples according to their geographical origin is clearly visible. Only samples from the seas around Ireland and Iceland seem to group together. Nitrogen and hydrogen isotope ratios in the North Sea European plaice were significantly different from the ratios in European plaice from other seas (Kruskal-Wallis $P < 0.05$). This was expected based on the results previously described on the carbon, hydrogen, nitrogen, and oxygen isotope ratios of various fish species [20, 21].

3.1.4. All data combined

All VOC, FA, and isotope ratio data acquired in this study were also combined and subjected to PCA plot (**Figure C**). Again, groups of samples from the same geographical origin cluster together, with 80% of the European plaice from the North Sea demonstrating high positive

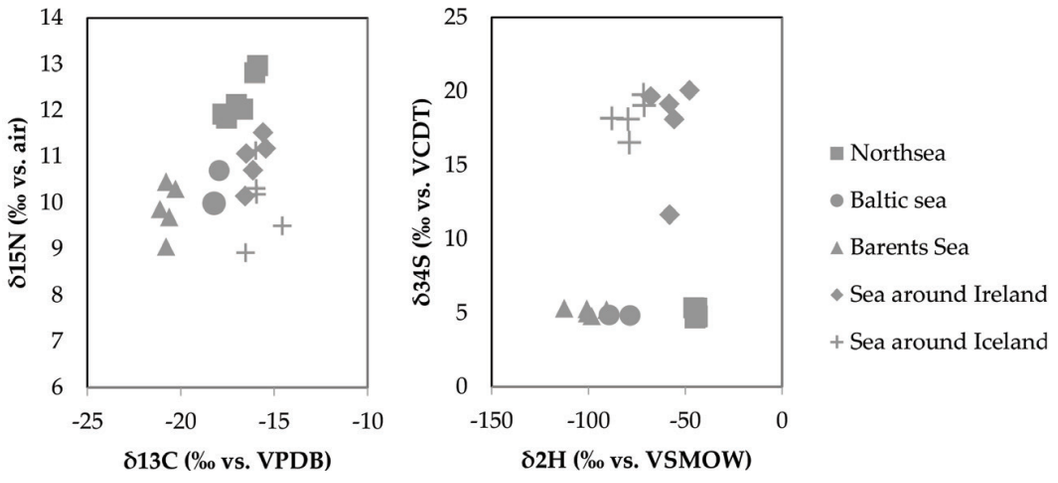

Figure. Scatter plot of IRMS data on European plaice: $\delta^{13}C$ versus δ15N (left) and δ^2H versus $\delta^{34}S$ (right) on European plaice from the North Sea (box), the Baltic Sea (dot), the Barents Sea (triangle), the sea around Ireland (diamond), and the sea around Iceland (plus).

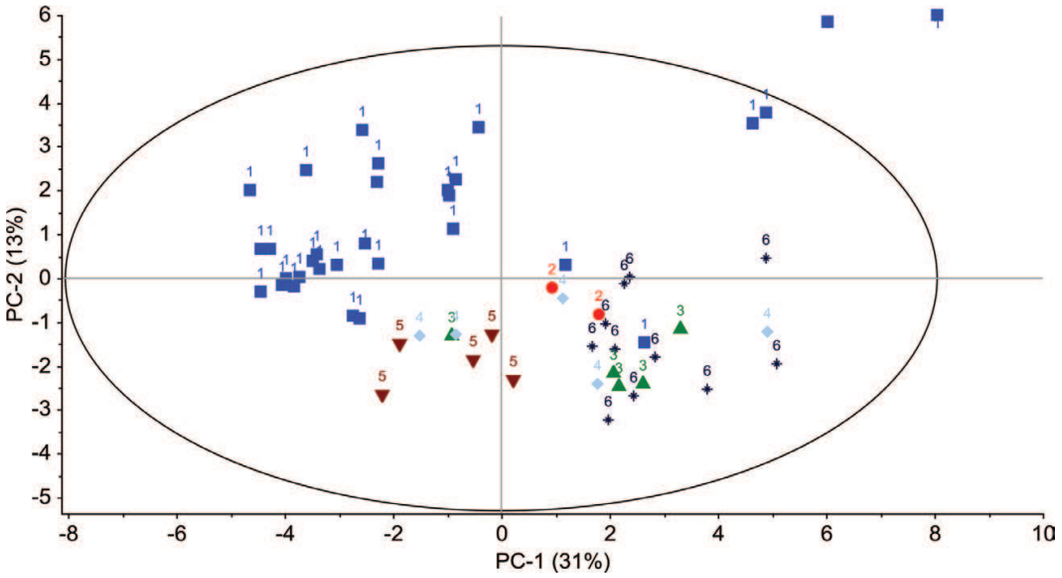

Figure. PCA plot of fatty acid profile of European plaice from the North Sea (1—box), the Baltic Sea (2—dot), the Barents Sea (3—triangle), the sea around Ireland (4—diamond), the sea around Iceland (5—inverted triangle), with projection of retail European plaice samples (6—star).

scores in the first dimension and samples from other seas clustering together with the rest from the North Sea and showing negative scores in the first PCA dimension. Distinction of North Sea European plaice from others appears less effective with all data combined in comparison to some of the individual datasets.

3.1.5. Retail European plaice samples

Eventually, a set of 11 non-breaded and non-marinated European plaice fillets from German, Italian, and Dutch supermarkets were analyzed for their FA profiles and compared with the reference set, since FA profiling showed a good distinction and is a robust method. They were projected on the PCA plot of the FA profiles as a sixth class (**Figure**). All retail samples clus-ter in the same region as European plaice samples from the Baltic Sea, the Barents Sea, and the sea around Ireland. According to their label, they originated from the North Sea specifically (n = 4), or more generally from FAO zone 27 (n = 7). In this phase of the research, it cannot be explained why all retail European plaice samples would cluster together away from the samples from the North Sea. This is possibly caused by the fact that samples were collected in a different year and season. However, more research would be needed to evaluate the seasonal effect on the FA composition.

4. Conclusion

This case study showed that the geographical origin of food products is reflected in its product characteristics, which can be measured by several analytical techniques. From the techniques evaluated in this study, it appears that VOC, FA, and isotope ratios all provide

relevant information regarding the provenance of European plaice. VOC analysis by PTR-MS allows rapid analysis, but may be influenced by factors like freshness and packaging. FA and isotope ratio analyses are more robust from that perspective, and both allow automated routine analyses. More samples should be collected to provide insight into differences between seasons and for building chemometric classification models. Nevertheless, the approaches are promising for future provenance verification of European plaice. The results may help to underpin sustainable fishing. Sustainable fishing is an important topic for governmental and possible certification organizations.

In a broader perspective, provenance of food products has become an important topic in food authenticity because of: (a) a quality perspective: the geographical origin of a food product influences its composition and thereby its quality and (b) an environmental perspective: some production locations are more sustainable than others. Consumer awareness on these topics is growing rapidly; they want their food to be of high quality and with a clear geographical identity.

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Conflict of interest

The authors declare no conflict of interest. The founding sponsors had no role in the design of the study; in the collection, analyses, or interpretation of data; in the writing of the manuscript; and in the decision to publish the results.

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References

[1] Moore JC, Spink J, Lipp M. Development and application of a database of food ingredient fraud and economically motivated adulteration from 1980 to 2010. Journal of Food Science. 2012;**77**(4):R118-RR26

[2] El Sheikha AF, Montet D. How to determine the geographical origin of seafood? Critical Reviews in Food Science and Nutrition. 2016;**56**(2):306-317

[3] Regulation (EU) No 1379/2013 of the European Parliament and of the Council of 11 December 2013 on the Common Organisation of the Markets in Fishery and Aquaculture Products, Amending Council Regulations (EC) No 1184/2006 and (EC) No 1224/2009 and Repealing Council Regulation (EC) No 104/2000; 2013

[4] Pustjens AM, Weesepoel Y, van Ruth SM, Leadley CE. Food fraud and authenticity: Emerging issues and future trends. In: Innovation and Future Trends in Food Manufacturing and Supply Chain Technologies. Sawston, Cambridge, UK: Woodhead Publishing; 2016. pp. 3-20

[5] Luykx DMAM, van Ruth SM. An overview of analytical methods for determining the geographical origin of food products. Food Chemistry. 2008;**107**(2):897-911

[6] Pustjens AM, Muilwijk M, Weesepoel Y, van Ruth SM. Advances in authenticity testing of geographical origin of food products. In: Downey G, editor. Advances in Food Authenticity Testing. Woodhead Publishing; 2016. pp. 339-367

[7] Lee WH, Choi S, Oh IN, Shim JY, Lee KS, An G, et al. Multivariate classification of the geographic origin of Chinese cabbage using an electronic nose-mass spectrometry. Food Science and Biotechnology. 2017;**26**(3):603-609

[8] Boggia L, Pignata G, Sgorbini B, Colombo ML, Marengo A, Casale M, et al. *Artemisia umbelliformis* Lam. and Génépi Liqueur: Volatile profile as diagnostic marker for geographic origin and to predict liqueur safety. Journal of Agricultural and Food Chemistry. 2017; **65**(13):2849-2856

[9] Masi E, Taiti C, Heimler D, Vignolini P, Romani A, Mancuso S. PTR-TOF-MS and HPLC analysis in the characterization of saffron (*Crocus sativus* L.) from Italy and Iran. Food Chemistry. 2016;**192**:75-81

[10] Li G, Cervelli C, Ruffoni B, Shachter A, Dudai N. Volatile diversity in wild populations of rosemary (*Rosmarinus officinalis* L.) from the Tyrrhenian Sea vicinity cultivated under homogeneous environmental conditions. Industrial Crops and Products. 2016;**84**:381-390

[11] Yener S, Sánchez-López JA, Granitto PM, Cappellin L, Märk TD, Zimmermann R, et al. Rapid and direct volatile compound profiling of black and green teas (*Camellia sinensis*) from different countries with PTR-ToF-MS. Talanta. 2016;**152**:45-53

[12] Condurso C, Mazzaglia A, Tripodi G, Cincotta F, Dima G, Maria Lanza C, et al. Sensory analysis and head-space aroma volatiles for the characterization of capers from different geographic origin. Journal of Essential Oil Research. 2016;**28**(3):185-192

[13] Araghipour N, Colineau J, Koot A, Akkermans W, Rojas JMM, Beauchamp J, et al. Geographical origin classification of olive oils by PTR-MS. Food Chemistry. 2008;**108**(1): 374-383

[14] Frank D, Poole S, Kirchhoff S, Forde C. Investigation of sensory and volatile characteristics of farmed and wild barramundi (*Lates calcarifer*) using gas chromatography–olfactometry mass spectrometry and descriptive sensory analysis. Journal of Agricultural and Food Chemistry. 2009;**57**(21):10302-10312

[15] Cossignani L, Blasi F, Simonetti MS, Montesano D. Fatty acids and phytosterols to discriminate geographic origin of *Lycium barbarum* berry. Food analytical methods. 2018;**11**(4):1180-1188

[16] Ricardo F, Maciel E, Domingues MR, Calado R. Spatio-temporal variability in the fatty acid profile of the adductor muscle of the common cockle *Cerastoderma edule* and its relevance for tracing geographic origin. Food Control. 2017;**81**:173-180

[17] Amorello D, Orecchio S, Pace A, Barreca S. Discrimination of almonds (*Prunus dulcis*) geographical origin by minerals and fatty acids profiling. Natural Product Research. 2016; **30**(18):2107-2110

[18] Happel A, Pattridge R, Walsh M, Rinchard J. Assessing diet compositions of Lake Ontario predators using fatty acid profiles of prey fishes. Journal of Great Lakes Research. 2017; **43**(5):838-845

[19] Colombo SM, Wacker A, Parrish CC, Kainz MJ, Arts MT. A fundamental dichotomy in long-chain polyunsaturated fatty acid abundance between and within marine and terrestrial ecosystems. Environmental Reviews. 2017;**25**(2):163-174

[20] Jardine TD, Gray MA, McWilliam SM, Cunjak RA. Stable isotope variability in tissues of temperate stream fishes. Transactions of the American Fisheries Society. 2005;**134**(5): 1103-1110

[21] Rossier JS, Maury V, de Voogd B, Pfammatter E. Use of isotope ratio mass spectrometry (IRMS) determination (18O/16O) to assess the local origin of fish and asparagus in western Switzerland. CHIMIA International Journal for Chemistry. 2014;**68**(10):696-700

[22] Pappalardo AM, Ferrito V. DNA barcoding species identification unveils mislabeling of processed flatfish products in southern Italy markets. Fisheries Research. 2015; **164**:153-158

[23] FAO. Aquatic Species Distribution Map Viewer: Fisheries and Aquaculture Department. 2017. Available from: http://www.fao.org/figis/geoserver/factsheets/species.html?species=PLE-m&prj=4326

[24] De Vlas J. Annual food intake by plaice and flounder in a tidal flat area in the dutch wadden sea, with special reference to consumption of regenerating parts of macrobenthic prey. Netherlands Journal of Sea Research. 1979;**13**(1):117-153

[25] Rijnsdorp AD, Vingerhoed B. Feeding of plaice *Pleuronectes platessa* L. and sole *Solea solea* (L.) in relation to the effects of bottom trawling. Journal of Sea Research. 2001;**45**(3):219-229

[26] Engelhard GH, Pinnegar JK, Kell LT, Rijnsdorp AD. Nine decades of North Sea sole and plaice distribution. ICES Journal of Marine Science. 2011;**68**(6):1090-1104

[27] van Keeken OA, van Hoppe M, Grift RE, Rijnsdorp AD. Changes in the spatial distribution of North Sea plaice (*Pleuronectes platessa*) and implications for fisheries management. Journal of Sea Research. 2007;**57**(2):187-197

[28] Petitgas P, Rijnsdorp AD, Dickey-Collas M, Engelhard GH, Peck MA, Pinnegar JK, et al. Impacts of climate change on the complex life cycles of fish. Fisheries Oceanography. 2013;**22**(2):121-139

[29] Hansel A, Jordan A, Holzinger R, Prazeller P, Vogel W, Lindinger W. Proton transfer reaction mass spectrometry: On-line trace gas analysis at the ppb level. International Journal of Mass Spectrometry. 1995;**149-150**:609-619

[30] Tres A, Van Ruth SM. Verification of organic feed identity by fatty acid fingerprinting. Journal of Agricultural and Food Chemistry. 2011;**59**(16):8816-8821

[31] Guardiola F, Codony R, Rafecas M, Boatella J, López A. Fatty acid composition and nutritional value of fresh eggs, from large- and small-scale farms. Journal of Food Composition and Analysis. 1994;**7**(3):171-188

[32] Olafsdottir G, Lauzon HL, Martinsdóttir E, Oehlenschláuger J, Kristbergsson K. Evaluation of shelf life of superchilled cod (*Gadus morhua*) fillets and the influence of temperature fluctuations during storage on microbial and chemical quality indicators. Journal of Food Science. 2006;**71**(2):S97-S109

[33] Phan N-T, Kim K-H, Jeon E-C, Kim U-H, Sohn JR, Pandey SK. Analysis of volatile organic compounds released during food decaying processes. Environmental Monitoring and Assessment. 2012;**184**(3):1683-1692

[34] Parlapani FF, Mallouchos A, Haroutounian SA, Boziaris IS. Volatile organic compounds of microbial and non-microbial origin produced on model fish substrate un-inoculated and inoculated with gilt-head sea bream spoilage bacteria. LWT- Food Science and Technology. 2017;**78**:54-62

[35] Karl H, Manthey-Karl M, Ostermeyer U, Lehmann I, Wagner H. Nutritional composition and sensory attributes of Alaskan flatfishes compared to plaice (*Pleuronectes platessa*). International Journal of Food Science and Technology. 2013;**48**(5):962-971

[36] Trueman CN, Mackenzie KM, Palmer MR. Identifying migrations in marine fishes through stable-isotope analysis. Journal of Fish Biology. 2012;**81**(2):826-847

[37] Sayle KL, Cook GT, Ascough PL, Hastie HR, Einarsson T, McGovern TH, et al. Application of 34S analysis for elucidating terrestrial, marine and freshwater ecosystems: Evidence of animal movement/husbandry practices in an early Viking community around Lake Mývatn, Iceland. Geochimica et Cosmochimica Acta. 2013;**120**:531-544

[38] Fuller BT, Müldner G, Van Neer W, Ervynck A, Richards MP. Carbon and nitrogen stable isotope ratio analysis of freshwater, brackish and marine fish from Belgian archaeological sites (1st and 2nd millennium AD). Journal of Analytical Atomic Spectrometry. 2012;**27**(5):807-820

Chapter 6

The Dairy Industry: Process, Monitoring, Standards, and Quality

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Abstract

Sampling and analysis occur along the milk processing train: from collection at farm level, to intake at the diary plant, the processing steps, and the end products. Milk has a short shelf life; however, products such as milk powders have allowed a global industry to be developed. Quality control tests are vital to support activities for hygiene and food standards to meet regulatory and customer demands. Multiples of chemical and microbiological contamination tests are undertaken. Hazard analysis testing strategies are necessary, but some tests may be redundant; it is therefore vital to identify product optimization quality control strategies. The time taken to undergo testing and turnaround time are rarely measured. The dairy industry is a traditional industry with a low margin commodity. Industry 4.0 vision for dairy manufacturing is to introduce the aspects of operational excellence and implementation of information and communications technologies. The dairy industries' reply to Industry 4.0 is represented predominantly by proactive maintenance and optimization of production and logistical chains, such as robotic milking machines and processing and packaging line automation reinforced by sensors for rapid chemical and microbial analysis with improved and real-time data management. This chapter reviews the processing trains with suggestions for improved optimization.

Keywords: dairy, processing, hygiene, analytical tests, automation

1. Introduction

The implementation of strategies to improve and strengthen milk process optimization is of vital importance within the dairy industry. The rapid deterioration of milk products forces

dairy processors to critically optimize and plan their production schedules. The business model is to look at the work force, to reduce or eliminate any time or/and resource wastage, unnecessary costs, bottlenecks, and mistakes while attaining the process objective of creating a quality product [1].

The global dairy sector is currently going through change. The Food and Agricultural Organization of the United Nations (FAO-UN), dairy price index shows prices 26% below its peak from February 2014 [2]. The demand for milk products from China is beginning to slow, trade sanctions on Russia and the end of "milk quotas" within the European Union (EU) has caused a period of excess supply and low prices [3]. Notwithstanding this, the dairy sector is expanding and projected to grow at a rate of 1.8% per year over the next 10 years, to 177 million tons of powdered milk by 2025 [4]. This increase is mainly due to rising urbanization and growing incomes in emerging markets [5]. In the EU, however, dairy farmers have used intervention stocks to shield themselves from poorer international prices. In September 2017, for instance, EU farmers consigned 16,597 tons of skimmed milk powder (SMP) to the interventions stock at Euro €1.698 [6].

In addition, changing consumer demand patterns are affecting food production. The "Traditional" value drivers of price, taste, and convenience have been complemented by newer and "Evolving" drivers such as health and wellness, safety, social impact, and experience. Central to all of these drivers is a need for transparency from food companies [5]. Given the ever-changing nature of the consumer food value drivers, dairy producers must look to their production processes to innovate with new products and to optimize output without compromising on quality and safety.

The world's milk is predominantly cow's milk, followed by buffalo milk. The leading producers include, Asia (30%), followed by the EU (28%), North and Central America (18%), South America (9%), other European countries (9%), Africa (5%), and Oceania (5%) [7]. To be named a dairy product, food must be produced from the milk of cows, buffalo, goats, etc. The dairy sector includes food such as liquid milk, milk powders, cheese, butter, and yogurt, as well as ice cream. Several factors including genetics, and breed of animal, environment, stages of lactation, parity, and nutrition, together determine the final composition of milk [8]. Milk and dairy products are significant sources of protein, essential minerals (calcium, potassium, magnesium, phosphorous, sodium, iodine) and several vitamins, (the fat-soluble vitamins A, D, E, K, and B1, B3, B6, B12). In a Western diet, dairy products provide between 40 and 70% of the recommended daily calcium intake. Cow's milk consists of about 87% water (**Table**), and 12–13% total solids. The solids consist of fat ~4% and solids-not-fat (SNF) ~9%, such as pro-teins, lactose, and various minerals and vitamins. Milk proteins consist of whey and caseins; caseins have four different species (α_{S1}, α_{S2}, β, and κ-caseins) which are separate molecules, but they do possess similarity in structure and they comprise around 80% of total milk protein. The major whey proteins in cow and sheep's milk are β-lactoglobulin and α-lactalbumin; the other proteins are serum albumin and immunoglobulins. Minor proteins include lactofer-rin (LF), an iron binding protein and β_2-Microglobulin—part of the Major Histocompatibility Complex II (MHC II), the rest are mostly enzymes including; lactoperoxidase, an enzyme that breaks down hydrogen peroxide, lysozyme which breaks down bacterial cell walls and has low activity in cow milk, proteases, protease activators, nucleases, glycosidases, and others.

	Cow's milk %	Skim milk powder (SMP) %	Whole milk powder (WMP) %	Acid whey powder (WP) %
Moisture	85.5–89.5	3.0–4.0	2.0–4.5*	3.5–5.0
Fat	2.5–6.0	0.6–1.5	26.0–42.0	1.0–1.5
Protein	2.9–5.0	34.0–37.00	24.5–27	11.0–14.5
Lactose	3.6–5.5	49.5–52.0	36.0–38.5	63.0–75.0
Minerals (ash)	0.8–0.9	8.2–8.6	5.5–6.5	8.2–8.8

*The moisture content does not include water of crystallization of the lactose, the milk solids-not-fat content includes the water of crystallization of the lactose (Source: [11, 12]).

Table. Average composition of milk and milk powders.

The milk proteins contain the nine essential amino acids required by humans, making it an important human food. The caseins are easily digested, while the whey proteins are relatively less digestible in the intestine.

The milk fat content varies within the same dairy products and between different dairy products. Raw farm milk, full-fat milk, semi-skimmed milk, and skimmed milk have their own percentage of fat. Raw milk normally has a fat content of ~4.4 g of milk fat per 100 g. This can be skimmed to obtain lower fat varieties. Full-fat milk is standardized to 3.5% of fat and semi-skimmed milk contains ~1.5% fat. Skimmed milk and buttermilk are very low in fat and, on average, contain 0.1 or 0.2% fat, respectively. The fat content of milk and cream is also known as butterfat, an important factor in determining the price to be paid for milk supplied by farmers in many countries. Milk sold to the consumer is standardized with a range of different fat content choices. However, international variances in standardization mean that the fat percentage for (semi)-skimmed, whole milk, and buttermilk can differ between countries. Modifications in the composition of milk are allowed, if they are indicated on the packing of the product, so that it can be easily seen and read, complying with the obligation as regards nutrition labeling, laid down by the countries regulations. In the case of the EU, regulation No. 1169/2011 applies on the provision of food information to consumers [9], plus providing an indication of origin, is considered of particular interest. The US Public Health Service (USPHS) Milk Ordinance and Code recommends a minimum of 3.25% butterfat in farm milk, as the official national standard [10].

Milk is not necessarily a local product and has developed into a global trade with the development of milk powders. In particular, whole milk powder (WMP) and skimmed milk powder (SMP) are the most traded agricultural commodities globally, as percentage of production traded, while fresh dairy products, with less than 1% of production traded are the least traded agricultural commodity [4]. The dairy industry, however, has been targeted in the climate change debate as it has been estimated that 14.5% of greenhouse gas emissions come from livestock with beef and milk production the main culprits [13, 14]. Extreme changes in climate can affect the microbiological safety of food. Wet conditions are favorable to pathogen growth and may result in increased risk of food contamination, including mycotoxin. Aflatoxin M1 is the most studied mycotoxin in milk and levels exceeding the EU maximum level (0.050 μg/kg) have been found [15]. Climate effects on animal diseases lead to increased use of veterinary

medicines resulting in drug resistance and anthropogenic (synthetic) chemicals with the potential for transmission of chemical residues into the food chain. The more frequent and intense rainfall that is predicted could encourage the spread of perchlorate through surface runoff with the potential to enter the food chain via cow's milk [16]. Perchlorate reduces thyroid hormone production in the thyroid gland [17].

2. Milk processing

The flow diagram for milk processing is presented in **Figure**. Milk arrives at the milk dairy processing plant over the weighbridge and the weight of milk is automatically recorded. At the same time, data from an on-board computer is downloaded wirelessly to a data capture system, which holds the records of the temperature and volumes of milk collected from each farm. The temperature should be at 4–6°C. Milk samples using sterile containers are collected automatically from each supplier at source and are delivered to a laboratory technician for detailed analysis. Milk that deviates in composition, taste, and smell from normal milk receives a lower quality rating. The technician also takes a composite sample, from each compartment in the refrigerated truck, which is compartmentalized to reduce sloshing of the milk. The samples from each compartment are tested for acidity, antibiotics, added water, fat, and protein content. These analytical tests and methods are determined by international standards as outlined in **Table**.

The ISO standards catalog ISO/TC34/SC5 [18] lists all milk and milk products standards, while other standard sets include, microbiology of the food chain, microbiological quality of milk, etc. The bacterial quality of the milk is also measured and these specify tests are outlined later.

The titratable acid test measures the acidity of the milk. Both titratable acidity (TA) and pH are measures of acid. TA is a more reliable indicator because relative to pH measurement, it is more sensitive to small changes in milk acidity, especially important in cheese making. The acidity of milk is of two types; natural acidity due to citrates and phosphates present in the milk and dissolved CO_2 during the processing of milking. The second is the developed acidity due to lactic acid produced by bacteria using the lactose in the milk as a nutrient, converting it to lactic acid. The acidity of milk measures the total acidity (natural acidity of milk and developed acidity). The International Standard Method for titratable acid is ISO 6091:2010 [19]. Titratable acidity is a measure of the buffering of milk between pH 6.6 and 8.3 (phenolphthalein endpoint) [26]. The appearance of a faint pink color, which signals the endpoint and the number of ml of NaOH used to reach the endpoint, is recorded. This value is called the "titer," titratable acidity is reported as percent lactic acid and is dependent on the volume of sample. As this test is dependent on the analyst reading eye measurement of the color change, it is prone to human error causing incorrect and unpredictable recording of results.

The antibiotic test uses kits known as Charm and Delvo tests. The Charm test is made by Charm Science Inc., e.g., one kit, the Charm Rosa TET–SL (www.charm.com), detects chlortetracycline, oxytetracycline, and tetracycline residues in raw milk in the initial assay at or below

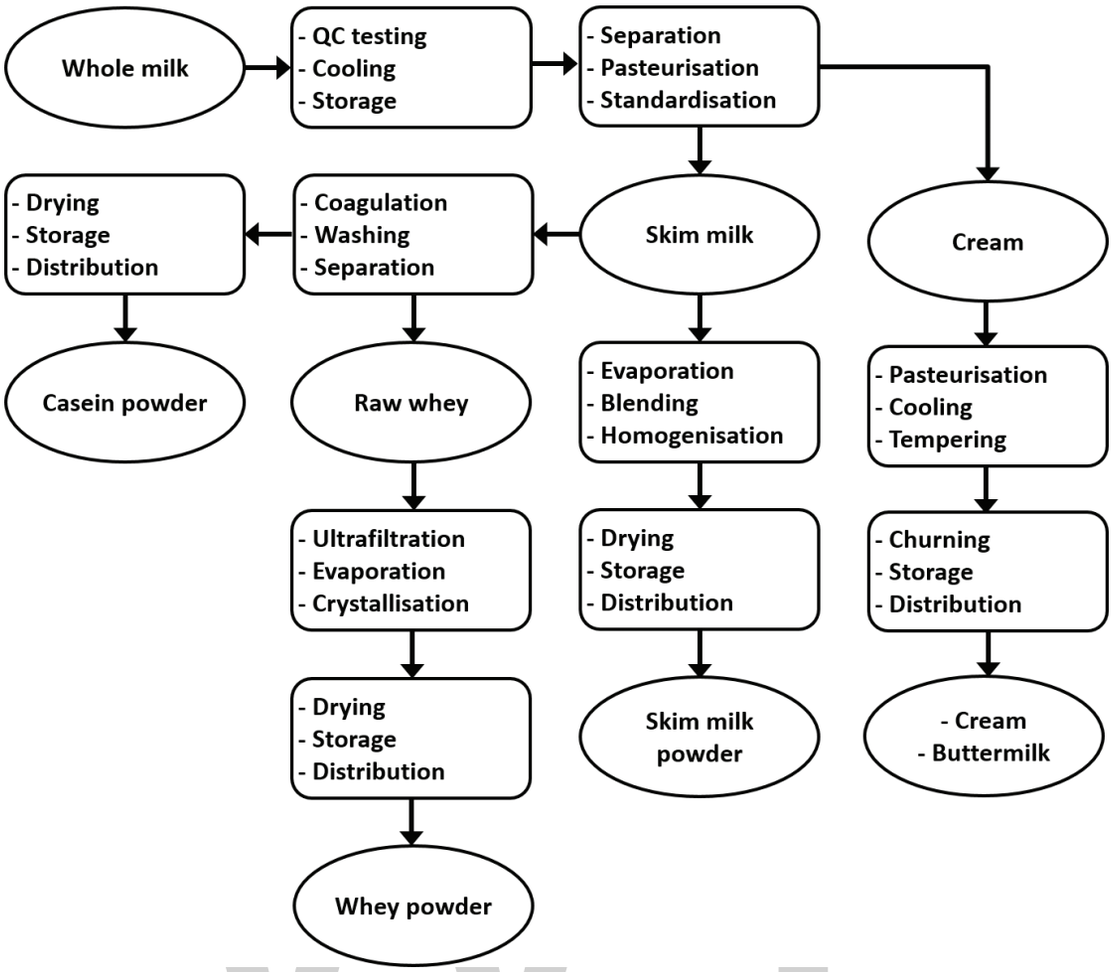

Figure. Milk processing stages.

Quality tests	Acceptable limits	Standards	Reference number
Acidity (Titratable)	≤0.18%	ISO 6091:2010	[19]
Antibiotic residues	Absent/0.1 g	ISO 26844:2006c	[20]
Freezing point (added water)	−0.54°C	ISO 5764:2009	[21]
Fat	0.8%	ISO 1736:2008	[22]
Protein	34%	ISO 8968–1/2:2014 and ISO 14891:2002	[23, 24]
Lactose	>4.2%	ISO 22662:2007	[25]

Table. Quality analytical tests for raw milk.

100 ppb, which complies with the EU regulation No 2377/90 [27], ISO 26844:2006 [20], and Codex Alimentarius regulations CAC/MRL 2-2015 [28]. The Delvo test (a rapid kit developed by DSM, food and beverage section; see, https://www.dsm.com) can be specific for residues of β-lactam or broad-spectrum antibiotics. Considerable concerns regarding antibiotics in the

food chain and antibiotic resistance transfer to human has been postulated, but in most cases, there is not sufficient evidence to demonstrate this conclusively [29].

Added water can be measured by changes in the freezing point of milk from its normal values, the current official freezing point limit is −0.525° Horvet or −0.505°C and was designed for whole-herd, bulk-tank samples, or processed milk samples. The freezing point of milk is the constant physical-chemical property of milk, which is determined only by its water-soluble components such as lactose, and salts, which in accordance with the Wigner law are held in milk at an approximately constant concentration. However, the mineral composition of milk depends on lactation, nutritional status of the animal, and environmental and genetic factors [30].

Adulteration of milk with water will cause a measureable rise of the freezing point of milk. The freezing point is also lowered by acidification of milk, which leads to protein denaturation. The freezing point is considered as an accurate and sensitive method, most laboratories use a cryoscopy, method that is the ISO reference method ISO 5764:2009 [21].

The average fat content of raw milk is ~4.4 g of milk fat per 100 g; with more than 400 various fatty acids (FA) being present in milk [31]. The milk fatty acids are derived almost equally from two sources, the feed and the microbial activity in the rumen of the cow [32]. A study of Swedish bovine milk found that the milk contained substantial quantities of unsaturated fatty acids with 4–10 carbon chains (C4:0–C10:0), about 2% each of saturated C18:2 and trans-C18:1, and almost no other long-chain polyunsaturated fatty acids. The most important fatty acid from a quantitative viewpoint was palmitic acid (C16:0), which accounted for approximately 30% by weight of the total fatty acids. Myristic acid (C14:0) and stearic acid (C18:0), made up 11 and 12% by weight, respectively [31]. Fatty acid composition can show rapid and significant variation in response to changes in diet. The ISO standard for fat determination is ISO 1736:2008 [22].

The fatty acid make-up of the milk can be altered by changes in diet [33], but are also affected by a number of factors, including diet composition, nutrient utilization, lactation cycle, breed of cows, with dietary variations changes up to 3% units, been reported [34]. Specific fatty acids produced during microbial fermentation of dietary fats in the rumen of the cows are responsible for low milk fat. 2–3 g of these fatty acids can decrease milk fat by 0.5% or more [35]. The Gerber method is a historic method still used today to find the fat content of milk in particular in milk powders. By using specific butyrometers designed especially for the different dairy products, e.g., for cream, ice cream, whole milk, or cheese butyrometers, with method modifications [36]. There are many suppliers of such analytical tools, e.g., Gerber instruments (http://www.gerber-instruments.com) or Brouwland instruments (https://www.brouwland.com). Infrared analysis (IR) is a commonly used method for the examination of milk and its components such as solid fat, milk proteins, or carbohydrates [37, 38]. An ISO standard 9622:2013 is available for IR applications for milk and milk products [39].

The protein fat and lactose content of milk has a bearing on the price the farmer achieves for its milk. Liquid milk contains around 3.4% protein. The proteins in milk were described

previously. The determination of protein content of milk and milk products underpins the international trade in dairy products. There are different analytical approaches for the determination of protein quality for nutrition purposes and chemically defined protein. These are divided into three broad categories: (i) determination of total nitrogen, (ii) direct protein determination, and (iii) indirect protein determination [40]. The Kjeldahl method ISO 8968/1:2014 [23] and Dumas method ISO 14891:2002 [24] are the current international standards, and use chemical digestion and combustion approaches respectfully. The advantage of these methods is that they have high reliability and accuracy. Using these methods, around 95% of nitrogen in milk is found to be present as proteins, with the remainder as nonprotein nitrogen sources such as urea. Together these tests and values form the basis for testing the quality of milk and milk products.

3. Dairy processing stages

The raw milk in the milk container truck, having passed the preliminary analytical tests, proceeds to whole milk intake bays and the milk hoses are connected up by the driver. The milk is pumped into bulk storage tanks called milk silos (capacity can be up to 300,000 l, plus). The driver enters the trucks identification number on the pump's control panel or uses a key fob (a passive wireless electronic device that usually uses radio frequency ID technology) to start pumping into the whole milk silos. Unloaded milk is cooled automatically to 4–6°C with a heat plate exchanger (HPE) while pumped into the silo. The offload time and setup time taken to couple and decouple the milk intake hoses are areas where processing monitoring can be implemented. The pumping time can be variable, indicating performance specific to each pump and the flow rate represents a reasonable performance indicator. Other significant factors that can influence pumping time include the volume of milk in the receiving silo, the number of bends and valves in each pipeline, and the associated backpressure variations. At milk offload, process optimization can be achieved by ensuring pumps are working effectively, efficiently, and planning truck supply due to intelligent time slot management.

3.1. Separation, clarification, and centrifugation

Different milk processing plants have their own process trains. In many cases, milk must be clarified on reception at the dairy, to remove particles of dirt such as sand, soil, dust, and precipitated protein, which will protect downstream processing equipment. In addition, removal of bacteria, spores, and somatic cells from milk can be achieved with centrifugation and microfiltration techniques [41]. Somatic cells such as leucocytes are removed, which will reduce the presence of Listeria trapped inside the leucocyte [42]. Reduction in the microbial load at this point can decrease the burden of biofilms [43], which leads to more efficient work of the HPE [44]. Milk bacterial clarification also avoids problems during cheese aging, and improves shelf life and organoleptic properties of the dairy products. A clarifier is a type of centrifugal separator, but clarifiers and milk separators serve slightly different duties. All

centrifuges can act as clarifiers; however, in general, only centrifuges with a high hydraulic capacity are used in this way. The clarifier can function with either cold (below 8°C) or hot milk (50–60°C).

The main use for centrifuges in diary processing plants is hot milk separation. The aim is to separate the globular milk fat from the serum, the skim milk. This process is known as skimming. This process is generally combined into the pasteurization line and joined with an in-line fat standardization system for both milk and cream. Separation normally takes place at 122–140°F (50–60°C). The fat content of the cream discharged from the separator can be controlled to a level of between 20 and 70%. The terminology for separation in the dairy industry includes continuous centrifugal separation of solid particles (Clarifier), separation of cream (Separator), or separation of bacteria (Bactofuge). The microbial quality of milk powders is highly significant and it is possible at this early phase of processing to remove 99.9% of the spore-forming bacteria by either bacto-fugation or microfiltration preceding heat treatment.

Standardization of milk is the alteration of fat and solids-not-fat (SNF) levels, i.e., raising or lowering of these levels. This is regularly carried out for the consumer market milk supply and in the production of other milk products including: condensed milk, milk powder, ice cream and cheese, etc. Standardization is typically carried out to create a uniform milk fat content in the final dairy product [45].

3.2. Pasteurization

Pasteurization was originally introduced to control *Mycobacterium bovis*, which causes tuberculosis (TB), which is no longer problematic as cows are tested for TB annually and removed from herds if they test positive for the disease [46]. The TB bacillus is a highly heat resistant microorganism; however, *Coxiella burnetii*, the cause of Q fever in humans [47], required pasteurization of 161°F (71.7°C) for 15 s, and is the current official standard for milk pasteurization [48], the standard vat pasteurization is 63°C (145°F) for 30 min. However, heat processing can result in the loss of subtle aroma and flavors components, loss of vitamins and natural antioxidants, the loss of texture and freshness, and the denaturation of proteins. The US Grade A pasteurization milk ordinance (PMO) is managed by the Departments of Health and Human Services and Public Health, and the Food and Drug Administration and gives the criteria concerning the milk parlor and processing plant design, milking practices, milk handling, sanitation, and standards for the pasteurization of Grade A milk products. Regulation of milk processing is controlled on each US state basis; however, all dairy products must meet the regulations outlined in the PMO for products that will be sold outside of that state [10].

The center for disease control (CDC) in the US, reported that unpasteurized milk is 150 times more likely to cause foodborne illness and results in 13 times more hospitalizations than illnesses involving pasteurized dairy products [49]. The dangerous bacteria include *Salmonella* spp., *Escherichia coli*, and *Listeria monocytogenes*; it is also for this reason milk is pasteurized. *E coli* 0157 emerged in 1982, while multidrug-resistant *Salmonella typhimurium* DT104 was reported in 1990 [50, 51] with some *E. coli* and *Salmonella* isolates resistant to seven antibiotics.

The EU Center for Disease Control and Infection (ECDC) reported Listeriosis cases of 2536 in 2016, of which *L. monocytogenes* was most frequently detected in both soft and semi-soft cheeses prepared from raw milk (2.5%), while 0.7% of raw milk ($n = 968$) samples tested positive [52]. These pathogens can also be found in multiple food products including meat [53]. However, postpasteurization contamination has been found to be the most causative factor in microbial outbreaks due to milk products [54, 55].

3.3. Verifying the pasteurization process

The PasLite test is an internationally accepted method used by dairies and food manufacturers to verify pasteurization for many types of dairy products. The PasLite test verifies the completeness of milk pasteurization by detecting alkaline phosphatase, a natural enzyme in milk that is destroyed by the heat and hold time of pasteurization. The test takes 3 min and multiple samples can be run simultaneously, however only one sample can be read at a time [56].

When a dairy sample is mixed with PasLite reagents and incubated, the resulting solution emits light in an amount directly proportional to the phosphatase enzyme present. The Charm nova LUM ATP detection system is used to measure the light emitted and coverts light readings to enzyme units. Phosphatase readings greater than 350 mU/L indicate product pasteurization issues, according to US and EU pasteurization requirements. The PasLite test detection limit for liquid dairy products is 20 milliunits per liter (mU/L) phosphatase (~0.002% raw milk). This is much lower than the 350 mU/L level (0.1% raw milk) mandated by nearly all public health agencies.

4. Milk powders

The development of milk powders has revolutionized the dairy industry and allowed for a highly nutritional foodstuff to be exported safely around the world. Milk contains 85–90% water (**Table**); it is reduced by removing the water and can reduce the milk weight to 12% w/v, allowed for cheaper and easier transport. History tells us that in the thirteenth century, Marco Polo reported that soldiers of Kublai Khan carried sun-dried milk on their expeditions [57]. In 2013, the world's largest dairy spray dryer was installed by Fonterra Dairy Co-Op in New Zealand that has a capacity to produce 30 tons of milk powder per hour, converting four and a half million liters of fresh milk each day [58]. Three years later, in 2016, a "second of its kind," world's largest spray dryer started production at another Fonterra milk powder plant, which illustrates that, the trend is toward maximized production of dairy powders.

Milk powders can include whole milk powder (WMP), skim milk powder (SMP), fat filled milk powder (FFMP), infant formula, and milk protein concentrate, which is 85% pure milk protein. Its uses include in bakery, confectionary, ice cream, and in fermented food such as yogurt. Many are advertised as nutritional supplements and are fortified with vitamins, folic acid, and iron.

4.1. Evaporation and drying

Milk powder is manufactured by spray-drying precondensed milk. A falling film evaporator is commonly used in the dairy industry to concentrate the milk from ~13% total solids (TS) to a target of up to 52%. Evaporation is simply the removal of a solvent from a solution or slurry. Milk itself is defined as a colloid with the solvent being the water. Other methods of removing water can include freeze-drying [59]. The constituents of milk can be seen in **Table**. As some products are sensitive to heat, the design of evaporators with respect to temperature and holding time is vital in order to achieve the desired effects on the one hand, but without causing heat damage and denaturation to the milk proteins. To minimize the thermal impact on the products from the heat applied, evaporation takes place in a vacuum at pressures of 160–320 hPa, equivalent to water boiling temperatures of 55–70°C. Energy efficiency is the main driving force in improved design and technologies in evaporation [60, 61]. Inside the evaporator are a bundle of tubes for the exchange of heat and these are enclosed in another steel cylinder, in evaporation parlance called a "calandria." The vaporized solvent is cooled to condensate, which is then removed. It can go to storage, be recirculated, recovered for heat transfer, or filtrated but this is secondary to the evaporation process itself. The main unit of an evaporator is called an "effect." Generally, more than one "effect" is used, to increase efficiency by using the heat from the vapor from the previous "effect" to heat the feed in the next. Steam economy is a term used to quantify how much original steam is used in ratio to vapor steam. If 1 Kg of steam produces 1 Kg of vapor in a single "effect" system, 1 Kg of steam will produce ~2 Kg of vapor in a two "effect" system. The specific steam consumption of the former is 100%, while it is 50% in the latter case. This cuts down the cost of generating original steam feed. A subsequent "effect" must have a lower pressure than the previous "effect," and a step-wise vacuum is applied to the whole evaporation process to achieve this. There are three main elements in evaporation: heat transfer, vapor-liquid separation, and energy efficiency [62].

When milk leaves the evaporator (**Figure**), it is passed through the spray dryer through small nozzles, which make small droplets or atomizing the liquid, the smaller the better. The drying chamber has a temperature of 160–205°C, the droplets are swirled around (1 l of concentrate is atomized to 1.2×10^{11} droplets with a diameter of 50 micron with a total surface of 120 m^2.). For effective drying, the air should be hot, dry (low humidity) and moving. The powder falls to the bottom where it is collected in a "fluid" bed under the cone of the drying chamber, where fine powder behave in an analogous manner to a liquid and it can be conveyed without forming clusters. Fluid beds permit mild second stage drying and cooling of delicate products. Agglomeration changes the bulk density of the product [12].

The bulk density of the powder can dictate how the milk powder dissolves in hot beverages including for tea, coffee, and chocolate. The particle size of the milk powders determines its reconstitution properties. Powders consisting of particles of <100 μm are difficult to wet with water and form lumps [63], in the case of full-fat milk powder (FFMP), which is difficult to wet, it is sprayed with lecithin or oils (e.g., palm) to improve reconstitution characteristics. The standard method for measuring bulk density is ISO 8966:2005 [64].

Milk powders can be classified accordingly to the heat treatment they receive. There are five levels of heat classification: ultra-low (<70°C/15 s) common low (70°C/15 s), medium (85–90°C/20–30 s), high (110–135°C/30 s), and high-heat stable (~135°C/30 s). The whey protein nitrogen index (WPNI) expresses the content of un-denatured whey protein (mg WPNI per gram of powder) and demonstrates the severity of the heat treatment. Low-heat WPNI >6.0 mg, while high-heat WPNI is <1.5 mg, values are expected [65]. An alternative heat classification of milk powder is by casein number (CN—total nitrogen precipitated at pH 4.7), this measure was introduced as the protein concentration in milk changes with the seasons and feeding patterns [66]. The CN number is not linked to the overall protein content of the milk. The CN value of high quality raw milk is in the range 80–82, expressed in percentages. The CN values in excess of 82 indicate that the denaturation of whey protein has taken place. Completely denatured milk has a CN value of 92.

The composition of and additives allowed in milk powders are regulated by the Codex Alimentarius Commission—Milk and Milk Products [67, 68]. The Codex standard stipulates that only milk and cream may be allowed in milk powders; though the protein content can be altered by adding lactose. Milk proteins include casein complexes and whey protein fractions. Casein is the most abundant with whey proteins in lower concentrations. The casein concentration in cow milk is 2.46–2.80/100 g and whey proteins in the range 0.55–0.70/100 g. The composition of milks from various animal species is well reviewed in Barłowska et al. [69].

The moisture content of milk powder must be controlled during milk processing, as it is a factor in the long-term quality of the product, and it influences the cost of production. The method for determination of the moisture content includes the ISO 5537: 2004 reference method [70] and IDF Provisional Standard 26A:1993 [71] and EU commission Directive method (79/1067) [72]. A test portion of milk powder is dried at 102 ± 2°C until constant mass is obtained, but this measurement can be affected by the relative humidity of the air in the laboratory where the test is carried out. Rapid methods and newly designed equipment are always being introduced to avoid air humidity interference in the measurement and one new method is by using a microwave cavity perturbation technique [73].

5. Microbiological quality analyses

Each step along the milk processing train can be contaminate by the air [74] and the water [75], used in the milk processing stages. Hygiene control at all stages, including hygienic design of the manufacturing equipment, is critically important.

5.1. Microbial quality analysis at farm level

The microbial quality of milk starts a farm level. Milk is sterile at secretion in the udder but is colonized by bacteria before it leaves the udder [76]. The temperature of milk expelled from the udder is approximately 35°C; to prevent microbial growth, rapid cooling, and storage to 4°C is necessary. The dairy farmer has the responsibility of managing and maintaining a

clean and hygienic milking parlor with a good milking and storage routine. The farmer can detect early signs of mastitis infection by using a somatic cell count (SSC) test. Low levels of SCC (<200,000/ml) are wanted to guarantee good extraction of protein from milk. High levels of SCC also reduce other levels of milk constituent including lactose. The California Mastitis Test (CMT) offers a quick and easy on-farm test; the test does not provide a specific SCC, but will give a positive result once a cow's SCC goes over 400,000 cells/mL. The addition of the CMT solution to milk samples with a high number of leukocytes/white blood cells causes the solution to become mucous like. This reaction is caused by the release of DNA from somatic cells, which are now higher due to the immune response of the cow to infections. Mastitis is caused by the microorganism *Staphylococcus aureus.* CMT test are available commercially from many companies.

The milk tanker driver can perform a few tests at the farm, but this is not often practical. The collector will also take a sample of raw milk and label it with a bar code identifier, to be brought back to the dairy processing plant. Composite samples are taken for the detection of inhibitory substances (e.g., antibiotics, antiseptics) to be tested later at the processing plant and if positive the individual suppliers samples are then analyzed.

5.2. Microbial quality testing at milk intake

At the milk intake point, the milk is tested before acceptance into the processing train. One such historic test described in 1929 [77, 78] is the Resazurin test, which determines the microbiological quality of the milk. The theory of this test is that Resazurin, a blue dye, is reduced in an oxidation-reduction reaction, as bacteria grow in the milk they use up oxygen and this can reduce the Resazurin dye to a pink color. All that is required is 10 ml of milk, 1 ml of resazurin solution (0.05%), mix well and incubate at 37°C for 2 min. The color changes from blue to mauve to purple to pink and lastly colorless and is compared to standardized color disks or measured in an instrument called a Comparator (developed by Lovibond, originally) which is a short path length instrument (up to 40 mm) for visually matching samples with relatively dark colors. A reading of ≥4, which is comparable to an estimate of a total bacterial count of 0.1–2 million cfu/ml, is a satisfactory milk quality result.

The milk density is another rapid test to determine adulteration of the milk and an indication for the deviations from the normal milk composition, for example, if it has been watered down or skimmed. In this test, a dipping lactodensimeter combined with a thermometer is used (Gerber instruments; Brouwland instruments), lactometers/milk hydrometers are calibrated in either grams per milliliter (g/cm^3), degrees specific gravity (SG), or Degrees Quevenne. 1° Quevenne = 0.001°SG. Density ranges for standard milk are between 1.026 and 1.034 g/cm^3. The adding of 10% water to milk will end up decreasing milk density by ~0.003 g/cm^3.

6. Microorganisms and milk

A wide variety of bacteria grow and survive in milk, including problematic spore-forming bacteria [79] and pathogens such as nontyphoid *Salmonella*, *Campylobacter*, *Listeria monocytogenes*, and Shiga toxin-producing *Escherichia coli* are also found [80]. In addition, *Cronobacter sakazakii* has been found in milk powder producing plants and is a particular risk to infants [81].

The common bacteria in milk are lactic acid bacteria (LAB), which can produce enough acid to reduce the pH of milk, and cause the coagulation of proteins, thus fermenting the milk [82]. The density test as previously described should be introduced at milk intake, as it can determine the degree of LAB growth. LAB can be divided into rods (*Lactobacillus* and *Carnobacterium*) and cocci (all other genera).

Psychrotrophic microorganisms are also present up to 80% in fresh collected milk, they are able to grow quickly below 7°C, and some contain heat-stable enzymes, which cause spoilage, including many Gram-negative bacteria, such as *Pseudomonas fluorescens*, *Pseudomonas fragi*, *Pseudomonas putida*, *Achromobacter*, *Aeromonas*, *Alcaligenes*, *Chromobacterium*, *Flavobacterium*, *Serratia*, and *Enterobacter* [83].

Thermoduric bacteria can survive pasteurization. They do this by forming spores, which can then carry over into the final product. This can cause quality defects in milk products such as decreasing the shelf life of pasteurized milk. They are represented mainly by Gram-positive bacteria, e.g., *Bacillus* and *Clostridium* spp., and the nonspore-forming genera, e.g., *Micrococcus*, *Streptococcus*, and *Corynebacterium.* Levels of greater than 1000 cfu/ml are normally the result of poor cow hygiene and milking equipment (particularly in the case of ineffective hot wash routines). Potential sources of thermoduric bacteria include silage, faces, animal bedding, and soil [84]. Thermophilic bacteria grow in milk held at raised temperatures (55°C or higher), including pasteurization, 62.8°C, they include the *Bacillus* spp. Thermophilic bacteria are monitored by standard plate count methods with incubation at 55°C [85]. However, obligate thermophiles, such as *Geobacillus stearothermophilus* and *Anoxybacillus flavithermus* tend to grow to high numbers in milk powder manufacturing plants [86]. Although these microorganisms generally are not pathogenic, there is evidence to show that they cause human diseases [87], their growth results in high bacterial numbers and their presence can be interpreted as an indicator of poor plant hygiene. Spores of *G. stearothermophilus* are also able to survive ultra-high-temperature (UHT; 134–145°C for 1–10 s) treatment [88]. A recent study outlined the prevalence of contaminated milk processing samples with spore-forming bacteria, which increased from 23% on farm, to up to 58% post pasteurization stage [89].

The total viable count (TVC), or total bacterial count (TBC), is used to indicate the overall level of microorganism in milk; *E. coli* and *coliforms* to indicate any fecal contamination; and *Pseudomonas* spp., to indicate any nonfecal contamination. EU legislation, describing precise hygiene rules for foods from animal origins (amended in 2017) lays down comprehensive criteria for milk quality [90]. The ruling indicates that TBC in raw milk should be less than 100,000 cfu/ml; however, a TBC of less than 15,000 cfu/ml is desired. A standard to aim for is <1000 cfu/ml as milk leaves the udder; <3000 cfu/ml as milk leaves the milking machine; and <5000 cfu/ml in the bulk tank. Further contamination takes place during storage and preprocessing activities.

7. Industry 4.0 in the dairy industry

New technologies in the dairy industry are slowly integrating both at farm level and in the dairy processing plant. At farm level, the introduction of robotics such as automated milking parlors developed by Lely and introduced in 1992 by Delaval (Sweden). The cows enter

the parlor without prompting and some cows are milked three times a day, with increased milk product for the farmer. The tags on the cows allow for integration into the machines which collect vast amounts of data, including number of steps, chewing the curd, etc. Robotic milking machines have a life span of approx. 13 years and then required further investment. Determination of when a cow is in heat for efficient reproduction is available with MooCow developed by Dairy Master (Ireland), together with MooMonitor to guide cows in the parlor. A separate company created MooCall, a sensor attached to the cow tail, which can monitor contractions during calf birth and send a SMS message to the farmer, the sensor can determine as close as 1 h to delivery [91]. Some of the more recent analytical instruments for milk analysis that has been introduced, but are not yet standard and include: Fourier transform MIR spectroscopy for milk-based quantitative, qualitative phenotypic and genomic analysis. Flow CYTOMETRY is a well-established technique for bacteria and somatic cells counting and differentiation [92], and companies making these include: Bentley (https://bentleyinstruments.com/), Foss (https://www.fossanalytics.com/en#) and Delta (http://www.deltainstruments.com/); ELISA (enzyme linked immunosorbent assay) for milk amyloid; milk amyloidA (MAA) biomarker is an early detection method for clinical and subclinical mastitis. MAA is the only acute phase protein produced in response to bacterial infection in the udder and is therefore an immediate and direct indicates of infection [93]. PCR (polymerase chain reaction) is used for bacteria identification and differentiation [94, 95]. Gas chromatography/mass spectrometry can be used for analysis for metabolomics characterization of milk [96, 97]. Liquid chromatography/electrospray ionization and mass spectroscopy can be used for the detection of peptides and glycopeptides. ICP-MS (Induction coupled plasma mass spectroscopy) for mineral and trace element analysis. Industry 4.0 technologies can be introduced at all stages of the plant manufacturing systems, including environment monitoring [98].

8. Conclusion

The milk processing chain demands accurate and quality products from farm to plate and for all of its products, e.g., fluid milk, milk powders, etc. It must start with the raw material at farm level including; dairy herd improvement testing, to payment parameters, and quality control of the raw milk. Optimization is important in the processing of milk in the dairy chain as 73 plus tests are carried out including chemical physical and microbiological tests, set against ISO standards, EU, USFDA regulations, and most countries internal regulations. Advances are slowly being made to have modern and optimized methodologies approved. The regulatory bodies are setting new standards from verified inter-laboratory studies, targeting the advancement in instrumentation and for at-line and in-line production analysis for improved predictability and control of manufacturing processes. The finished product must be safe and comply with regulatory requirements.

At a conference in Glasgow (Semex Dairy Conference, Jan 2018), it was questioned whether the dairy industry could cease to exist after approximately 10 years, due to the interest in vegan alternatives and the increased population who are lactose intolerant [99, 100]. A business model to address this alternative has resulted in a cow-free milk product called Perfect

Day, an animal-free milk made by using yeast and fermentation techniques to produce a product with equivalent dairy proteins (http://www.perfectdayfoods.com/). It is unlikely that nondairy products will overtake real-natural product in the short term. Milk powders are still a big business and the optimization approach to change the paradigm from inspect and reject to predict and prevent is developing increased interest for the factory of the future [101], and is an approach that cannot be ignored. Regardless of developments, a quality milk product must be the result. There can be no food security without food safety.

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Conflict of interest

There is no conflict of interest.

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References

[1] Ahern J. Applying lean techniques to dairy farming. Acountance Plus. 2017;**03**(Sept):26-27

[2] FAO-UN. Food and Drug Organisation of the United Nations [Internet]. 2017. Milk and Milk Products: Price and Trade Update Price Index. Available from: http://www.fao.org/3/a-i8326e.pdf [Accessed: April 3, 2018]

[3] DeLoitte. The Global Dairy Sector—Trends and Opportunities [Internet]. 2017. Deloitte Development LLC. Available from: https://www2.deloitte.com/ie/en/pages/consumer-industrial-products/articles/global-dairy-trends-2017.html [Accessed: February 24, 2018]

[4] FAO-UN. Global Dairy Sector: Facts. 2016. Food and Agriculture Organisation of the United Nations. Available from: https://www.fil-idf.org/wp-content/uploads/2016/12/FAO-Global-Facts-1.pdf [Accessed: February 24, 2018]

[5] Deloitte. Capitalizing on the Shifting Consumer Food Value Equation [Internet]. 2015. Deloitte Development LLC. Available from: https://www2.deloitte.com/content/dam/Deloitte/us/Documents/consumer-business/us-fmi-gma-report.pdf [Accessed: February 24, 2018]

[6] FAO-UN. Gateway to Dairy Production and Products [Internet]. 2018. Available from: http://www.fao.org/dairy-production-products/en/ [Accessed: June 9, 2018]

[7] Eurostat. Milk and Milk Product Statistics [Internet]. 2018. Available from: http://ec.europa.eu/eurostat/statistics-explained/index.php/Milk_and_milk_product_statistics [Accessed: April 3, 2018]

[8] Jenkins TC, McGuire MA. Major advances in nutrition: Impact of milk composition. Journal of Dairy Science. 2006;**89**:1302-1310

[9] EU Council Regulations No. 1169/2011 of the European Parliament and of the Council of 25 October 2011. On the vision of food information to consumers. Official Journal of the European Union. 2011;**L304**:18-63

[10] US-FDA. Grade "A" Pasteurized Milk 2015 Revision U.S. Department of Health and Human Services, Public Health Service, Food and Drug Administration [Internet]. Available from: https://www.fda.gov/downloads/Food/GuidanceRegulation/Guidance-DocumentsRegulatoryInformation/Milk/UCM513508.pdf

[11] Tetra Pak. The Chemistry of Milk. Chapter 2. In: Dairy Processing Handbook: Tehnology, Engineering, Agriculture [Internet]. 2015. Ch2. Tetra Pak International. Available from: http://dairyprocessinghandbook.com/chapter/chemistry-milk [Accessed: May 21, 2018]

[12] Tetra Pak. Milk and Whey Powder. In: Dairy Processing Handbook: Technology, Engineering, Agriculture [Internet]. 2015. Tetra Pak International. Ch17. Available from: http://dairyprocessinghandbook.com/chapter/milk-and-whey-powder [Accessed: May 21, 2018]

[13] FAO-UN. Livestock Solutions for Climate Change [Internet]. 2017. Food and Agriculture Organisation of the United Nations. Available from: http://www.fao.org/3/a-i8098e.pdf [Accessed: June 9, 2018]

[14] Safefood. Impact of Climate Change on Dairy Productoin [Internet]. 2017. Safefood, Little Island, Ireland. Available from: http://www.safefood.eu/Publications/Research-reports/The-impact-of-climate-change-on-dairy-production.aspx [Accessed: May 22, 2018]

[15] Flores-Flores ME, Lizarraga E, López de Cerain A, González-Peñas E. Presence of mycotoxins in animal milk: A review. Food Control. 2015;**53**:163-176

[16] EPA. US Environmental Protection Agency. Technical Fact Sheet—Perchlorate [Internet]. 2014. Available from: https://www.epa.gov/sites/production/files/2014-03/documents/ffrrofactsheet_contaminant_perchlorate_january2014_final.pdf

[17] Leung AM, Pearce EN, Braverman LE. Perchlorate, iodine and the thyroid. Best Practice & Research. Clinical Endocrinology & Metabolism. 2010;**24**(1):133-141. DOI: 10.1016/j.beem.2009.08.009

[18] ISO/TC34/SC5. Milk and Milk Products, (Calalogue of Standards). Geneva, Switzerland: International Organization for Standardization; 2018

[19] ISO 6091. Dried Milk. Determination of Titratable Acidity (Reference Method). Geneva, Switzerland: International Organization for Standardization; 2010

[20] ISO 26844. Milk and Milk Products. Determination of Antimicrobial Residues. Tube Diffusion Test. Geneva, Switzerland: International Organization for Standardization; 2006

[21] ISO 5764. Milk—Determination of Freezing Point—Thermistor Cryoscopy Method (Reference Method). Geneva, Switzerland: International Organization for Standardization; 2009

[22] ISO 1736. Dried Milk and Dried Milk Products. Determination of Fat Content. Gravimetric Method (Reference Method). Geneva, Switzerland: International Organization for Standardization; 2008

[23] ISO 8968-1. Milk and Milk Products. Determination of Nitrogen Content—Part 1: Kjeldahl Principle and Crude Protein Calculation. Geneva, Switzerland: International Organization for Standardization; 2014

[24] ISO 14891. Milk and Milk Products. Determination of Nitrogen Content. Routine Method by Combustion According to the Dumas Principle. Geneva, Switzerland: International Organization for Standardization; 2002

[25] ISO 22662. Milk and Milk Products. Determination of Lactose Content by High Preformance Liquid Chromatography (Reference Method). Geneva, Switzerland: International Organization for Standardization; 2007

[26] van der Have AJ, Deen JR, Mulder H. The composition of cow's milk. 4. The calculation of the titratable acidity studied with separate milkings of individual cows. Netherlands Milk and Dairy Journal. 1979;**33**:164-171

[27] EU Council Regulation (EEC) No 2377/90 of 26 June 1990. Laying down a community 'procedure for the establishment of maximum residue limits of veterinary medicinal products in foodstuffs of animal origin'. Official Journal of the European Union. 1990;**L224**:1-136

[28] Codex CAC/MRL 2-2015. Maximum Residue Limis (MRL's) and Riskmanagement Recommendatins (RMM's) for Residues of Veterinary Drugs in Foods. Rome: Codex

Alimentarius World Health Organisation, Food and Agriculture Organisation of the United Nations (FAO/WHO); 2015

[29] Adley CC, Dowling A, Handschue H, Ryan MP. Emerging policy on antibiotic resistance, the consumption of antimicrobial agents and occurrence of antimicrobial resistance in bacteria from humans and food producing animals. In: Mendez-Vilas A, editor. The Battle against Microbial Pathogens, Basic Science, Technological Advances and Educational Programs. Microbiology Book Series 5. Vol. 2. Badajoz: Formatex Research Centre; 2015. pp. 913-922

[30] Zamberlin Š, Antunac N, Havranek J, Samaržija D. Mineral elements in milk and dairy products. Mljekarstvo/Dairy. 2012;**62**(2):111-125

[31] Månsson HL. Fatty acids in bovine milk fat. Food & Nutrition Research. 2008;**52**:3. DOI: 10.3402/fnr.v52i0.1821

[32] Parodi P. Milk fat in human nutrition. Australian Journal of Dairy Technology. 2004; **59**:3-59

[33] Shingfield KJ, Reynolds CK, Hervas G, Griinari JM, Grandison AS, Beever DE. Examination of the persistency of milk fatty acid composition responses to fish oil and sunflower oil in the diet of dairy cows. Journal of Dairy Science. 2006;**89**(2):714-732

[34] Ranjan A, Sahoo B, Singh VK, Singh SP. Effect of rumen protected fat supplementation on nutrient utilization and production performance of lactating buffaloes. Animal Nutrition and Feed Technology. 2013;**13**:205-2014

[35] Pitta DW, Indugu N, Vecchiarelli B, Rico DE, Harvatine KJ. Alterations in ruminal bacterial populations at induction and recovery from diet-induced milk fat depression in dairy cows. Journal of Dairy Science. 2018;**10**(1):295-309

[36] James CS. Experiment procedures—Estimation of major food constitutions. In: Analytical Chemistry of Foods. Chapter 5. Heidelberg: Springer; 1994. pp. 71-135

[37] Brandão MCMP, Carmo AP, Bell MJV, Anjos VC. Characterization of milk by infrared spectroscopy. Review Instituto de Laticinios'Cândido Tostes'. 2010;**65**(373):30-33

[38] Meagher LP, Holroyd SE, Illingworth D, van de Ven F, Lane S. At-line near-infrared spectroscopy for prediction of the solid fat content of milk fat from New Zealand butter. Journal of Agricultural and Food Chemistry. 2007;**55**(8):2791-2796. DOI: 10.1021/jf063215m

[39] ISO 9622. Milk and liquid milk products. In: Guidelines for the Application of Mid Infared Spectrometry. Geneva, Switzerland: International Organization for Standardization; 2013

[40] IDF. Protein Determination. International Dairy Federation Fact Sheet [Internet]. 2013. Available from: https://www.fil-idf.org/wp-content/uploads/2016/04/Protein-Determination.pdf [Accessed: May 20, 2018]

[41] Gésan-Guiziou G. Removal of bacteria, spores and somatic cells from milk by centrifugation and microfiltration techniques. In: Griffiths MW, editor. Improving the Safety and Quality of Milk: Milk Production and Processing. Cambridge: Woodhead Publishing; 2010. pp. 349-372

[42] Doyle MP, Glass KA, Beery JT, Garcia GA, Pollard DJ, Schultz RD. Survival of *Listeria monocytogenes* in milk during high-temperature, short-time pasteurization. Applied and Environmental Microbiology. 1987;**53**(7):1433-1438

[43] Smeets E, Kooman J, van der Sander F, Stobberingh E, Frederik P, Claessens P, Grave W, Schot A, Leunissen K. Prevention of biofilm formation in dialyssis water treatment systems. Kidney International. 2003;**63**:1574-1576

[44] Kananeh AB, Peschel J. Fouling in plate heat exchangers: Some practical experience. In: Mitrovic J, editor. Heat Exchangers—Basics Design Applications. Chapter 20. Croatia: InTech. Available from: http:www.intechopen.com/books/heat-exchangers-basics-design-applications/fouling-in-plate-heat-exchanges-some-practical-experience

[45] Bird J. Milk standardisation. International Journal of Dairy Technology. 1993;**46**(2):35-37

[46] Holsomger VH, Rajkowski KT, Stabel JR. Milk pasteurisation and safety; brief history and update. Review Scientific and Technical Review of the Office International des Epizooties (Paris). 1997, 1997;**16**(2):441-451

[47] Enright JB, Sadler W, Thomas RC. Pasteurization of milk containing the organism of Q fever. American Journal of Public Health. 1957;**47**:695-700

[48] Pexara A, Solomakos N, Govaris A. Q fever and prevalence of *Coxiella burnetii* in milk. Trends in Food Science & Technology. 2018;**71**:65-72

[49] US–FDA. United States Food and Drug association. Food Facts. The Dangers of Raw Milk. Center for Food Safety and Applied Nutrition Food [Internet]. 2012. Available from: https://www.fda.gov/downloads/Food/FoodborneIllnessContaminants/UCM239493.pdf [Accessed: May 17, 2018]

[50] Gorman R, Adley CC. Characterisation of *Salmonella enterica* serotype Typhimurium isolates from human, food and animal sources in the Republic of Ireland. Journal of Clinical Microbiology. 2004;**42**(5):2314-2316

[51] USDA-FSIS. *Salmonella* Typhimurium DT104 Situation Assessment (Review of Information as of December, 1997) [Internet]. United Stated Departmentof Agricultuer and Food Safety and Inspection Services. Available from: https://www.aphis.usda.gov/animal_health/emergingissues/downloads/dt104.pdf [Accessed: May 19, 2018]

[52] EU-ECDC. European Union summary report on trends and sources of zoonoses, zoonotic agents and food-borne outbreaks in 2016. European Food Safety Association Journal. 2017;**15**(12):5077. (228 pages)

[53] Adley CC, Dillon C. Listeriosis, salmonellosis and verocytoxigenic *E. coli*: significance and contamination in processed meats. In: Kerry JP, Kerry JF, editors. Processed Meats: Improving Safety, Nutrition and Quality. Chapter 4. Oxford: Woodhead Publishing Ltd; 2011. pp. 72-108

[54] Olsen SJ, Ying M, Davis MF, Deasy M, Holland B, Iampietro L, et al. Multidrug-resistant *Salmonella typhimurium* infection from milk contaminated after pasteurization. Emerging Infectious Diseases. 2014;**10**(5):932-935. DOI: 10.3201/eid1005.030484

[55] Murphy S, Martin NH, Barbano DM, Wiedmann M. Influence of raw milk quality on processed dairy products: How do raw milk quality test results relate to product quality and yield? Journal of Dairy Science. 2016;**99**(12):10128-10147

[56] Albillos SM, Reddy R, Salters R. Evaluation of alkaline phosphatase detection in dairy products using a modified rapid chemiluminescent method and official methods. Journal of Food Protection. 2011;**74**(7):1144-1154

[57] Yule H. Concerning the tartar customsof war. In: The Book of Ser Marco Polo, the Venetian; Concerning the Kingdoms and Marvels of the East. Book 1, Ch LIV, 260-266. Translated by Sir Henry Yule. 3rd ed. London: John Murray; 1903

[58] GEA. GEA group Aktiengesellschaft [Internet]. 2014. Building the World's Largest Spray Dryer. Available from: https://www.gea.com/en/stories/building-the-worlds-largest-dairy-spray-dryer.jsp [Accessed: May 21, 2018]

[59] Sánchez J, Hernández E, Auleda JM, Raventós M. Freeze concentration technology applied to dairy products. Food Science and Technology International. 2011;**17**(1):5-13

[60] Moejes SN, van Boxtel AJB. Energy saving potential of emerging technologies in milk powder production. Trends in Food Science and Technology. 2017;**60**:31-42

[61] Tetra Pak. Centrifugal seperators and milk standardisation. In: Dairy Processing Handbook: Tehnology, Engineering, Agriculture [Internet]. 2015. Tetra Pak International. Ch 6.2. Available from: https://www.tetrapak.com/about/tetra-pak-dairy-processing-handbook [Accessed: May 21, 2018]

[62] Minton PE. Evaporator elements. In: Handbook of Evaporation Technology. New Jersey: Noyes; 1986. p. 5

[63] Tuohy JJ. Some physical properties of milk powders. Irish Journal of Food Science and Technology. 1989;**13**(2):141-152

[64] ISO 8967. Dried Milk and Dried Milk Products. Determination of Bulk Density. Geneva, Switzerland: International Organization for Standardization; 2005

[65] Tetra Pak. Recombined milk products. In: Dairy Processing Handbook: Tehnology, Engineering, Agriculture [Internet]. 2015. Tetra Pak International. Ch 18. Available from: http://dairyprocessinghandbook.com/chapter/recombined-milk-products chapter 18 [Accessed: May 21, 2018]

[66] Sweetsur AWM. The stability of instantizeo skimmed milk powder to hot coffee. International Journal of Dairy Technology. 1976;**29**(3):157-160

[67] Codex. Codex standard 207-1999 for Milk Powders and Cream Powder. Rome: Codex Alimentarius World Health Organisation, Food and Agriculture Organisation of the United Nations (FAO/WHO); 1999

[68] Codex, editor. Milk and Milk Products. 2nd ed. Rome: Codex Alimentarius World Health Organisation, Food and Agriculture Organisation of the United Nations (FAO/WHO); 2011. Available from: http://www.fao.org/docrep/015/i2085e/i2085e00.pdf

[69] Barłowska J, Szwajkowska M, Litwińczuk Z, Król J. Nutritional value and technological suitability of milk from various animal species used for dairy production. Comprehensive Reviews in Food Science and Food Safety. 2011;**10**:291-301

[70] ISO 5537. Dried Milk. Determination of Moisture Content (Reference Method). Geneva, Switzerland: International Organization for Standardization; 2004

[71] IDF. Dried Milk and Cream—Determination of the Water Content of Dried Milk and Cream. Provisional Standard 26A. Brussels: International Dairy Federation; 1993

[72] EU First Commission Directive of 13 November 1979. Laying down Community 'methods of analysis for testing certain partly or wholly dehydrated preserved milk for human consumption' (79/1067/EEC). Official Journal of the European Union. 1979;**L327**:29-53. (p 34)

[73] Yang J, Huang M, Peng J, Shi J. Rapid determination of the moisture content of milk powder by microwave sensor. Measurement. 2016;**87**:83-86

[74] Zacharski KA, Ryan A, Southern M, Adley CC. Examination of the microbiological status of air and surfaces in a dairy plant environment. Journal of Food Protection. 2018; **81**(7):1108-1116

[75] Burke N, O'Dwyer J, Southern M, Adley CC. An analysis of the microbial quality of water in a milk production plant. LWT - Food Science and Technology. 2017;**78**:41-46

[76] Evans AC. The bacteria of milk freshly drawn from the normal udders. The Journal of Infectious Diseases. 2016;**18**(5):437-476

[77] Pesch KL, Simmert U. Combined assays for lactose and galactose by enzymatic reactions. In: Grimmer WE, editor. Dairying. Milchwirtschaftliche Forschungen. Vol. 8. Berlin: Springer; 1929. p. 551

[78] Silanikove N, Shapiro F. Combined assays for lactose and galactose by enzymatic reactions. In: Preedy VR, editor. Food and Nutritional Components in Focus No 3: Dietary Sugars: Chemistry, Analysis, Function and Effects. Chapter 23. London: Royal Society for Chemistry; 2012. pp. 395-404

[79] Pereira APM, Sant'Ana AS. Diversity and fate of spore forming bacteria in cocoa powder, milk powder, starch and sugar during processing: A review. Trends in Food Science and Technology. 2018;**76**:101-118

[80] Adley CC, Ryan MP. The nature and extent of foodborne disease. In: Barros-Velázquez J, editor. Antimicrobial Food Packaging. Ch1. San Diego: Academic Press; 2016. pp. 1-10

[81] Jacobs C, Braun P, Hammer P. Reservoir and routes of *Enterobacter sakazakii* (*Cronobacter* spp.) in a milk powder-producing plant. Journal of Dairy Science. 2011;**94**(8):3801-3810

[82] Tsakalidou E, LML N. In: Toldrá F, editor. Handbook of Dairy Foods Analysis. Boca Raton: CRC Press; 2009. pp. 647-664

[83] Sørhaug T, Stepaniak L. Psychrotrophs and their enzymes in milk and dairy products: Quality aspects. Trends in Food Science and Technology. 1997;**8**(2):35-41

[84] Khanal SN, Anand S, Muthukumarappan K, Huegli M. Inactivation of thermoduric aerobic spore formers in milk by ultrasonication. Food Control. 2014;**37**:232-239

[85] Burgess SA, Flint SH, Lindsay D. Characterization of thermophilic bacilli from a milk powder processing plant. Journal of Applied Microbiology. 2014;**11**:350-359

[86] Scott SA, Brooks JD, Rakonjac J, Walker KMR, Flint SH. The formation of thermophilic spores during the manufacture of whole milk powder. International Journal of Dairy Technology. 2007;**60**:109-117

[87] Rabkin CS, Galaid EI, Hollis DG, Weaver RE, Dees SB, Kai A, Moss CW, Sandhu KK, Broome CV. Thermophilic bacteria: A new cause of human disease. Journal of Clinical Microbiology. 1985;**21**(4):553-557

[88] Hill BM, Smythe BW. Endospores of thermophilic bacteria in ingredient milk powders and their significance to the manufacture of sterilized milk products: An industrial perspective. Food Reviews International. 2012;**28**(3):299-312

[89] Ortuzar J, Martinez B, Bianchini A, Stratton J, Rupnow J, Wang B. Quantifying changes in spore-forming bacteria contamination along the milk production chain from farm to packaged pasteurized milk using systematic review and meta-analysis. Food Control. 2018;**86**:319-331

[90] EU Council Regulation No. 853/2004 of the European Parliment and of the Council of 29 April 2004. Laying down 'specific hygienee rules for food of animal origin'. Official Journal of the European Union. 2004;**L139**:55. Last Amended in 2017;**L285**:10

[91] Kegan M. Connected Cows: A Game Changer in the Dairy Industry [Internet]. 2018. Available from: https://www.ptc.com/en/product-lifecycle-report/connected-cows-a-game-hanger-in-the-dairy-industry [Accessed: February 26, 2018]

[92] Li N, Richoux R, Perruchot MH, Boutinaud M, Mayol JF, Gagnaire V. Flow cytometry approach to quantify the viability of milk somatic cell counts after various physico-chemical treatments. PLoS One. 2015;**10**(12):e0146071. DOI: 10.1371/journal.pone.0146071

[93] Miglio A, Moscati L, Fruganti G, Pela M, Scoccia E, Valoani A, Maresca C. Use of milk amyloid A in the diagnosis of subclinical mastitis in dairy ewes. Journal of Dairy Research. 2013;**80**(4):496-502

[94] Oliwa-Stasiak K, Kolaj-Robin O, Adley CC. Development of real-time PCR assays for detection and quantification of the *Bacillus cereus* group sp: Differentiation of *B. pseudomycoides* and *B. weihenstephanensis* from species with non rhizoid growth isolated from milk. Applied and Environmental Microbiology. 2011;**77**(1):80-88

[95] Oliwa-Stasiak K, Molnar C, Arshak K, Baroszcze M, Adley CC. Development of a PCR assay for identification of the *Bacillus cereus* group species. Journal of Applied Microbiology. 2010;**108**(1):266-273

[96] Scano P, Murqia A, Pirisi FM, Caboni P. A gas chromatography-mass spectrometry-based metabolomic approach for the characterization of goat milk compared with cow milk. Journal of Dairy Science. 2014;**97**(10):6057-6066

[97] Dallas DC, Lee H, Le Parc A, de Moura-Bell JMN, Barile D. Coupling mass spectrometry-based "Omic" sciences with bioguided processing to unravel milk's hidden bioactivities. Advances in Dairy Research. 2013;**1**(2):1-9

[98] Zacharski ZA, Southern M, Adley CC. Industry 4.0 vision considerations in a quality control sampling regime in a diary manufacturing plant. In: Proceedings of the 33rd International Manufacturing Conference (IMC33); 31 August–1 September 2016. Ireland: University of Limerick

[99] Pevreall K. 'Perfect' high protein vegan diet created with out use of cows [Internet]. 2017. Livekindly. Available from: www.livekindly.co/vega-milk-protein/ [Accesssed: June 19, 2018]

[100] Silanikove N, Leitner G, Merin U. The Interrelationships between lactose intolerance and the modern dairy industry: Global perspectives in evolutional and historical backgrounds. Nutrients. 2015;**7**:7312-7331

[101] Petrusich J. Leveraging Big Data in Real Time: Changing the Paradigm from Inspect and Reject to Predict and Prevent [Internet]. 2014. InTech Magazine. Available from: https://www.scribd.com/document/228808622/Intech-Marabr-2014

Chapter 7

Chemical Constituents of Fruit Wines as Descriptors of their Nutritional, Sensorial and Health-Related Properties

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Ilija Klarić, Vlatka Petravić Tominac and Ana Mornar

Abstract

Functional foods are foods that provide positive health effects apart from the provision of essential nutrients. Along with nutraceuticals, they represent the top trends in the food industry. Fruit wines are considered functional foods. When assessing the fruit wine quality, a wide range of descriptors are taken into consideration, namely physico-chemical and sensorial properties of fruit wine. Furthermore, within the context of the new food products development (e.g. functional products), functional properties of fruit wines are also taken into consideration. Functional properties are determined by the content of the biologically active components, such as polyphenols, vitamins and micro- and macrominerals. It is also important to consider the food-safety issues regarding the fruit wines consummation, that is, the presence of pesticides, mycotoxins and biogenic amines in different fruit wines. This chapter aims to give an overview of various factors used to evaluate the quality and the functional properties of fruit wines.

Keywords: fruit wine, functional food, biologically active compounds, phenolic compounds, mineral composition, volatile compounds, food safety

1. Introduction

The past decade has seen the rapid development of fruit wine production in many countries, which may be attributed to the enhanced consumers' interest in functional foods that may help to promote health and help reduce the risk of disease. However, there is literature gap data

regarding the production, properties and health benefits of different fruit wines, compared to grape wines. With the ageing of the population, the incidence of chronic diseases that can be linked to caloric excess and the cost of health-care both increase, and so is the consumers' preference towards self-care, instead of pharmaceutical management of disease [1]. The utilisation of functional foods for health-care can lead to the reduction of the use of pharmaceuticals or their replacement. According to the Functional Food and Nutraceuticals market report [2], many consumers were prompted to turn to preventive or alternative health-care practices due to the increase of health-care costs [3]. Consequently, the consummation of functional foods and nutraceuticals increased substantially. Moderate consumption of grape wine (mainly red) has a beneficial health effects when combined with balanced diet, which is confirmed by many studies [4–9]. Some of the reported effects regarding health effects include the protection of the incidence of cardiovascular diseases, ischaemic stroke, hypertension, diabetes, dietary cancers, peptic ulcers, kidney stones and macular degeneration, as well as stimulation of resistance to infection and bone density retention [10–14]. However, apart from grapes, a whole range of other fruits that differ in shape, taste, colour and nutritive value can also be utilised for the production of wine [9], which are nowadays gaining the full acceptance at the market [15]. Fruit wines have proved to be an excellent dietary source of antioxidants, phytonutrients and minerals [16]. According to the European regulations, fruit wines must be obtained by the fermentation of fruits (or respective juices) other than grape. Based on the carbon dioxide retention and content, fruit wines can be classified either as still or sparkling, while their permitted alcoholic strength lies between 1.2 and 14% by volume [14]. Winemaking technology of fruit wines is mostly similar to that of grape wines, except for the variations based on the fruit used, that usually involves the adjustment of fruit juice for winemaking, for example, sugar content or acidity. Geographical area, that is, the fruit cultivars typically grown in that specific area, mainly determines the production and consumption of different fruit wines. Non-grape fruits successfully used for the production of fruit wines in different parts of the world include blackberries, strawberries, currants, apples, wild apricots, pears, kiwifruit, plums, peaches, cherries, bananas, pineapples, cashew nuts, pomegranates, lemons, tangerines, oranges, dates, figs, and so on [17, 18]. There is a large volume of published studies describing chemical composition, with the particular emphasis on various bioactive compounds, of different fruits and fruit juices [19–32]. However, comprehensive reviews of respective fruit wines are still scarce, as well as the comparison of different fruit wines regarding their bioactive compounds. The primary focus of this chapter includes three most popular fruit wines in Croatia, produced from three different groups of fruit, namely berries (blackberry wine), stone fruit (sour cherry wine) and pome fruit (apple wine), in respect to chemical constituents affecting their nutritional, sensorial and health-related properties. Furthermore, food-safety issues, that is, the presence of pesticides, mycotoxins and biogenic amines, are also tackled.

2. Chemical constituents of fruit wines

2.1. Alcohol, sugars and organic acids

Fruit wine consists of two primary ingredients—water and ethanol, the latter being the primary product of alcoholic fermentation, essential for both flavour and stability of wine.

Fermentation is an anaerobic process carried out by yeast in which yeast converts the relatively high level of sugars present in fruits into ethanol and carbon dioxide [14]. Ethanol production is controlled by three main factors: sugar content, fermentation temperature and yeast strain. Particular types of yeast are used in wine production that can both tolerate and produce high alcohol levels (in some cases up to 14%, or even higher). The alcohol content is usually expressed as a volume percentage. Under standard fermentation conditions, ethanol can accumulate to up to 14–15%, while higher ethanol levels can be achieved by the sequential addition of sugar during fermentation. However, to achieve ethanol concentrations above 15%, the wine has to be fortified [33]. The role of ethanol in wine is multiple: it acts as an essential cosolvent (along with water) in extracting fruits constituents, it serves as a reactant in the generation of important volatile compounds (e.g. ethyl esters) and is crucial to the sensory properties, stability and ageing of wine [33]. The secondary products of yeast metabolism include higher alcohols (containing more than two carbons), also known as fusel alcohols or fusel oils [34]. Some of the representatives of higher alcohols are *n*-propanol, isobutyl alcohol (2-methyl-1-propanol), 2-methyl butanol (optically active amyl alcohol), isoamyl alcohol and 2-phenyl ethanol. Glycerol is the primary fermentation product of yeast, besides ethanol and carbon dioxide, and can indirectly contribute to the sensory character of the wine [35]. It is a colourless, odourless, non-volatile compound, without aromatic properties. However, it contributes significantly to the sweetness, as well as to the full and round mouth-feel of wine and wine texture [36]. There is a difference in the levels of glycerol formed by various yeast strains, and therefore glycerol production should be considered in the selection of wine yeast strains [37, 38]. Glycerol is mainly produced during glyceropyruvic fermentation at the start of alcoholic fermentation, and its degradation can be detrimental to the wine quality—partly because of the decrease of its concentration and partly because of the resulting metabolic products [39]. Glycerol concentrations of 5.976 and 4.491 were determined in two blackberry wines, produced on a small scale (in microfermentation experiments) by two commercial wine yeasts [40]. The reported glycerol concentrations in cider are 3–6 g/L [41]. Furthermore, glycerol can be metabolised by lactic acid bacteria during cider maturation, decreasing its final sensorial quality. Selection of yeast strains for secondary fermentation of sparkling ciders has been made by Suárez Valles et al. [42]. Glycerol level was 4.7 g/L in base cider while sparkling ciders produced using four different yeast strains for secondary fermentation contained 5.1–5.2 g/L. Besides ethanol and glycerol, methanol can also be present in measurable concentrations in some types of fruit wines (e.g. plum wine). However, being toxic to humans, methanol content of commercially available fruit wines should not exceed 200–250 mg/L, as regulated in different countries [14]. Fructose, glucose and, in some fruits, sucrose are the major sugars present in fruits. They are fermented by yeast during fruit wine fermentation, producing previously mentioned major metabolites: ethanol, carbon dioxide and glycerol. However, fruits other than grapes are often much lower in sugar content compared to grapes, which makes them unsuitable for winemaking unless sugar content is adjusted by the addition of (most often) sucrose [14]. Sugars may also be metabolised to higher alcohols, fatty acid esters, and aldehydes, which mostly define the primary aromatic character of wine [33]. The sugars that remain unfermented (i.e. the sugars in wine) are referred to as residual sugars. Amidžić Klarić et al. [43] reported the wide range of residual (reducing) sugars from 13.5 to 177.6 g/L in investigated blackberry wines. The authors noted that sugar (mostly sucrose) is often added during different stages of the blackberry wine production, so the wide range

of reducing sugars concentration found in investigated samples is probably the result of the applied technological procedure. Sun et al. [44] studied the suitability of different yeast strains for the production of cherry wine and reported the concentration of total reducing sugars in wine ranging from 0.7 to 1 g /L. Initial analysis of must revealed the initial concentration of total sugars of 165 g/L, achieved by the addition of sucrose to the crushed and deseeded cherries. The discrepancy in the residual sugar content between the blackberry wines and cherry wines can be attributed to the production technology and consumers' preferences—low acidity and high sugar fruit wines seem to be the most acceptable [14]. Fruit species, climate and geomorphological character of soil determine the content of organic acids in fruit. Consequently, the acidity of fruits affects the fruit wine acidity. Total acidity is the wine-quality parameter that gives the measure of the wine acidity, a parameter influencing the wine taste and the overall quality [43]. The total acidity of blackberry wines reported by Amidžić Klarić et al. [43] ranged from 6.7 to 18.1 g/L (as tartaric acid), while the reported total acidities of cherry wines ranged from 5.94 to 6.71 g/L [44]. When it comes to grape wine tasting, a high level of acidity refers to excessively tart, sour and sharp wine attributes, while a low total acidity results in a flat-tasting wine that is more susceptible to infection and spoilage by microorganisms [45]. Organoleptic properties of musts and fruit wines are strongly influenced by organic acids [46]. The major organic acid present in blackberry, cherry and apple wines is malic acid, with reported concentrations of 3.5, 6.8 and 6.2 g/L, respectively. The second most abundant is citric acid [14], while the predominant volatile acid is acetic acid, often expressed as the wine-quality parameter called volatile acidity. Acetic acid is a secondary metabolite derived from a pyruvic acid, which is always formed during alcoholic fermentation. Its accumulation in wine is usually the result of the secondary infection of the fruit, that is, the acetic acid bacteria activity, which is promoted by excessive oxygen uptake. The secondary infection can occur during the vinification process or after bottling [47]. Higher concentrations of acetic acid can detrimentally affect the organoleptic properties of wine, because of bitter taste and smell-like vinegar [39]. Besides acetic acid, as already mentioned, malic acid also contributes to the acidity of fruit wine. However, its degradation by malolactic fermentation (MLF) can reduce the acidity of the wine. MLF can occur spontaneously during or at the end of alcoholic fermentation or can be induced by the addition of lactic acid bacteria starter cultures, namely *Oenococcus oeni* as the primary species used in MLF. Compared to malic acid, lactic acid has a softer flavour, which results in the more desirable flavour profile of wine [48]. Furthermore, the conversion of malic acid to lactic acid results in reduced wine acidity and improved stability and quality of high-acid wines [49].

2.2. Volatile (aroma) compounds

Knowledge of the volatile composition of wine is of great interest; since these compounds are responsible for the quality of wine aroma. The aroma of fruit wines is mainly determined by volatile compounds produced by the fruit itself (varietal wine aroma), as by-products of alcoholic and malolactic fermentation (fermentative wine aroma), and formed during bottling, ageing and storing (post-fermentative wine aroma) [50]. Esters, higher alcohols, acetates, organic acids and other compounds are the groups of volatile compounds that most commonly contribute to the flavour and/or aroma profile of fruit. Apart from the listed groups of

volatiles, there are also many minor volatile and non-volatile compounds adding to the aroma of fruit wines, such as aldehydes, ketones, lactones, terpenes and phenols [51]. A fast, selective and sensitive method for the determination of volatile compounds in blackberry wine samples using an HSS-GC-FID method was presented by Mornar et al. [52]; still, the study encompassed a limited number of compounds as well as samples. Therefore, Amidžić Klarić et al. [53] analysed volatile compounds in the 15 blackberry wine samples produced from conventionally and organically grown blackberries using a GC-FID method. The amount of ethyl acetate in the investigated samples ranged from 53.8 to 188.4 mg/L, while propane-1-ol was found in three organic samples. Although isoamyl alcohol was found in all samples, the measured values ranged from 56.7 to 226.9 mg/L. A GC–MS technique was used by Wang et al. [54] for the investigation of the fermentation process influence (primary and secondary) on volatile compounds of blackberry wine. Fifty-five volatile compounds were detected in blackberry fruit juice before fermentation, while nine new aroma components such as octanoate, benzenepropanoic acid ethyl ester, ethyl benzoate, dodecyl ethyl, *n*-propanol, *n*-butanol, D-citronellol, benzaldehyde and cedrol were detected in natural ageing wine which appeared during secondary fermentation. These findings emphasise the importance of natural ageing for the formation of aroma components of blackberry wine. Due to the high popularity of cherry wine, the volatile compounds of this fruit wine were in focus of several investigations as indicators of aroma profile of fruit wines. Niu et al. [55] have applied descriptive sensory analysis to describe the aroma attributes of different cherry wines by GC-olfactometry, an analytical technique that uses human assessors as a sensitive and selective detector for odour-active compounds. Fifty-one compounds were detected and subsequently quantified by GC–MS. The conducted research revealed that the aroma-active compound profiles were dominated by ethyl 2-methyl propionate, 2,3-butanedione, ethyl butyrate, ethyl pentanoate, 3-methyl-1-butanol, ethyl hexanoate, 3-hydroxy-2-butanone, ethyl lactate, 1-hexanol, (Z)-3-hexen-1-ol, ethyl hydroxyacetate, acetic acid, furfural, 2-ethyl-1-hexanol, benzaldehyde, propanoic acid, butanoic acid, guaiacol, beta-citronellol, hexanoic acid, 2-methoxy-4-methylphenol, 2-ethyl-3-hydroxy-4H-pyran-4-one, ethyl cinnamate and 2-methoxy-4-vinylphenol. Afterwards, Xiao et al. [56] have determined 75 volatiles in 9 cherry wine samples using an improved sample preparation technique HS-SPME. The cluster analysis results suggested that esterification reactions and fermentation process were more extended during the ageing period and the production process could have a significant influence on volatile profile of cherry wines. The similar conclusion was obtained by Xiao et al. [57] using multivariate classification of cherry wines. The research revealed the major volatile components of investigated cherry wines: isoamylol, ethyl acetate, benzyl alcohol, benzaldehyde and diethyl succinate. More recently, the research of Xiao et al. [58] have shown that the Chinese cherry wines from different price segments have the different aroma-active compounds profile as well as various aroma attributes. Furthermore, the correlation between the composition of aroma-active compounds and cherry wine from different regions was established by Xiao et al. [59]. Wang et al. [60] have proposed a new, rapid method for analysis of volatile compounds in apple wine using HS-SPME-GC-MS technique. Forty-three volatile compounds (alcohols, esters, lower fatty acids, carbonyls, alkenes, terpenes and phenols) were quantified in `Fuji´ apple wine. The most abundant aroma compounds were esters, alcohols and lower fatty acids; total concentrations were 242.1, 479.3 and 297.4 mg/mL, respectively. Still, the

dominant aroma component was isoamyl alcohol (232.0 mg/mL). The same technique was used for the investigation of the temperature effect during apple winemaking on both the critical aroma compounds and sensory properties of wine. The concentration of all aroma compounds was changed with a temperature increase, and sensory analysis showed the highest acceptance of apple wine fermented at 20°C [61]. The influence of apple variety (Šampion, Idared and Gloster) harvested from the orchard in Poland on the volatile composition, and sensory characteristics of apple wine were investigated by Satora et al. [62]. The high concentration of acetaldehyde, ethyl acetate and methanol was found in Šampion wines, while Gloster wines contained a higher concentration of fusel alcohols. The Idared wines had the best results of the sensory evaluation and high levels of butanol and acetic acid. As mentioned earlier, the majority of investigations were performed using sample preparation procedures such as HS and/or SPME, while gas chromatography with FID and MS detectors was shown to be the analytical technique of choice for the determination of volatile compounds in fruit wine. Ye et al. [63] have developed and applied a new rapid method for the identification of volatile compounds in apple wine using FT-NIR spectroscopy. To provide the greater insight into yeast metabolism and flavour formation of mulberry wine, Butkhup et al. [64] have developed a new HS-SPME-GC-MS method to characterise various volatile compounds of in-house-made mulberry wine. Eighty volatile compounds belonging to groups of higher alcohols, fatty acids, esters and phenols were quantified. These compounds were present in various amounts from 0.1 mg/L (benzene carboxaldehyde) to 138.4 mg/mL (isoamyl alcohol). Feng et al. [65] have extended their research on other fruit wines produced from mulberry as well as raspberry and strawberry. Alcohols formed the most abundant group, followed by esters and acids. Comparing to investigated grape wine, two alcohols, 4-methyl-2-pentanol and 2,3-butanediol, were not found in the three fruit wines. While the number of esters in raspberry (1.5%) and mulberry (2.1%) wines were higher than those of strawberry (0.8%) wine, there were no significant differences in acid content. Song et al. [66] have used the same sample preparation and analytical techniques to determine 78 volatile compounds in wild strawberry wine sample from a southern region of China. Odour activity values were detected for 21 compounds while 6 of them were identified as the particular aroma substances for wild strawberry wine, in particular, methyl 2-methylbutyrate, ethyl 2-methylbutyrate, methyl 3-methylbutyrate, (E)-3-hexen-1-ol, 1-octen-3-ol and phenylacetaldehyde.

2.2.1. Volatile compounds containing sulphur

The formation of off-flavours represents the core problem of high-quality fruit wine production. The main compounds responsible for off-flavours of fruit wines are sulphur-containing volatiles, acetic acids and free amino nitrogen [14]. The volatile compounds containing sulphur, formed as a part of sulphur metabolism, include both molecules positively correlated to the aromatic profile of the wine (volatile thiols), as well as those responsible for wine defects, such as notes described as cabbage, onion, rotten egg, garlic, sulphur and rubber. Hydrogen sulphide and mercaptans (e.g. dimethyl sulphide, dimethyl disulphide, dimethyl trisulphide) are the most often linked to the formation of off-odours in wine, and they are usually present in very low concentration [67]. However, their perception threshold is also low [68].

2.3. Phenolic compounds

Wine quality is significantly influenced by phenolic compounds, namely anthocyanins, flavonols, catechins and other flavonoids since they have an enormous impact on the sensory characteristics of wines, mainly colour and astringency [69]. Furthermore, they exhibit a range of antioxidant and pharmacological effects. Phenolic compounds can be classified in different ways because they are constituted at a large number of heterogeneous structures that range from simple molecules to highly polymerised compounds. A review of the composition and content of phenolic compounds in various fruit wines is presented in **Table**. Based on the total phenolic content, wines are commonly categorised to three major groups: (i) high in total phenolics, (ii) moderately high in total phenolics and (iii) low in total phenolics. The total phenolic content of cherry, raspberry, black currant, bilberry, elderberry and sea buckthorn fruit wines proved to be comparable or even higher than that of grape red wines, while apple, plum and peach fruit wines had a lower total phenolic content than red grape wines [20, 87–89]. Among the phenolic compounds with known antioxidant activity, flavonoids, phenolic acids and tannins are highlighted [26].

Flavonoids, the most studied phenolics, are important components of the human diet. They are also the most widely present in plants and are among the most potent antioxidants in plants. Different members of certain classes of flavonoids can be either colourless or coloured (e.g. anthocyanins), such as pigments of flowers and other plant parts [26]. Important subclasses of flavonoids are, among others, anthocyanins and flavanols. Anthocyanins are important polyphenolic components of fruits, especially berries [90]. The anthocyanins are conjugated anthocyanidins, and they provide the unique colours of dark berries. There are six anthocyanidins distributed throughout the plant kingdom: cyanidin, malvidin, delphinidin, peonidin, pelargonidin and petunidin. The significant anthocyanins in blackberries are cyanidine derivatives. In nature, anthocyanins are mainly found as heterosides. The aglycone form of anthocyanin, known as anthocyanidin, is structurally based on the flavilium or 2-phenylbenzopyrilium cation, with methoxyl and hydroxyl groups present at different positions of the basic structure. Numerous studies have shown that anthocyanins are absorbed in their original glycosylated forms in humans [14, 91, 92]. Anthocyanins are predominant phenolic components or comprise a considerable portion of the total polyphenol content of blackberry as well as cherry juice [76, 84, 93–95]. They are found in berry wines and some fruit wines and presented as total anthocyanin content (TAC). The TAC values found in some blackberry and blueberry wines were 75.56 and 20.82 mg/L (expressed as ellagic acid equivalents), respectively [79]. Mudnić et al. [96] determined high TAC values in blackberry wines, comparable to that measured in red grape wine and ranging from 134 to 164 mg/L (expressed as malvidin 3-glucoside equivalents). Flavonols, another important class of flavonoids, are characteristic for the cool climate fruits and their respective wines [14]. Myricetin and quercetin have been recognised as the first flavonols detected in red berry wines [5]. The results of a study conducted by Mudnić et al. [73] that compared flavanol and procyanidin B_2 contents of several grape wines (red and white), with four commercially available blackberry wines, revealed that the highest concentrations of catechin (45.2 mg/L) and epicatechin (34.7 mg/L) were determined in two blackberry wines. The content of procyanidin B_2 (77.1 mg/L) of the

Phenolic compound	Blackberry wine (no. of samples) [Ref.]	Cherry wine (no. of samples) [Ref.]	Apple wine (no. of samples) [Ref.]	Wine [Ref.]
Total phenolics*	733–2698 (17) [70]	584–743 (6) [44]	160–470 (4) [71]	932–1055 [72]
	1697–22,789 (4) [73]	1081–2711 (9) [74]	225–645 (3) [74]	190–1215 [75]
	1055–2704 (13) [74]	850–1300 (5) [76]	451 (6) [20]	
	1052–3621 (6) [73]	991 (6) [20]	228–639 (3) [62]	
	1122–1400 (10) [77]	1940 (1) [78]	471–801 (40) [77]	
Total anthocyanins	1.3–125.3 (17) [70]		NF	61–125 [72]
	13–164 (4) [73]	17.9–27.9 (6) [44]		
	23–217 (13) [74]	55–483 (9) [74]		
	18.5–192.0 (6) [79]	120 (1) [78]		
	52–105 (19) [77]			
Flavonoids*	924–1417 (4) [73]	NF	NF	NF
Tannin*	1010–1312 (10) [77]	741–901 (6) [44]	NF	266–414 [72]
Individual phenolic compounds				
Flavonol				
Quercetin	0.4–17.9 (13) [74]	0.3–3.9 (9) [74]	0.4–0.9 (3) [74]	<LOD-13.4 [80–83]
	0.8–21.7 (15) [43]	ND (1) [78]		
Quercetin-4-glucoside	ND-2.6 (4) [73]	NF	<LOD-0.1 (3) [62]	NF
Quercetin-3-O-rutinoside (Rutin)	NF	0.2 (1) [78]	NF	6.8–20.2 (67) [82]
Flavan-3-ol				
(+)-Catechin	1.7–10.3 (13) [74]	0.4–11.2 (9) [74]	1.5–15.2 (3) [74]	13.4–95.8 (6) [81]
	9.1–45.2 (4) [73]	12.0–15.2 (4) [84]	1.6–3.5 (3) [79]	
		1.3 (1) [78]		
(−)-Epicatechin	ND-34.7 (4) [73]	0.5–24.1 (9) [74]	4.1–25.9 (3) [74]	4.4–68.5 [80–83]
	0.1–61.4 (13) [74]	10.3–20.91 (4) [84]	3.5–6.6 (3) [62]	
		3.91 (1) [78]		
Epigallocatechin gallate	ND-148.8 (4) [73]	ND (1) [78]	NF	<LOD-15.6 [85]
(−)-Epigallocatechin	NF	1.01 (1) [78]	NF	NF
Procyanidin B2	6.1–77.1 (4) [73]	24.7–69.1 (4) [84]	2.9–7.4 (3) [62]	3.0–83.2 (67) [82]
Flavanone				
Naringenin	NF	0.15 (1) [78]	NF	NF
Flavone				
Apigenin	NF	0.06 (1) [78]	NF	NF

Phenolic compound	Blackberry wine (no. of samples) [Ref.]	Cherry wine (no. of samples) [Ref.]	Apple wine (no. of samples) [Ref.]	Wine [Ref.]
Anthocyanins				
Cyanidin	<LOD-3.2 (15) [43]	ND-4.2 (5) [84]	NF	<0.4–12.3 [86]
Cyanidin 3-glucoside	0.39–25.51 (13) [74]	0.36–0.68 (6) [44]	NF	NF
	8–18 (10) [77]	0.78–12.07 (9) [74]		
		<LOQ-3.78 (5) [84]		
Cyanidin 3-rutinoside	1.9–119.3 (13) [74]	10.7–15.1 (6) [44]	NF	NF
	25–69 (10) [77]	3.2–91.4 (9) [74]		
		1.3–10.2 (5) [84]		
Cyanidin 3-glucosylrutinoside	NF	17.6–25.4 (6) [44]	NF	NF
		7.4–361.3 (9) [74]		
		10.1–44.6 (5) [84]		
Pelargonidin	<LOD-1.46 (15) [43]	NF	NF	NF
Peonidin 3-rutinosid	NF	1.06–1.64 (6) [44]	NF	11.4–128.0 (67) [82]
Phenolic acids				
Gallic acid	45.4–59.0 (4) [73]	<LOQ–5.7 (9) [74]	0.1–1.4 (3) [74]	6.7–104.8 [80–83]
	10.8–52.3 (13) [74]	1.1 (1) [78]		
	28.1–122.4 (17) [70]			
Caffeic acid	2.0–4.8 (17) [70]	0.4–1.7 (6) [44]	1.1–5.4 (3) [74]	0.2–30.8 [80–83]
		1.7–46.3 (9) [74]	0.6–2.5 (3) [62]	
		13.9 (1) [78]		
		16.3–25.6 (5) [76]		
Chlorogenic acid	1.0–3.9 (17) [70]	21.7–29.6 (6) [44]	13.3–25.7 (3) [74]	NF
		12.2–110. 5 (9) [74]		
		3.6 (1) [78]		
		27.3–81.0 (5) [76]		
Neochlorogenic acid	NF	12.8–18.5 (6) [44]	NF	NF
		44.5–71.4 (5) [76]		
p-Hydroxybenzoic acid	NF	4.0–4.7 (6) [44]	NF	0.4–0.7 (6) [81]
		6.7 (1) [78]		
p-Coumaric acid	0.1–79.7 (13) [74]	3.3–7.0 (6) [44]	<LOQ-0.2 (3) [74]	0.2–14.2 [80–83]
	1.0–4.4 (17) [70]	0.2–22.2 (9) [74]	0.1–0.9 (3) [62]	
		23.4 (1) [78]		
		0.6–15.2 (5) [76]		

Phenolic compound	Blackberry wine (no. of samples) [Ref.]	Cherry wine (no. of samples) [Ref.]	Apple wine (no. of samples) [Ref.]	Wine [Ref.]
p-Coumarylquinic acid	NF	NF	0.3–4.3 (3) [62]	NF
Protocatechuic acid	13.8–62.6 (13) [74]	<LOQ–15.4 (9) [74]	NF	NF
		23.9 (1) [78]		
Ferulic acid	NF	ND-4.2 (5) [76]	NF	0.1–0.2 (6) [81]
		1.9–13.4 (9) [74]		
Stilbene				
trans-Resveratrol	0.5–0.9 (4) [73]	NF	ND (1) [78]	0.02–1.46 (6) [81]
cis-Resveratrol	ND-1.5 (4) [73]	NF	NF	NF
Astringin	ND-12.1 (4) [73]	NF	NF	NF
(E)-Piceid	2.0–6.5 (4) [73]	NF	NF	NF

All concentrations are expressed in mg/L.

ND, not detected; NF, not found; LOD, limit of detection; LOQ, limit of quantification.

*Values are expressed as gallic acid equivalents (milligrams of gallic acid per litre of fruit wine).

Table. A review of the composition and content of phenolic compounds in various fruit wines.

same wines was sevenfold higher than in red grape wine (12.3 mg/L). Cherry wines proved to be a much more potent source of flavonols than grape wines, with almost 10 times higher values (from 24.2 to 69.08 g/L for procyanidin B_2) [84]. The flavanol profile of cherry wine is similar to its grape counterparts, with values up to 3.92 mg/kg for epicatechin, followed by smaller amounts of catechin and epigallocatechin [78]. The major flavanol in apple wines is epicatechin, followed by catechin and their polymerisation product procyanidin B_2 [62].

Phenolic acids. P (Phenolic acids) are usually divided into hydroxybenzoic acid derivatives (such as gallic, hydroxybenzoic, salicylic and protocatechuic) and diverse hydroxycinnamic acids and their derivatives. Blackberry and wine made from this edible fruit are known to have high concentrations of benzoic and cinnamic acids [19, 21], especially gallic acid, chlorogenic acid, *p*-coumaric acid and caffeic acid [70]. Cherry wines have a significantly higher content of phenolic acids in comparison to grape wine and almost 10 times higher content of caffeic acid. The prevalent hydroxycinnamic acids in apple wines are chlorogenic and *p*-coumaroylquinic acid. However, they are susceptible to very fast oxidation catalysed by enzyme polyphenol oxidase, giving *o*-quinone, a compound responsible for the browning of apple must, as the principal product [62]. It has been reported in the literature that organically produced fruits may have a higher level of phenolic compounds. This can probably be explained by the enhanced synthesis of endogenous natural defence substances (phenolics) enriching plant defence mechanisms, that is, produced as a response to biotic and abiotic stress [97], in the absence of fertilisers and pesticides, commonly used during conventional production [98–100]. Even though there are generally recognised differences between the conventionally and organically grown food, namely the yield, the size and the pesticide residues, the data on the impact of the practices mentioned earlier on nutritional quality are scarce

and often contradictory. Amidžić Klarić et al. [43] evaluated the quercetin content, colour and selected physicochemical quality parameters of Croatian blackberry wines produced using organically and conventionally grown blackberries. Quercetin content of organic wine samples group was slightly higher than that of conventional wine samples group. No significant overall differences were found in case of colour. Phenolic compounds, in general, are not well absorbed, and it is supposed that the presence of ethanol enhances their bioavailability. This can explain the reported results, indicating that (grape) wines offer somewhat higher health benefits than the preparation of isolated phenolic extracts alone or dealcoholised wine [101, 102]. Similar investigations of fruit wines are still lacking. A progressive loss of phenolic compounds can occur during the winemaking process, as a result of chemical reactions, such as degradation, oxidation, precipitation of phenolics with polysaccharides, condensation with tannins and the formation of other stable anthocyanin-derived pigments. All these reactions can result in significant changes in colour and flavour of red (grape) wines [103, 104]. The same could be applied to other fruit wines. Rommel et al. [93] investigated the processing and storage effects on the composition of anthocyanin, colour and appearance of blackberry juice and wine. They reported 85–100% degradation of juice total anthocyanins, caused by fermentation in combination with depectinising and storage time.

2.4. Vitamins

Vitamins are compounds essential for both plant and animal metabolism, and their deficiencies in humans often lead to different disorders and diseases. Because of their ability to synthesise vitamins, plants (namely fruits and vegetables) are an excellent source of the vitamins in the diet. The stability of vitamins in different foods is often at risk due to various technological practices used during the processing of food, namely changes in temperature regimes (e.g. thermal treatments) and oxygen levels [105, 106]. Since fruits are a good source of vitamins, consequently the vitamins can also be found in fruit juices. However, the concentration of vitamins decreases during the winemaking (fermentation and ageing), so their levels in wine are inadequate to be of significance in human nutrition. On the other hand, their levels are usually enough or more than enough to support microbial growth. Biotin (vitamin H) and nicotinic acid (niacin, vitamin B_3) contents are adequate for most yeast strains [33]. Low levels of vitamins determined in grape wines are probably the reason for the absence of relevant studies regarding the vitamins in fruit wines. Grape wines are reported to contain some B vitamins (thiamine, riboflavin and vitamin B_{12}) and very small amounts of vitamin C and fat-soluble vitamins A, D and K. Some vitamins, such as vitamin C, may be primarily destroyed during processing and storage, whereas others, for example, B vitamins, are also produced by the action of yeast and may be present in measurable quantities in the final product [107]. Thiamine (vitamin B_1) is utilised by yeast during fermentation, so its levels in wine are insignificant. In addition, thiamine levels are lowered by reaction with SO_2 during fermentation as well as the absorption by bentonite. Riboflavin (vitamin B_2) is oxidised on exposure to light [33]. Free riboflavin (RF) is naturally present in raw and processed fruits and fermented beverages. Flavin-adenine dinucleotide (FAD) and sometimes flavin mononucleotide (FMN) are present together with RF in significant amounts in fruit juices, while RF is the only form of riboflavin present in important quantities in wine [108]. Since some yeasts synthesise RF

during cell growth as an extracellular by-product of the fermentation process, its content can increase in wine. *p*-aminobenzoic acid (PABA), classified into a group of vitamin-like substances, although often referred to as a vitamin [109], is the only compound to increase substantially during fermentation [33]. Folic acid (pteroylglutamic acid) is a water-soluble B vitamin, essential for healthy growth and development of humans, can be found in significant amounts in berries [110] and can reach up to 1 mg/100 mL in some berry wines [14]. During the process of alcoholic fermentation, this vitamin is not subject to change because it has no significant effect on yeast, while for lactic acid bacteria, it is essential [111]. Vitamin C is one of the essential nutritional quality factors of fruits and has many vital biological roles in the human body, such as cell division and proliferation, photosynthesis, hormone biosynthesis and signalling [112, 113]. It is a crucial antioxidant that is produced by plants response to various biotic and abiotic stresses. Vitamin C plays a fundamental role in scavenging reactive oxygen species (ROS) due to its potent antioxidant properties [113]. Fruits and vegetables provide more than 90% of the vitamin C in human diets [112]. The reported vitamin C concentration ranges in blackberries, cherries and apples are 87–696 mg/L [114], 43–177 mg/L [115] and 3–25 mg/100 mg of fresh weight [116], respectively. Previous investigation has indicated that the content of ascorbic acid varied for organically and conventionally cultivated fruits and vegetables [117], as well as for different environmental conditions under which the plant was grown, such as temperature, water availability, pathogenic attack and nutrients [118]. Taken together, the facts listed earlier lead to a conclusion that moderate wine consumption is very far from providing an adequate intake of vitamins for maintaining optimal nutritional status and overall health [14].

2.5. Antioxidant capacity

Fruits contain various dietary phytonutrients with strong antioxidant capacities, such as phenolics (which include flavonoids and phenolic acids), carotenoids and vitamins. Many phenolic substances present in fruits influence sensory properties of fruit wine such as taste, astringency, bitterness as well as colour. Since fruit wines are processed in the same way as wine made from grapes, significant compositional changes take part in winemaking [119]. Both fermentation and ageing result in the transformation of starting compounds present in musts into secondary metabolites that determine the quality of the final product [119, 120]. Berries, grape and their processed products such as wines contain a wide range of flavanoids and other phenolic compounds that possess antioxidant activity. Heinonen et al. [71] evaluated the antioxidant activity of over 44 different fruit wines, mainly berry wines, but also apple wines. The results of the study showed that all the investigated wines possessed a significant antioxidant activity. However, the total phenolic content did not correlate with the antioxidant activity. On the other hand, several other studies confirmed a strong positive correlation between the total antioxidant activity of fruit wines and total phenolics [26, 28, 31, 69, 95, 121]. When compared to red (grape) wines, blackberry, blueberry and sour cherry wines proved to have 30–40% more superoxide radical-scavenging activity [122], which makes them very potent natural antioxidants beneficial to human health [74, 76, 84, 94]. Yoo et al. [12] compared the levels of enzymes superoxide dismutase (SOD) and catalase in fresh cherries,

cherry juice and cherry wines and concluded that cherry wine had enhanced levels of the mentioned enzymes. Superoxide dismutases (SODs) are the major antioxidant defence systems against superoxide anion, the most common free radical in the body [123]. SODs reduce the cellular damage caused by superoxide anion. When comparing different fruit wines, it has been reported that berry fruit wines (blackberry, elderberry, blueberry and raspberry) in general had higher total antioxidant capacities than apple and pear wines (as representatives of pome wines) [14, 20].

2.6. Mineral composition

Minerals are inorganic ingredients of food, required by the body in small amounts for a variety of different metabolic processes important for the functioning of human body. Since some minerals are needed in more substantial quantities than others, they can be divided to three groups, based on their recommended daily intake: macrominerals (over 50 mg/day), trace minerals and ultratrace minerals (lower than 50 mg/day) [124–126]. Nowadays, a mineral deficiency occurs very often without its noticeable signs. The sufficient intake of minerals depends on their quantities consumed with food, but even more on their bioavailability, which depends on the food composition and properties. The mineral content of fruits can vary significantly depending on the genetic and climate factors, cultivation procedures, soil composition, ripeness and many other factors. Furthermore, changes in mineral content usually occur as a result of applied technological procedures, for example, the removal of a portion of the raw material [125, 126]. The sources of minerals (metals) in alcoholic beverages are various: raw materials, process equipment, applied technological procedures, fermentation, bottling, ageing, storage and adulteration [127]. The soil on which fruits are grown is a source of metals of natural origin (primary metals) present in wine. The concentration of primary metals is characteristic and comprises the most significant part of the total metal content in wine [128, 129]. External impurities present in fruits, as well as those that occur during different stages of winemaking, are the source of metals of secondary origin. For example, the prolonged contact of wine and the construction materials used for the oenological equipment (stainless steel, aluminium, brass, glass and wood) can be a source of contamination of wine with Al, Cd, Cr, Cu, Fe and Zn [128–130]. Fining and clarifying substances (e.g. bentonites) used during winemaking can be a source of contamination with Na, Ca or Al [129–132]. Furthermore, individual metals have a significant effect on the organoleptic properties of wine (i.e. Al, Zn, Cu and Fe) and, therefore, their concentration must be monitored [133]. The total amount of minerals in a sample of fruits and fruit wines can be expressed as the ash content. Ash is the inorganic residue remaining after the water, and organic matter has been removed. A higher ash content in a fruit wine implies a higher amount of minerals and a higher quality of the wine [134]. The literature regarding grape wines proposes that the mineral profile of wines could be used as a 'fingerprint', used for the characterisation of wines based on their geographical origin [20, 135]. While there is a significant volume of available data on the mineral content of blackberries, cherries and apples, far too little attention has been paid to the mineral composition of respective fruit wines. A review of metal concentrations in fruit wines is given in **Table**. As it could be seen from the table,

Metal	Blackberry wine (no. of samples) [Ref]	Cherry wine (no. of samples) [Ref]	Apple wine (no. of samples) [Ref]	Grape wine [Ref]
Ag	0.34–2.76 μg/L (15) [134]	NF	NF	0.008–2.77 μg/L (31) [128]
Al	6–16 mg/L (15) [134] 37–1110 μg/L (22) [136]	0.200 mg/kg (1) [78]	NF	0.017–14.3 μg/mL [129]
As	<0.9 μg/L (15) [134]	0.093 μg/kg (1) [78]	NF	1.7–15.2 μg/L (31) [128]
B	NF	2.760 mg/kg (1) [78]	NF	2.1–12.1 mg/L (68) [137]
Ba	0.48–1.46 mg/L (15) [134]	NF	NF	0.01–0.48 μg/mL [129]
Ca	86–457 mg/L (32) [134, 138] 115–555 mg/L (22) [136]	0.084 g/kg (1) [78] 69 μg/g (6) [20]	8–28 mg/L (3) [9] 45 μg/g (6) [20]	7–241 μg/mL [129]
Cd	0.3–9.9 μg/L (32) [134, 138]	0.093 μg/kg (1) [78]	<0.026–3.72 μg/L (3) [9]	ND-0.054 μg/mL [129]
Co	1.3–11.9 μg/L (32) [134, 138] 2–40 μg/L (22) [136]	0.577 μg/kg (1) [78]	NF	ND-0.045 μg/mL [129]
Cr	2.3–18.7 μg/L (32) [134, 138]	0.016 mg/kg (1) [78]	NF	ND-0.2 μg/mL [129]
Cu	0.06–0.77 mg/L (32) [134, 138] 0.05–3.83 mg/L (22) [136]	0.030 mg/kg (1) [78]	NF	ND-2.60 μg/mL [129]
Fe	0.082–8.4 mg/L (32) [134, 138] 0.093–5.49 mg/L (22) [136]	2.192 mg/kg (1) [78] 1.2 μg/g (6) [20]	<0.027–0.508 mg/L (3) [9] 0.4 μg/g (6) [20]	0.06–23.7 μg/mL [129]
Hg	NF	NF	<0.036 μg/L (3) [9]	NF
K	564–2014 mg/L (32) [134, 138] 615–1760 mg/L (22) [136]	1.373 g/kg (1) [78] 834 μg/g (6) [20]	233–353 mg/L (3) [9] 958 μg/g (6) [20]	265–3056 μg/mL [129]
Li	13–21 μg/L (15) [134] 1–40 μg/L (22) [136]	0.678 μg/kg (1) [78]	NF	0.002–0.098 μg/mL [129]
Mg	706–381 mg/L (32) [134, 138] 77–238 mg/L (22) [136]	0.072 g/kg (1) [78] 50 μg/g (6) [20]	13–19 mg/L (3) [9] 38 μg/g (6) [20]	7–718 μg/mL [129]
Mn	0.7–11.5 mg/L (32) [134, 138] 0.5–11.3 mg/L (22) [136]	0.632 mg/kg (1) [78] 0.2 μg/g (6) [20]	0.2 μg/g (6) [20]	ND-5.5 μg/mL [129]
Mo	NF	0.093 μg/kg (1) [78]	NF	0.7–64 μg/L (88) [139]
Na	12–213 mg/L (32) [134, 138] 3–13 mg/L (22) [136]	1.65 mg/kg (1) [78] 38 μg/g (6) [20]	31 μg/g (6) [20]	ND-310 μg/mL [129]
Ni	60–278 μg/L (15) [134]	0.054 mg/kg (1) [78]	NF	ND-0.5 μg/mL [129]
P	32–119 mg/L (15) [43]	0.179 g/kg (1) [78] 54 μg/g (6) [20]	68 μg/g (6) [20]	0.3–47.3 mg/L (31) [128]
Pb	13.6–52.8 μg/L (15) [134] 17–54 μg/L (22) [136]	4.4 μg/kg (1) [78]	75.3–116.3 μg/L (3) [9]	ND-1.1 μg/mL [129]
Rb	90–1470 μg/L (22) [136]	NF	NF	0.03–9.90 μg/mL [129] 96–2470 μg/L (31) [128]

Metal	Blackberry wine (no. of samples) [Ref]	Cherry wine (no. of samples) [Ref]	Apple wine (no. of samples) [Ref]	Grape wine [Ref]
S	NF	0.089 g/kg (1) [78] 136 μg/g (6) [20]	80 μg/g (6) [20]	NF
Sb	NF	0.093 μg/kg (1) [78]	NF	0.18–29.7 μg/L (31) [128]
Se	<1.5 μg/L (15) [134]	0.011 mg/kg (1) [78]	NF	NF
Si	1.82–8.24 mg/L (15) [134]	NF	NF	5.30–33.3 mg/L (68) [137]
Sn	12.7–22.0 μg/L (15) [134]	NF	<32.5 μg/L (3) [9]	0.02–1.0 mg/L (68) [137]
Sr	165–1445 μg/L (22) [136]	NF	NF	0.12–3.22 μg/mL [129] 0.27–1.84 mg/L (54) [140]
V	NF	6.86 μg/kg (1) [78]	NF	ND-0.40 μg/mL [129]
Zn	0.25–6.65 mg/L (32) [134, 138] 0.30–1.96 mg/L (22) [136]	0.3 mg/kg (1) [78] 0.1 μg/g (6) [20]	36–105 μg/L (3) [9] 0.2 μg/g (6) [20]	ND-8.9 μg/mL [129]

ND, not detected; NF, not found.

Table. A review of metal concentrations in blackberry, cherry and apple wines.

the mineral content of blackberry, cherry and apple wine is in accordance with that of grape wines. Predominant metal in all reviewed fruit wines is K, its concentration being the highest in blackberry wine. Na is a widespread metal in food since the industrially processed food is a particularly important source of this macroelement. The sodium concentrations of blackberry wines varied over a wide range when compared to other fruit wines (**Table**). Still, the low-sodium/high-potassium levels found in all three types of fruit wine indicate that fruit wines can be considered as effective sources of potassium. Due to irregular diet and large intake of refined sugars, coffee and alcohol, the insufficient intake of magnesium may occur. Amidžić Klarić et al. [141] investigated the mineral composition of commercially available Croatian blackberry wines. The results indicated that 17 investigated Croatian blackberry wines could be considered as an excellent additional source of magnesium, manganese and potassium. When compared to cherry and apple wines (**Table**), blackberry wine seems to be a better source of Ca, Mg, Fe and Mn. The literature data show that calcium and phosphorus absorp-tion is closely related, but due to an unbalanced diet dominated by a high phosphorus intake, homeostasis is compromised and contributes to low serum calcium levels (hypocalcaemia). For this reason, it has been suggested that the calcium-phosphorus ratio in foods should be between 1:1 and 2:1. As can be seen from **Table**, the calcium-phosphorus ratio of reviewed blackberry wine samples was higher than 1 in favour of calcium. Iron deficiency is one of the most common nutritional deficiencies, characterised by signs and symptoms such as unusual tiredness, paleness, shortness of breath and anaemia. In Croatia, blackberry wine is tradition-ally called 'ferrous wine', and it has been used as a popular medicine for anaemia and iron deficiency. Some of the metals exhibit not only beneficial effects on the human body but also deleterious ones that made them a cause of concern for wine producers and consumers for years now. Heavy metals are some of the most critical chemicals found in the environment,

and the exposure through the consumption of food is frequent, regardless of the mode of food production (conventional or organic) [142]. Amidžić Klarić et al. [134] examined mineral and heavy metal content of blackberry wines made from conventionally and organically grown blackberries. The comparison between these two groups of investigated blackberry wines showed the statistically significant difference in the content of Si and Li, whereas the organic wines group contained higher levels of these compounds. Toxic metals (As, Cd, Hg, Pb) are frequently found to be the primary food contaminants [126], and they can be poisonous and harmful. Therefore, the metal content of grape wine is regulated according to the national legislation and the European Union legislation [141, 143]. Above optimal level, elements, such as Al, Cu, K, Fe, Mn, Zn, may have detrimental effects on wine stability and its commercial acceptability [144].

3. Food-safety issues regarding fruit wines

3.1. Pesticides residues

Pesticides comprise a numerous and diverse group of chemical compounds with a common characteristic of eliminating pest in agriculture and households. They are widely used in agricultural practice, not only during cultivation but also in post-harvest storage. Pesticide residues may end up in fruit pulp and juices since they can penetrate plant tissues. However, their concentrations are lower than those in the intact fruit [145]. Pesticide residues in fruit wine, like those found in juice, can also be introduced by planting and preservation process [146]. In winemaking, the activity of yeast can be affected by pesticides. The presence of pesticides has been associated with stuck and sluggish fermentation [147]. Most of the pesticides can pass from fruit/grape to must and wine, but the extent to which they pass is different [148, 149]. Organic products, compared to those produced by conventional practices, present some advantages in respect to well-known toxicants, such as pesticides and nitrates [142]. However, caution is needed concerning the problem of pesticide because the number of chemicals to trace is very high [142]. The research, which included the analysis of 25 pesticides in Croatian grape wines, conducted by Vitali Čepo et al. [150], indicated significantly lower total pesticide concentrations and the average number of pesticides per sample of organic grape wines, compared to conventional wines. Pesticide residues not only result in potential health risks for the consumers but also lead to a decrease in the fruit wine quality. Because of the health risk of pesticide residues in juice and fruit wine, it is of particular importance to provide the precise, accurate and reliable result of residues as the scientific basis for ensuring food safety. Determination of pesticides is challenging, because of their chemical diversity and lack of collective analytical methods, which probably explains the fact that the reports regarding the pesticide residues in different fruit wines are still missing. Since the diet is the primary source of exposure of the general population to pesticide residues, the regulatory controls on pesticides by different organisations aim at minimisation of exposure to pesticide residues in food. The great variety of applied pesticides, both within European Union countries (EU) and non-EU countries, as well as the arrival of new plant protectors and chemicals, calls for an ever-expanding list of pesticides along with their accompanying maximum residue limits (MRLs) [151].

3.2. Mycotoxins

Mycotoxins are secondary metabolites of filamentous fungi that naturally occur in food. Mycotoxins can be present in food when fungi are no longer present, which means that the fungal growth is not necessarily associated with the mycotoxins synthesis [152]. Fruits are susceptible to fungal growth, whose development occurs in between harvest and fermentation process. During fermentation, the fungal growth is inhibited by ethanol and the anaerobic conditions [153]. Organic acids present in fruits (malic, citric and tartaric acids) slow down the bacterial spoilage of fruits by lowering the pH. However, the acidic pH of fruit that varies from <2.5 to 5.0 is tolerable for many fungal species [152]. Different practices applied in fruit cultivation (such as the use of pesticides and different cultivars) and in winemaking (period and conditions of storage, type of maceration, time and temperature of fermentation) can have an impact on mycotoxins accumulation [154]. It seems that organic production could be more affected by mycotoxins since no synthetic fungicides are applied [142]. Several mycotoxins have been reported in fruit juices and wine (the list, however, is not exhaustive): *Alternaria* mycotoxins are produced by *A. alternata*, byssochlamic acid produced by *Byssochlamys nivea* in prune, grape and apple juices, citrinin produced by *Penicillium expansum* in apple juice, ochratoxin A, the most crucial mycotoxin in wine, secreted by *Aspergillus carbonarius* present in harvested grapes and juices and finally patulin (PAT), the most critical mycotoxin in fruit juices, produced by several species such as *B. fulva*, *B. nivea* and *P. expansum* [155]. The most critical mycotoxin in grape wine is the ochratoxin A (OTA), which is produced by *A. carbonarius* and is not degraded during winemaking, fermentation process and storage [155]. OTA has been detected in cherries and strawberries and their associated juices. Since the fungus grows on the exterior surfaces of the fruit, the concentration of OTA tends to be higher in wines that are produced by the increased skin contact that is necessary to extract pigments and tannins. OTA concentration in wine is highest after maceration and tends to diminish during the yeast and malolactic fermentations, probably due to adsorption to yeast cells or degradation by lactic acid bacteria [156]. Toxicological studies have determined that OTA may have several effects on health, such as genotoxicity, carcinogenicity, immunosuppressive properties and nephrotoxicity, and for that reason, the EU regulation (EC no. 1881/2006) [157] has set the acceptable limit for OTA in wine to 2 μg/L. Patulin (PAT), which is the most important mycotoxin in fruit juices, has been studied mainly in fruit juices and particularly in apple juices, although the presence of the compound was also described in the brown rot of other fruits [155]. It is produced by the fungi belonging to the genera *Penicillium*, *Aspergillus* and *Byssochlamys,* and its presence in foodstuffs may be a health hazard since this mycotoxin can cause severe acute (convulsions, nausea and ulceration) and chronic (carcinogenic, genotoxic and immunotoxic) effects in humans [158]. The removal of the damaged or rotten fruit could help reduce the levels of PAT in the juices, but the complete elimination of PAT is not possible since PAT diffuses into the healthy parts of the fruit [152]. Stinson et al. [159] reported the 99% reduction of PAT levels as a result of alcoholic fermentation of apple juice. The Codex Alimentarius [160] and the EU Commission Regulation (EC no. 1881/2006) [157] have established limits for patulin maximum level of 50 μg/kg for apple products intended for human consumption. When it comes to winemaking, it seems that the use of sound manufacturing practices (e.g. fruit selection, handling, sorting, storage, culling and washing) could keep the residual mycotoxin levels of the fruit juices and wine under the maximum allowed limits [155].

3.3. Biogenic amines

Fermentation by-products, biogenic amines, are low molecular nitrogen compounds formed mainly by decarboxylation of amino acids or by amination and transamination of aldehydes and ketones. In food and beverages, they are synthesised by the enzymes from raw material or are generated by decarboxylation of amino acids by microorganisms [161, 162]. Biogenic amines are undesirable in all foods and beverages because if absorbed at too high concentrations, they may cause adverse physiological effects in sensitive humans, especially in the presence of alcohol and acetaldehyde. The most studied biogenic amine is histamine [33, 163]. Biogenic amines, particularly histamine, the most frequently found in wines, often reduce the sensory quality of grape wines. Different microorganisms present in wine can synthesise biogenic amines from their respective amino acid precursors, at any stage of wine production, ageing or storage. Therefore, their presence in wines can serve as an indicator of spoilage and authenticity [164, 165]. Biogenic amines associated with grape wine are putrescine, histamine, tyramine and cadaverine, followed by phenylethylamine, spermidine, spermine, agmatine and tryptamine [162]. Lactic acid bacteria present in wine (e.g. *Lactobacillus*, *Leuconostoc* and *Oenococcus* spp.) are mainly responsible for the occurrence of biogenic amines in wine (and in other fermented products) since they produce enzymes that decarboxylate the respective precursor amino acids [166]. To prevent the problem of a high biogenic amine concentration in wines, the length of the processes of maceration and the contact of wine and lees should be reduced to a minimum, because those are the processes that incorporate amino acids to must or wine. This is hard to achieve when age wines are produced. Another way of solving the problem is to inhibit the growth of indigenous lactic acid bacteria and use the selected *O. oeni* strains unable to produce biogenic amine [167]. Biogenic amines present in fruits also contribute to their level in the wine. It has been reported that fruit cultivation conditions and management technique play essential roles in the accumulation of biogenic amines in fruits, for example, the accumulation of putrescine in grapes as a response to a potassium deficiency in soil [168]. While data are available on the concentrations of biogenic amines in grape wines, rare investigations have been conducted to evaluate their concentrations in fruit wines. Ouyang et al. [169] observed a noticeable difference in the profile of biogenic amines in the wines made from different fruits. A high level of the total biogenic amines (28.11–67.48 mg L^{-1}) was detected in red grape wines, followed by the strawberry wine (14.60 mg L^{-1}) and the raspberry wine (8.75 mg L^{-1}). The total content of BA in the white grape wines ranged from 5.42 to 7.21 mg L^{-1}, while the level of total biogenic amines of other fruit wines was below 2.5 mg L^{-1}. When individual biogenic amines were analysed, the results revealed that spermidine was present in most of the wines, while putrescine was present in all the grape wine samples. However, the levels of putrescine in blueberry, raspberry, schisandrae and strawberry wines were higher than that in the grape wines. Blueberry wine and raspberry wine also contained tyramine.

3.4. Sulphur dioxide

The most common preservative in wine production used to control both oxidative processes and unwanted spontaneous fermentations is sulphur dioxide (SO_2) [39]. This is due to its potent antioxidant and antimicrobial properties. However, when present in high concentrations in wine, SO_2 can have unfavourable health effects, for example, diarrhoea, urticaria and abdominal pain [170]. The International Organisation of Vine and Wine (OIV) has been gradually reducing the maximum advisable levels of total SO_2 in wines [143]. The maximum acceptable limit for

total SO_2 in grape wine is set between 150 and 300 mg/L depending on the wine type and the level of reducing substances in wine. One of the challenges for scientists and winemakers who are striving to meet today's consumer demands for SO_2-free high-quality wines is the application of new technologies to replace the use of SO_2 [171]. The alternatives to SO_2 investigated so far include treatments with lysozyme, dimethyl dicarbonate (DMDC), the addition of reduced glutathione (up to 20 mg/L) and the addition of commercial oenological tannins [170, 172–176].

4. Conclusions

The role of fruits in the human diet is well established due to various important nutritive and biologically active components intrinsic to fruits. Consequently, fruit wines tend to preserve these components, along with developing new desirable ones in the final product. Fruit wines proved to be a good source of different antioxidants, phytonutrients and minerals, which classify them with the functional food. The production of fruit wines is growing steadily in recent years, probably driven by the demand for new functional products. However, having in mind the fact that most fruits can be used for fruit wines production, it is evident that they comprise a vast and diverse group of fermented beverages. Compared to grape wines, the comprehensive reviews of specific fruit wines are still scarce, as well as the comparison of different fruit wines regarding their production, composition, nutritional, functional and health-related properties. Hopefully, this chapter will contribute to the body of knowledge, as well as to diversification, standardisation and, consequently, commercialisation of quality fruit wine production.

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References

[1] Hasler C, Bloch A, Thomson C. Position of the American dietetic association: Functional foods. Journal of the American Dietetic Association. 2004;**104**(5):814-826

[2] Global Functional Food and Nutraceuticals Market 2016-2020; March 2016

[3] Basu SK, Thomas JE, Acharya SN, Canada A. Prospects for growth in global nutraceutical and functional food markets: A Canadian perspective. Australian Journal of Basic and Applied Sciences. 2007;**1**(4):637-649

[4] Goldberg DM, Bromberg IL. Health effects of moderate alcohol consumption: A paradigmatic risk factor. Clinica Chimica Acta. 1996;**246**(1-2):1-3. DOI: 10.1016/0009-8981(96)06222-5

[5] Vuorinen H, Määttä K, Törrönen R. Content of the flavonols myricetin, quercetin, and kaempferol in Finnish berry wines. Journal of Agricultural and Food Chemistry. 2000;**48**(7):2675-2680. DOI: 10.1021/jf991388o

[6] Teissedre PL, Landrault N. Wine phenolics: Contribution to dietary intake and bioavailability. Food Research International. 2000;**3**(6):461-467. DOI: 10.1016/S0963-9969(00)00070-3

[7] Ortuño J, Covas MI, Farre M, Pujadas M, Fito M, Khymenets O, et al. Matrix effects on the bioavailability of resveratrol in humans. Food Chemistry. 2010;**120**(4):1123-1130. DOI: 10.1016/j.foodchem.2009.11.032

[8] Motilva MJ, Macià A, Romero MP, Rubió L, Mercader M, González-Ferrero C. Human bioavailability and metabolism of phenolic compounds from red wine enriched with free or nano-encapsulated phenolic extract. Journal of Functional Foods. 2016;**25**:80-93. DOI: 10.1016/j.jff.2016.05.013

[9] Płotka-Wasylka J, Rutkowska M, Cieślik B, Tyburcy A, Namieśnik J. Determination of selected metals in fruit wines by spectroscopic techniques. Journal of Analytical Methods in Chemistry. 2017:1-9. DOI: 10.1155/2017/5283917

[10] Ferreira RB, Monteiro SS, Piçarra-Pereira MA, Teixeira AR. Engineering grapevine for increased resistance to fungal pathogens without compromising wine stability. Trends in Biotechnology. 2004;**22**(4):168-173. DOI: 10.1016/j.tibtech.2004.02.001

[11] Shahidi F. Nutraceuticals and functional foods: Whole versus processed foods. Trends in Food Science and Technology. 2009;**20**(9):376-387. DOI: 10.1016/j.tifs.2008.08.004

[12] Yoo KM, Al-Farsi M, Lee H, Yoon H, Lee CY. Antiproliferative effects of cherry juice and wine in Chinese hamster lung fibroblast cells and their phenolic constituents and antioxidant activities. Food Chemistry. 2010;**123**(3):734-740. DOI: 10.1016/j.foodchem.2010.05.043

[13] Jagtap UB, Waghmare SR, Lokhande VH, Suprasanna P, Bapat VA. Preparation and evaluation of antioxidant capacity of jackfruit (*Artocarpus heterophyllus* lam.) wine and its protective role against radiation induced DNA damage. Industrial Crops and Products. 2011;**34**(3):1595-1601. DOI: 10.1016/j.indcrop.2011.05.025

[14] Kosseva MR, Joshi VK, Panesar, PS. Science and Technology of Fruit Wine Production. Amsterdam: Elsevier Inc., Academic Press; 2017. DOI: 10.1016/B978-0-12-800850-8.01001-2

[15] Rivard D. The Ultimate Fruit Winemaker's Guide: The Complete Reference Manual for all Fruit Winemakers. 2nd ed. Ontario, Canada: Bacchus Enterprises Ltd.; 2009

[16] Rupasinghe HPV, Joshi VK, Smith A, Parmar I. Chemistry of Fruit Wines. In: Kosseva MR, Joshi VK, Panesar PS, editors. Science and Technology of Fruit Wine Production. Amsterdam: Elsevier Inc., Academic Press; 2017. p. 105-176. DOI: 10.1016/B978-0-12-800850-8.01001-2

[17] Joshi VK. Production of wines from non-grape fruit. In: Natural Product Radiance Special Issue, July–August. New Delhi: NISCARE. p. 2009

[18] Joshi VK, Sharma S, Thakur AD. Wines: White, red, sparkling, fortified, and cider. In: Pandey et al., editors. Current Developments in Biotechnology and Bioengineering. Food Beverges Industry, Elsevier B.V.; 2017. pp. 353-406. DOI: 10.1016/B978-0-444-63666-9.00013-3

[19] Siriwoharn T, Wrolstad RE. Polyphenolic composition of Marion and evergreen blackberries. Journal of Food Science. 2004;**69**:C233-C240. DOI: 10.1111/j.1365-2621.2004.tb06322.x

[20] Rupasinghe HPV, Clegg S. Total antioxidant capacity, total phenolic content, mineral elements, and histamine concentrations in wines of different fruit sources. Journal of Food Composition and Analysis. 2007;**20**(2):133-137. DOI: 10.1016/j.jfca.2006.06.008

[21] Szajdek A, Borowska EJ. Bioactive compounds and health-promoting properties of berry fruits: A review. Plant Foods for Human Nutrition. 2008;**63**(4):147-153. DOI: 10.1007/s11130-008-0097-5

[22] McDougall GJ, Ross HA, Ikeji M, Stewart D. Berry extracts exert different antiproliferative effects against cervical and colon cancer cells grown in vitro. Journal of Agricultural and Food Chemistry. 2008;**56**(9):3016-3023. DOI: 10.1021/jf073469n

[23] Jeong JH, Jung H, Lee SR, Lee HJ, Hwang KT, Kim TY. Anti-oxidant, anti-proliferative and anti-inflammatory activities of the extracts from black raspberry fruits and wine. Food Chemistry. 2010;**123**(2):338-344. DOI: 10.1016/j.foodchem.2010.04.040

[24] Bornsek SM, Ziberna L, Polak T, Vanzo A, Ulrih NP, Abram V, et al. Bilberry and blueberry anthocyanins act as powerful intracellular antioxidants in mammalian cells. Food Chemistry. 2012;**134**(4):1878-1884. DOI: 10.1016/j.foodchem.2012.03.092

[25] Manganaris GA, Goulas V, Vicente AR, Terry LA. Berry antioxidants: Small fruits providing large benefits. Journal of the Science of Food and Agriculture. 2014;**94**(5):825-833. DOI: 10.1002/jsfa.6432

[26] De Lourdes Reis-Giada M. Food phenolic compounds: Main classes, sources and their antioxidant power, In: Morales-Gonzalez JA, editor. Oxidative Stress and Chronic Degenerative Diseases - A Role for Antioxidants. London: InTech; 2013. pp. 87-112. DOI: 10.5772/51687

[27] Fredes C, Montenegro G, Zoffoli JP, Santander F, Robert P. Comparison of the total phenolic content, total anthocyanin content and antioxidant activity of polyphenol-rich fruits grown in Chile. Ciencia e Investigación Agrarian. 2014;**41**(1):9-10. DOI: 10.4067/S0718-16202014000100005

[28] Skrovankova S, Sumczynski D, Mlcek J, Jurikova T, Sochor J. Bioactive compounds and antioxidant activity in different types of berries. International Journal of Molecular Sciences. 2015;**16**(10):24673-24706. DOI: 10.3390/ijms161024673

[29] Diaconeasa Z, Leopold L, Rugină D, Ayvaz H, Socaciu C. Antiproliferative and antioxidant properties of anthocyanin rich extracts from blueberry and blackcurrant juice. International Journal of Molecular Sciences. 2015;**16**(2):2352-2365. DOI: 10.3390/ijms16022352

[30] Olas B. The multifunctionality of berries toward blood platelets and the role of berry phenolics in cardiovascular disorders. Platelets. 2017;**28**(6):540-549. DOI: 10.1080/09537104.2016.1235689

[31] Barros RGC, Andrade JKS, Denadai M, Nunes ML, Narain N. Evaluation of bioactive compounds potential and antioxidant activity in some Brazilian exotic fruit residues. Food Research International. 2017;**102**:84-92. DOI: 10.1016/j.foodres.2017.09.082

[32] Gutierrez E, García A, Velasco V, Lucas JA, Mañero FJG, Solano BR. The flavonol-anthocyanin pathway in blackberry and arabidopsis: State of the art. In: Goncalo CJ, editor. Agricultural and Biological Sciences; Flavonoids - From Biosynthesis to Human Health. London: InTech; 2017. pp. 129-150

[33] Jackson RS. Wine Science Principles and Applications. 4th ed. USA: Academic Press; 2014

[34] Amerine MA, Kunkee RE, Ough CS, Singleton VL, Webb AD. Technology of Wine Making. USA, Westport: Connecticut: AVI Publ. Co; 1980. pp. 185-703

[35] du Toit M, Engelbrecht L, Lerm E, Krieger-Weber S. Lactobacillus: The next generation of malolactic fermentation starter cultures-an overview. Food and Bioprocess Technology. 2011;**4**(6):876-906. DOI: 10.1007/s11947-010-0448-8

[36] Nieuwoudt HH, Prior BA, Pretorius IS, Bauer FF. Glycerol in South African table wines: An assessment of its relationship to wine quality. South African Journal of Enology and Viticulture. 2002;**23**(1):22-30. Available from: http://www.sawislibrary.co.za/dbtextimages/NieuwoudtHH1.pdf [Accessed: 2018-02-03]

[37] Petravić-Tominac V, Blagojević K, Novak S, Zechner-Krpan V, Marić V. Fermentative properties of some *Saccharomyces cerevisiae* wine yeasts isolated from Istria region. Periodicum Biologorum. 2005;**107**(1):59-65

[38] Suárez-Lepe JA, Morata A. New trends in yeast selection for winemaking. Trends in Food Science and Technology. 2012;**23**(1):39-50. DOI: 10.1016/j.tifs.2011.08

[39] Ribéreau-Gayon P, Dubourdieu D, Doneche B, Lonvaud A. Handbook of Enology: The Microbiology of Wine and Vinifications. 2nd ed. Vol. 1. England, Chichester, West Sussex: John Wiley & Sons Ltd.; 2006. pp. 193-201

[40] Petravić-Tominac V, Mesihović A, Mujadžić S, Lisičar J, Oros D, Velić D, Velić N, Srečec S, Zechner-Krpan V, Petrović Z. Production of blackberry wine by microfermentation using commercial yeasts Fermol rouge® and Fermol Mediterranée®. Agriculturae Conspectus Scientificus. 2013;**78**(1):49-55

[41] Garai-Ibabe G, Ibarburu I, Berregi I, Claisse O, Lonvaud-Funel A, Irastorza A, et al. Glycerol metabolism and bitterness producing lactic acid bacteria in cidermaking. International Journal of Food Microbiology. 2008;**121**(3):253-261. DOI: 10.1016/j.ijfoodmicro.2007.11.004

[42] Suárez Valles B, Pando Bedriñana R, Lastra Queipo A, Mangas Alonso JJ. Screening of cider yeasts for sparkling cider production (Champenoise method). Food Microbiology. 2008;(5, 5):690-697

[43] Amidžić Klarić D, Klarić I, Velić D, Velić N, Marček T. Evaluation of quercetin content, colour and selected physico-chemical quality parameters of Croatian blackberry wines. Polish Journal of Food and Nutrition Sciences. 2017;**67**(1):75-83. DOI: 10.1515/pjfns-2016-0010

[44] Sun SY, Jiang WG, Zhao YP. Evaluation of different *Saccharomyces cerevisiae* strains on the profile of volatile compounds and polyphenols in cherry wines. Food Chemistry. 2011;**127**(2):547-555. DOI: 10.1016/j.foodchem.2011.01.039

[45] Huerta MD, Salinas MR, Masoud T, Alonso GL. Wine differentiation according to color using conventional parameters and volatile components. Journal of Food Composition and Analysis. 1998;**11**(4):363-374. DOI: 10.1006/jfca.1998.0601

[46] Kelebek H, Selli S, Canbas A, Cabaroglu T. HPLC determination of organic acids, sugars, phenolic compositions and antioxidant capacity of orange juice and orange wine made from a Turkish cv. Kozan. Microchemical Journal. 2009;**91**(2):187-192. DOI: 10.1016/j.microc.2008.10.008

[47] Jackson RS. Wine Tasting. A Professional Handbook. Food Science and Technology. 3rd ed. London: Elsevier-Academic Press; 2017. pp. 78-79

[48] Herrero M, Noriega E, García LA, Díaz M. Influence of a malolactic starter on the quality of the cider produced on an industrial scale. European Food Research and Technology. 2005;**221**(1-2):168-174. DOI: 10.1007/s00217-005-1134-3

[49] Davis CR, Wibowo D, Eschenbruch R, Lee TH, Fleet GH. Practical implications of malolactic fermentation: A review. American Journal of Enology and Viticulture. 1985;**36**(4):290-301

[50] Lambrechts MG, Pretorius IS. Yeast and its importance to wine aroma—A review. South African Journal of Enology and Viticulture. 2000;**21**:97-129. Available from: http://www.sawislibrary.co.za/dbtextimages/LambrechtsMG.pdf [Accessed: 2017-12-21]

[51] Zhu F, Du B, Li J. Aroma Compounds in Wine, Grape and Wine Biotechnology, Morata A, editor. London: InTech; 2016. DOI: 10.5772/65102. Available from: https://mts.intechopen.com/books/grape-and-wine-biotechnology/aroma-compounds-in-wine [Accessed: 2018-3-15]

[52] Mornar A, Amidžić Klarić D, Nigović B. Quality assessment of blackberry wines by HSS-GC-FID. Farmaceutski Glasnik. 2011;**67**:741-746

[53] Amidžić Klarić D, Klarić I, Mornar A, Nigović B. Evaluation of volatile compound and food additive contents in blackberry wine. Food Control. 2015;**50**:714-721. DOI: 10.1016/j.foodcont.2014.10.013

[54] Wang Y, Li P, Singh NK, Shen T, Hu H, Li Z, Zhao Y. Changes in aroma composition of blackberry wine during fermentation process. African Journal of Biotechnology. 2012;**11**(99):16504-16511

[55] Niu Y, Zhang X, Xiao Z, Song S, Eric K, Jia C, Yu H, Zhu J. Characterization of odor-active compounds of various cherry wines by gas chromatography–mass spectrometry, gas chromatography–olfactometry and their correlation with sensory attributes. Journal of Chromatography B. 2011;**879**(23):2287-2293. DOI: 10.1016/j.jchromb.2011.06.015

[56] Xiao Z, Liu S, Gu Y, Xu N, Shang Y, Zhu J. Discrimination of cherry wines based on their sensory properties and aromatic fingerprinting using HS-SPME-GC-MS and multivariate analysis. Journal of Food Science. 2014;**79**(3):C284-C294. DOI: 10.1111/1750-3841.12362

[57] Xiao ZB, Zhang N, Niu YW, Feng T, Tian HX, Zhu JC, Yu HY. Multivariate classification of cherry wines based on headspace solid phase microextraction and gas chromatography-mass spectrometry of volatile compounds. International Journal of Food Properties. 2015;**18**(6):1272-1287. DOI: 10.1080/10942912.2012.710286

[58] Xiao Q, Zhou X, Xiao Z, Niu Y. Characterization of the differences in the aroma of cherry wines from different price segments using gas chromatography–mass spectrometry, odor activity values, sensory analysis, and aroma reconstitution. Food Science and Biotechnology. 2017;**26**(2):331-338. DOI: 10.1007/s10068-017-0045-y

[59] Xiao Z, Zhou X, Niu Y, Zhao F. The analysis of the characteristic aroma components of cherry wine by gas chromatography-olfactometry (GC-O) and odor activity values (OAV). Journal of Chinese Institute of Food Science and Technology. 2017;**17**:246-254. DOI: 10.16429/j.1009-7848.2017.08.033

[60] Wang L, Xu Y, Zhao G, Li J. Rapid analysis of flavor volatiles in apple wine using headspace solid-phase microextraction. Journal of Institute of Brewing. 2004;**110**(1):57-65. DOI: 10.1002/j.2050-0416.2004.tb00181.x

[61] Peng B, Li F, Cui L, Guo Y. Effects of fermentation temperature on key aroma compounds and sensory properties of apple wine. Journal of Food Science. 2015;**80**(12):S2937-S2943. DOI: 10.1111/1750-3841.13111

[62] Satora P, Sroka P, Duda-Chodak A, Tarko T, Tuszyński T. The profile of volatile compounds and polyphenols in wines produced from dessert varieties of apples. Food Chemistry. 2008;**111**(2):513-519. DOI: 10.1016/j.foodchem.2008.04.007

[63] Ye M, Gao Z, Li Z, Yuan Y, Yue T. Rapid detection of volatile compounds in apple wines using FT-NIR spectroscopy. Food Chemistry. 2016;**190**(1):701-708. DOI: 10.1016/j.foodchem.2015.05.112

[64] Butkhup L, Jeenphakdee M, Jorjong S, Samappito S, Samappito W, Chowtivannakul S. HS-SPME-GC-MS analysis of volatile aromatic compounds in alcohol related beverages made with mulberry fruits. Food Science and Biotechnology. 2011;**20**(4):1021-1032. DOI: 10.1007/s10068-011-0140-4

[65] Feng Y, Liu M, Ouyang Y, Zhao X, Ju Y, Fang Y. Comparative study of aromatic compounds in fruit wines from raspberry, strawberry, and mulberry in central Shaanxi area. Food and Nutrition Research. 2015;**59**(1):29290. DOI: 10.3402/fnr.v59.29290

[66] Song Y, Y-jie Z, Liu N, Ye D-q, Gong X, Qin Y, Liu Y. Volatile compounds in wild strawberry and their odorants of wild strawberry wines: Effects of different stages of fermentation. International Journal of Food Properties. 2017;**20**(1):S399-S415. DOI: 10.1080/10942912.2017.1297951

[67] Robinson AL, Boss PK, Solomon PS, Trengove RD, Heymann H, Ebeler SE. Origins of grape and wine aroma. Part 1. Chemical components and viticultural impacts. American Journal of Enology and Viticulture. 2014;**56**:1-24. DOI: 10.5344/ajev.2013.12070

[68] Bartowsky EJ, Pretorius IS. Microbial formation and modification of flavour and off-flavour compounds in wine. In: König H, Unden G, Fröhlich J, editors. Biology of 238 Grapes and Wines—Advances in Production, Processing, Analysis and Valorization Microorganisms on Grapes, in Must and Wine. Heidelberg: Springer; 2008. pp. 211-233. DOI: 10.1007/978-3-540-85463-0

[69] Kalkan Yildirim H. Evaluation of colour parameters and antioxidant activities of fruit wines. International Journal of Food Sciences and Nutrition. 2006;**57**(1-2):47-63. DOI: 10.1080/09637480600655993

[70] Amidžić Klarić D, Klarić I, Mornar A, Klarić I, Mornar A. Polyphenol content and antioxidant activity of commercial blackberry wines from Croatia: Application of multivariate analysis for geographic origin differentiation. Journal of Food and Nutrition Research. 2011;**50**(4):199-209

[71] Heinonen IM, Lehtonen PJ, Hopia AI. Antioxidant activity of berry and fruit wines and liquors. Journal of Agricultural and Food Chemistry. 1998;**46**(1):25-31. DOI: 10.1021/jf970489o

[72] Cliff MA, King MC, Schlosser J. Anthocyanin, phenolic composition, colour measurement and sensory analysis of BC commercial red wines. Food Research International. 2007;**40**(1):92-100. DOI: 10.1016/j.foodres.2006.08.002

[73] Mudnić I, Budimir D, Modun D, Gunjača G, Generalić I, Skroza D, et al. Antioxidant and vasodilatory effects of blackberry and grape wines. Journal of Medicinal Food. 2012;**15**(3):315-321. DOI: 10.1089/jmf.2011.0129

[74] Ljevar A, Ćurko N, Tomašević M, Radošević K, Srček VG, Ganić KK. Phenolic composition, antioxidant capacity and in vitro cytotoxicity assessment of fruit wines. Food Technology and Biotechnology. 2016;**54**(2):145-155. DOI: 10.17113/ftb.54.02.16.4208

[75] Zoecklein B, Fugelsang K, Gump B, Nury F. Wine Analysis and Production. New York: Chapman & Hall; 1995

[76] Czyzowska A, Pogorzelski E. Changes to polyphenols in the process of production of must and wines from blackcurrants and cherries. Part I. Total polyphenols and phenolic acids. European Food Research and Technology. 2002;**214**:148-154. DOI: 10.1007/s00217-001-0422-9

[77] Ortiz J, Marín-Arroyo MR, Noriega-Domínguez MJ, Navarro M, Arozarena I. Color, phenolics, and antioxidant activity of blackberry (*Rubus glaucus* Benth.), blueberry (*Vaccinium floribundum* Kunth.), and apple wines from Ecuador. Journal of Food Science. 2013;**78**(7):985-993. DOI: 10.1111/1750-3841.12148

[78] Pantelić M, Dabić D, Matijašević S, Davidović S, Dojčinović B, Milojković-Opsenica D, Tešić Ž, Natić M. Chemical characterization of fruit wine made from Oblačinska sour cherry. The Scientific World Journal. 2014;**2014**;9. Article ID: 454797. DOI: 10.1155/2014/454797. https://www.hindawi.com/journals/tswj/2014/454797/

[79] Johnson MH, Mejia EG. Comparison of chemical composition and antioxidant capacity of commercially available blueberry and blackberry wines in Illinois. Journal of Food Science. 2012;**71**:C141-C148

[80] De Dias FS, David JM, David JP. Determination of phenolic acids and quercetin in Brazilian red wines from vale do São Francisco region using liquid-liquid ultrasound-assisted extraction and HPLC-DAD-MS. Journal of the Brazilian Chemical Society. 2016;**27**(6):1055-1059. DOI: 10.5935/0103-5053.20150363

[81] Minuti L, Pellegrino R. Determination of phenolic compounds in wines by novel matrix solid-phase dispersion extraction and gas chromatography/mass spectrometry. Journal of Chromatography A. 2008;**1185**(1):23-30. DOI: 10.1016/j.chroma.2008.01.039

[82] Nikfardjam MSP, Márk L, Avar P, Figler M, Ohmacht R. Polyphenols, anthocyanins, and trans-resveratrol in red wines from the Hungarian Villány region. Food Chemistry. 2006;**98**(3):453-462. DOI: 10.1016/j.foodchem.2005.06.014

[83] Sladkovský R, Solich P, Urbánek M. High-performance liquid chromatography determination of phenolic components in wine using off-line isotachophoretic pretreatment. Journal of Chromatography A. 2004;**1040**(2):179-184. DOI: 10.1016/j.chroma.2004.04.009

[84] Czyzowska A, Pogorzelski E. Changes to polyphenols in the process of production of must and wines from blackcurrants and cherries. Part II. Anthocyanins and flavanols. European Food Research and Technology. 2004;**218**(4):355-359. DOI: 10.1007/s00217-003-0857-2

[85] Fernández-Pachón MS, Villaño D, Troncoso AM, García-Parrilla MC. Determination of the phenolic composition of sherry and table white wines by liquid chromatography and their relation with antioxidant activity. Analytica Chimica Acta. 2006;**563**(1-2):101-108. DOI: 10.1016/j.aca.2005.09.057

[86] Gambelli L, Santaroni GP. Polyphenols content in some Italian red wines of different geographical origins. Journal of Food Composition and Analysis. 2004;**17**(5):613-618. DOI: 10.1016/j.jfca.2003.09.010

[87] Dey G, Negi B, Gandhi A. Can fruit wines be considered as functional food? —An overview. Indian Journal of Natural Products and Resources. 2009;**8**(4):314-322

[88] Negi B, Dey G. Comparative analysis of total phenolic content in sea buckthorn wine and other selected fruit wines. World Academy of Science, Engineering and Technology. 2009;**54**(6):99-102

[89] Klopotek Y, Otto K, Böhm V. Processing strawberries to different products alters contents of vitamin C, total phenolics, total anthocyanins, and antioxidant capacity. Journal of Agricultural and Food Chemistry. 2005;**53**(14):5640-5646. DOI: 10.1021/jf047947v

[90] Peña-Sanhueza D, Inostroza-Blancheteau C, Ribera-Fonseca A, Reyes-Díaz M. Anthocyanins in berries and their potential use in human health. In: Shiomi N, Waisundara V, editors. Superfood and Functional Food¨CThe Development of Superfoods and their Roles as Medicine. Chapter 8. London: InTech; 2017. pp. 155-172. DOI: 10.5772/67104

[91] Amrani Joutei K, Glories Y. Tannins and anthocyanins of grape berries: Localization and extraction technique. Revue Francaise d'Oenologie. 1995;**153**:28-31

[92] Joshi VK, Bhutani VP, Thakur NK. Composition and nutrition of fermented products. In: Joshi VK, Pandey A, editors. Biotechnology: Food Fermentation, Vol. I. New Delhi: Educational Publishers and Distributors; 1999. pp. 259-320

[93] Rommel A, Wrolstad RE, Heatherbell DA. Blackberry juice and wine: Processing and storage effects on anthocyanin composition, color and appearance. Journal of Food Science. 1992;**57**(2):385-391. DOI: 10.1111/j.1365-2621.1992.tb05500.x

[94] Ferretti G, Bacchetti T, Belleggia A, Neri D. Cherry antioxidants: From farm to table. Molecules. 2010;**15**(10):6993-7005. DOI: 10.3390/molecules15106993

[95] Jakobek L, Šeruga M, Medvidović-Kosanović M, Novak I. Anthocyanin content and antioxidant activity of various red fruit juices. Deutsche Lebensmittel Rundschau. 2007; **103**(2):58-64

[96] Mudnić I, Modun D, Rastija V, Vuković J, Brizić I, Katalinić V, Kozina B, Medić-Šaric M, Boban M. Antioxidative and vasodilatory effects of phenolic acids in wine. Food Chemistry. 2010;**119**(3):1205-1210. DOI: 10.1016/j.foodchem.2009.08.038

[97] Letaief H, Zemni H, Mliki A, Chebil S. Composition of *Citrus sinensis* (L.) Osbeck cv "Maltaise demi-sanguine" juice. A comparison between organic and conventional farming. Food Chemistry. 2016;**194**:290-295. DOI: 10.1016/j.foodchem.2015.08.025

[98] Carbonaro M, Mattera M. Polyphenoloxidase activity and polyphenol levels in organically and conventionally grown peach (*Prunus persica* L., cv. Regina bianca) and pear (*Pyrus communis* L., cv. Williams). Food Chemistry. 2001;**72**(4):419-424. DOI: 10.1016/S0308-8146(00)00248-X

[99] Faller ALK, Fialho E. The antioxidant capacity and polyphenol content of organic and conventional retail vegetables after domestic cooking. Food Research International. 2009;**42**(1):210-215. DOI: 10.1016/j.foodres.2008.10.009

[100] Popa M, Mitelut A, Popa E, Stan A, Popa V. Organic foods contribution to nutritional quality and value. Trends in Food Science & Technology. DOI: 10.1016/j.tifs.2018.01.003

[101] Manach C, Scalbert A, Morand C, Remesy C, Jimenez L. Polyphenols: Food sources and bioavailability. The American Journal of Clinical Nutrition. 2004;**79**:727-747

[102] Manach C, Williamson G, Morand C, Scalbert A, Rémésy C. Bioavailability and bioefficacy of polyphenols in humans. I. Review of 97 bioavailability studies. The American Journal of Clinical Nutrition. 2005;**81**:230S-242S

[103] Fulcrand H, Dueñas M, Salas E, Cheynier V. Phenolic reactions during winemaking and aging. American Journal of Enology and Viticulture. 2006;**57**(3):289-297

[104] Raposo R, Chinnici F, Ruiz-Moreno MJ, Puertas B, Cuevas FJ, Carbú M, et al. Sulfur free red wines through the use of grapevine shoots: Impact on the wine quality. Food Chemistry. 2018;**243**:453-460. DOI: 10.1016/j.foodchem.2017.09.111

[105] Gironés-Vilaplana A, Villanõ D, Marhuenda J, Moreno DA, Garciá-Viguera C. Vitamins. In: Galanakis C, editor Nutraceutical and Functional Food Components: Effects of Innovative Processing Techniques. 1st ed. Amsterdam: Academic Press; 2017. pp. 159-201. DOI: 10.1016/B978-0-12-805257-0.00006-5

[106] Moreno P, Salvadó V. Determination of eight water- and fat-soluble vitamins in multivitamin pharmaceutical formulations by high-performance liquid chromatography. Journal of Chromatography A. 2000;**870**(1-2):207-215. DOI: 10.1016/S0021-9673(99)01021-3

[107] Fuller NJ, Lee SH, Buglass AJ. Nutritional and health aspects-residues in alcoholic beverages. In: Buglass AJ, editor. Handbook of Alcoholic Beverages: Technical, Analytical and Nutritional Aspects. West Sussex, UK: John Wiley & Sons, Ltd.; 2011. pp. 933-1110. DOI: 10.1002/9780470976524

[108] Mattivi F, Monetti A, Vrhovšek U, Tonon D, Andrés-Lacueva C. High-performance liquid chromatographic determination of the riboflavin concentration in white wines for predicting their resistance to light. Journal of Chromatography A. 2000;**888**(1-2): 121-127. DOI: 10.1016/S0021-9673(00)00561-6

[109] McDowell LR, Vitamins in Animal and Human Nutrition. 2nd ed. Ames: Iowa State University Press; 2000. pp. 659-674. DOI: 10.1002/9780470376911

[110] Beattie J, Crozier A, Duthie G. Potential health benefits of berries. Current Nutrition & Food Science. 2005;**1**(1):71-86. DOI: 10.2174/1573401052953294

[111] Amidžić Klarić D. The influence of organically grown fruit on the nutritional and biologically active components of blackberry wine [Thesis]. Zagreb: Faculty of Pharmacy and Biochemistry, University of Zagreb; 2011

[112] Lee SK, Kader AA. Preharvest and postharvest factors influencing vitamin C content of horticultural crops. Postharvest Biology and Technology. 2000;**20**(3):207-220. DOI: 10.1016/S0925-5214(00)00133-2

[113] Fang T, Zhen Q, Liao L, Owiti A, Zhao L, Korban SS, Han Y. Variation of ascorbic acid concentration in fruits of cultivated and wild apples. Food Chemistry. 2017;**225**:132-137. DOI: 10.1016/j.foodchem.2017.01.014

[114] Kafkas E, Koşar M, Türemiş N, Başer KHC. Analysis of sugars, organic acids and vitamin C contents of blackberry genotypes from Turkey. Food Chemistry. 2006;**97**(4):732-736. DOI: 10.1016/j.foodchem.2005.09.023

[115] Bonerz D, Würth K, Dietrich H, Will F. Analytical characterization and the impact of ageing on anthocyanin composition and degradation in juices from five sour cherry cultivars. European Food Research and Technology. 2007;**224**(3):355-364. DOI: 10.1007/s00217-006-0328-7

[116] Planchon V, Lateur M, Dupont P, Lognay G. Ascorbic acid level of Belgian apple genetic resources. Scientia Horticulturae. 2004;**100**(1-4):51-61. DOI: 10.1016/j.scienta.2003.08.003

[117] Olsson ME, Andersson CS, Oredsson S, Berglund RH, Gustavsson KE. Antioxidant levels and inhibition of cancer cell proliferation in vitro by extracts from organically and conventionally cultivated strawberries. Journal of Agricultural and Food Chemistry. 2006;**54**(4):1248-1255. DOI: 10.1021/jf0524776

[118] You Q, Wang B, Chen F, Huang Z, Wang X, Luo PG. Comparison of anthocyanins and phenolics in organically and conventionally grown blueberries in selected cultivars. Food Chemistry. 2011;**125**:201-208. DOI: 10.1016/j.foodchem.2010.08.063

[119] Duarte WF, Dias DR, Oliveira JM, Vilanova M, Teixeira JA, e Silva JBA, Schwan RF. Raspberry (*Rubus idaeus* L.) wine: Yeast selection, sensory evaluation and instrumental analysis of volatile and other compounds. Food Research International. 2010;**43**(9):2303-2314. DOI: 10.1016/j.foodres.2010.08.003

[120] Ginjom I, D'Arcy B, Caffin N, Gidley M. Phenolic compound profiles in selected Queensland red wines at all stages of the wine-making process. Food Chemistry. 2011; **125**(3):823-834. DOI: 10.1016/j.foodchem.2010.08.062

[121] Sánchez-Moreno C, Cao G, Ou B, Prior RL. Anthocyanin and proanthocyanidin content in selected white and red wines. Oxygen radical absorbance capacity comparison with nontraditional wines obtained from highbush blueberry. Journal of Agricultural and Food Chemistry. 2003;**51**(17):4889-4896. DOI: 10.1021/jf030081t

[122] Pinhero RG, Paliyath G. Antioxidant and calmodulin-inhibitory activities of phenolic components in fruit wines and its biotechnological implications. Food Biotechnology. 2001;**15**(3):179-192. DOI: 10.1081/FBT-100107629

[123] Fukai T, Ushio-Fukai M. Superoxide dismutases: Role in redox signaling, vascular function, and diseases. Antioxidants & Redox Signaling. 2011;**15**(6):1583-1606. DOI: 10.1089/ars.2011.3999

[124] Brody T. Nutritional Biochemistry. San Diego: Academic Press; 1994. pp. 693-878

[125] Reilly C. The Nutritional Trace Metals. Oxford: Blackwell Publishing; 2004. pp. 35-81

[126] Belitz HD, Grosch W, Schieberle P. Food Chemistry. 4th revised and extended ed. Berlin, Germany: Springer; 2009. pp. 498-520

[127] Ibanez JG, Carreon-Alvarez A, Barcena-Soto M, Casillas N. Metals in alcoholic beverages: A review of sources, effects, concentrations, removal, speciation, and analysis. Journal of Food Composition and Analysis. 2008;**21**(8):672-683. DOI: 10.1016/j.jfca.2008.06.005

[128] Kment P, Mihaljevič M, Ettler V, Šebek O, Strnad L, Rohlov L. Differentiation of Czech wines using multielement composition—A comparison with vineyard soil. Food Chemistry. 2005;**91**(1):157-165. DOI: 10.1016/j.foodchem.2004.06.010

[129] Pohl P. What do metals tell us about wine? Trends in Analytical Chemistry. 2007;**26**:41-949. DOI: 10.1016/j.trac.2007.07.005

[130] Lara R, Cerutti S, Salonia JA, Olsina RA, Martinez LD. Trace element determination of argentine wines using ETAAS and USN-ICP-OES. Food and Chemical Toxicology. 2005;**43**(2):293-297. DOI: 10.1016/j.fct.2004.10.004

[131] Sauvage L, Frank D, Stearne J, Millikan MB. Trace metal studies of selected white wines: An alternative approach. Analytica Chimica Acta. 2002;**458**(1):223-230. DOI: 10.1016/S0003-2670(01)01607-5

[132] Díaz C, Conde JE, Estévez D, Olivero SJP, Trujillo JPP. Application of multivariate analysis and artificial neural networks for the differentiation of red wines from the canary. Agriculture and Food Chemistry. 2003;**51**:4303-4307. DOI: 10.1021/jf0343581

[133] Gennaro MC, Mentasti E, Sarzanini C, Pesticcio A. Undesirable and harmful metals in wines—Determination and removal. Food Chemistry. 1986;**19**(2):93-104. DOI: 10.1016/0308-8146(86)90103-2

[134] Amidžić Klarić D, Klarić I, Mornar A, Velić D, Velić N. Blackberry wines mineral and heavy metal content determination after dry ashing: Multivariate data analysis as a tool for fruit wine quality control. International Journal of Food Sciences and Nutrition. 2016;**67**(5):514-523. DOI: 10.1080/09637486.2016.1181159

[135] Taylor VF, Longerich HP, Greenough JD. Multielement analysis of Canadian wines by inductively coupled plasma mass spectrometry (ICP-MS) and multivariate statistics. Journal of Agricultural and Food Chemistry. 2003;**51**:856-860. DOI: 10.1021/jf025761v

[136] Alpeza I, Varga T, Kubanović V. Composition and content of selected elements of Croatian blackberry wines. Journal of Food, Agriculture and Environment. 2014;**12**(3-4):100-103

[137] Marengo E, Aceto M. Statistical investigation of the differences in the distribution of metals in Nebbiolo-based wines. Food Chemistry. 2003;**81**(4):621-630. DOI: 10.1016/S0308-8146(02)00564-2

[138] Amidžić Klarić D, Klarić I, Velić D, Vedrina Dragojević I. Evaluation of mineral and heavy metal content in Croatian blackberry wines. Czech Journal of Food Sciences. 2011;**29**:260-267

[139] Thiel G, Geisler G, Blechschmidt I, Danzer K. Determination of trace elements in wines and classification according to their provenance. Analytical and Bioanalytical Chemistry. 2004;**378**(6):1630-1636. DOI: 10.1007/s00216-003-2444-6

[140] Moreno IM, González-Weller D, Gutierrez V, Marino M, Cameán AM, González AG, Hardisson A. Differentiation of two canary DO red wines according to their metal content from inductively coupled plasma optical emission spectrometry and graphite furnace atomic absorption spectrometry by using probabilistic neural networks. Talanta. 2007;**72**(1):263-268. DOI: 10.1016/j.talanta.2006.10.029

[141] Galani-Nikolakaki S, Kallithrakas-Kontos N, Katsanos AA. Trace element analysis of Cretan wines and wine products. Science of the Total Environment. 2002;**285**(1-3):155-163. DOI: 10.1016/S0048-9697(01)00912-3

[142] Pussemier L, Larondelle Y, Van Peteghem C, Huyghebaert A. Chemical safety of conventionally and organically produced foodstuffs: A tentative comparison under Belgian conditions. Food Control. 2006;**17**(1):14-21. DOI: 10.1016/j.foodcont.2004.08.003

[143] Organisation Internationale de la Vigne et du Vin (O.I.V.) Compendium of International Méthods of Wine and Must Analysis. Vol. 1, Paris: OIV; 2016. http://www.oiv.int/public/medias/2624/compendium-2016-en-vol1.pdf

[144] Monaci F, Bargagli R, Focardi S. Element concentrations in chianti classico appellation wines. Journal of Trace Elements in Medicine and Biology. 2003;**17**:45-50

[145] Albero B, Sanchez-Brunete C, Tadeo JL. Multiresidue determination of pesticides in juice by solid-phase extraction and gas chromatography–mass spectrometry. Talanta. 2005;**66**(4):917-924. DOI: 10.1016/j.talanta.2004.12.046

[146] Lazić S, Komlen V, Šunjka D, Grahovac N, Pejčić J, Rahmić A, Blesić M. Determination of Fungicide Residues in Grape by GC/MS. In: Proceedings of the International Symposium: Current Trends in Plant Protection. 25-28 September 2012; Belgrade; 2012. pp. 401-409

[147] Jin B, Xie L, Guo Y, Pang G. Multi-residue detection of pesticides in juice and fruit wine: A review of extraction and detection methods. Food Research International. 2012;**46**(1):399-409. DOI: 10.1016/j.foodres.2011.12.003

[148] Cabras P, Angioni A. Pesticide residues in grapes, wine, and their processing products. Journal of Agricultural and Food Chemistry. 2000;**48**(4):967-973. DOI: 10.1021/jf990727a

[149] Navarro S, Barba A, Navarro G, Vela N, Oliva J. Multiresidue method for the rapid determination—In grape, must and wine—Of fungicides frequently used on vineyards. Journal of Chromatography A. 2000;**882**:221-229. DOI: 10.1016/S0021-9673(00)00337-X

[150] Vitali Čepo D, Pelajić M, Vinković Vrček I, Krivohlavek A, Žuntar I, Karoglan M. Differences in the levels of pesticides, metals, sulphites and ochratoxin A between organically and conventionally produced wines. Food Chemistry. 2018;**246**:394-403. DOI: 10.1016/j.foodchem.2017.10.133

[151] Kmellár B, Pareja L, Ferrer C, Fodor P, Fernández-Alba AR. Study of the effects of operational parameters on multiresidue pesticide analysis by LC-MS/MS. Talanta. 2011; **84**(2):262-273. DOI: 10.1016/j.talanta.2010.12.006

[152] Fernández-Cruz ML, Mansilla ML, Tadeo JL. Mycotoxins in fruits and their processed products: Analysis, occurrence and health implications. Journal of Advanced Research. 2010;**1**(2):113-122. DOI: 10.1016/j.jare.2010.03.002

[153] Otteneder H, Majerus P. Occurrence of ochratoxin A (OTA) in wines: Influence of the type of wine and its geographical origin. Food Additives and Contaminants. 2000;**17**(9): 793-798. DOI: 10.1080/026520300415345

[154] Zimmerli B, Dick R. Ochratoxin A in table wine and grape-juice: Occurrence and risk assessment. Food Additives and Contaminants. 1996;**13**(6):655-668. DOI: 10.1080/02652039609374451

[155] Delage N, d'Harlingue A, Colonna Ceccaldi B, Bompeix G. Occurrence of mycotoxins in fruit juices and wine. Food Control. 2003;**14**(4):225-227. DOI: 10.1016/S0956-7135(03)00010-0

[156] Fiori S, Urgeghe PP, Hammami W, Razzu S, Jaoua S, Migheli Q. Biocontrol activity of four non- and low-fermenting yeast strains against *Aspergillus carbonarius* and their ability to remove ochratoxin A from grape juice. International Journal of Food Microbiology. 2014;**189**:45-50. DOI: 10.1016/j.ijfoodmicro.2014.07.020

[157] European Commission, Commission regulation no. 1881/2006, no. L364/5. Official Journal of the European Union. 2006;**49**:5-24

[158] Moake MM, Padilla-Zakour OI, Worobo RW. Comprehensive review of patulin control methods in foods. Comprehensive Reviews in Food Science and Food Safety. 2005;**4**(1):8-21. DOI: 10.1111/j.1541-4337.2005.tb00068.x

[159] Stinson EE, Osman SF, Huhtanen CN, Billis DD. Disappearance of patulin during alcoholic fermentation of apple juice. Applied and Environmental Microbiology. 1978;**36**(4):620-622

[160] Codex Committee on Food Additives and Contaminants. Maximum level for patulin in apple juice and apple juice ingredients and other beverages. Codex Alimentarius. 2003;**235**:1

[161] Moreno-Arribas MV, Smit AY, du Toit M. Biogenic ammines and the winemaking process. In: Reynols AG, editor. Managing Wine Quality. Cambridge: Woodhead Publishing Limited; 2010. pp. 494-522. DOI: 10.1016/B978-1-84569-798-3.50017-6

[162] Smit AY, du Toit WJ, du Toit M. Biogenic amines in wine: Understanding the headache. South African Journal of Enology and Viticulture. 2008;**29**:109-127. DOI: 10.21548/29-2-1444

[163] Hernández-Orte P, Lapeña AC, Peña-Gallego A, Astrain J, Baron C, Pardo I, Polo L, Ferrer S, Cacho J, Ferreira V. Biogenic amine determination in wine fermented in oak barrels: Factors affecting formation. Food Research International. 2008;**1**(7):697-706. DOI: 10.1016/j.foodres.2008.05.002

[164] Kovačević Ganić K, Gracin L, Komes D, Ćurko N, Lovrić T. Changes of the content of biogenic amines during winemaking of Sauvignon wines. Croatian Journal of Food Science and Technology. 2009;**1**(2):21-27. https://hrcak.srce.hr/file/81239

[165] Košmerl T, Šućur S, Prosen H. Biogenic amines in red wine: The impact of technological processing of grape and wine. Acta Agriculturae Slovenica. 2013;**101**(2):249-261. DOI: 10.2478/acas-2013-0021

[166] Halasz A, Barath A, Simon-Sarkadi L, Holzapfel W. Biogenic amines and their production by microorganisms in food. Trends in Food Science & Technology. 1994;**5**:42-49. DOI: 10.1016/0924-2244(94)90070-1

[167] Landete JM, Ferrer S, Pardo I. Biogenic amine production by lactic acid bacteria, acetic bacteria and yeast isolated from wine. Food Control. 2007;**18**(12):1569-1574. DOI: 10.1016/j.foodcont.2006.12.008

[168] Bover-Cid S, Iquierdo-Pulido M, Mariné-Font A, Vidal-Carou MC. Biogenic mono-, di- and polyamine contents in Spanish wines and influence of a limited irrigation. Food Chemistry. 2006;**96**(1):43-47. DOI: 10.1016/j.foodchem.2005.01.054

[169] Ouyang X, Wang S, Yuan G, Liu Y, Gu P, Zhang B, Zhu B. Comparison of amino acids, biogenic amines and ammonium ion of wines made of different types of fruits. International Journal of Food Science & Technology. 2017;**52**(2):448-456. DOI: 10.1111/ijfs.13300

[170] Ferrer-Gallego R, Puxeu M, Nart E, Martín L, Andorrà I. Evaluation of Tempranillo and Albariño SO_2-free wines produced by different chemical alternatives and winemaking procedures. Food Research International. 2017;**102**:647-657. DOI: 10.1016/j.foodres.2017.09.046

[171] Costanigro M, Appleby C, Menke SD. The wine headache: Consumer perceptions of sulfites and willingness to pay for non-sulfited wines. Food Quality and Preference. 2014;**31**(1):81-89. DOI: 10.1016/j.foodqual.2013.08.002

[172] Abramovič H, Košmerl T, Poklar Ulrih N, Cigić B. Contribution of SO_2 to antioxidant potential of white wine. Food Chemistry. 2015;**174**:147-153. DOI: 10.1016/j.foodchem.2014.11.030

[173] Nagy B, Soós J, Horvath M, Nyúl-Pühra B, Nyitrai-Sárdy D. The effect of fine lees as a reducing agent in Sur lie wines, aged with various sulphur dioxide concentrations. Acta Alimentaria. 2017;**46**:109-115. DOI: 10.1556/066.2017.46.1.14

[174] Lužar J, Košmerl T, Jamnik P, Korošec M. Wine ageing as alternative to addition SO_2. In: Trends and Challenges in Food Technology, Nutrition, Hospitality, Tourism, Education and Training: Collection Proceedings of the 4th International Professional Conference; Biotechnical Educational Center; Ljubljana, Slovenia. 2017. pp. 71-78

[175] Santos MC, Nunes C, Saraiva JA, Coimbra MA. Chemical and physical methodologies for the replacement/reduction of sulfur dioxide use during winemaking: Review of their potentialities and limitations. European Food Research and Technology. 2012;**234**(1):1-12. DOI: 10.1007/s00217-011-1614-6

[176] Sonni F, Chinnici F, Natali N, Riponi C. Pre-fermentative replacement of sulphur dioxide by lysozyme and oenological tannins: Effect on the formation and evolution of volatile compounds during the bottle storage of white wines. Food Chemistry. 2011;**129**:1193-1200. DOI: 10.1016/j.foodchem.2011.05.104

Chapter 8

Quality of Reduced-Fat Dairy Coffee Creamer: Affected by Different Fat Replacer and Drying Methods

Simin Hedayatnia and Hamed Mirhosseini

Abstract

This work aims to investigate the effects of inulin (0, 2.5, 5 and 7.5%, w/w) and maltodextrin (0, 15, 20 and 25%, w/w) as wall materials and fat replacers and drying techniques (i.e. spray drying and fluidized-bed drying) on physicochemical properties of regular and instant reduced-fat dairy creamers. The regular reduced-fat dairy creamer was produced by one-stage drying (i.e. spray drying), while the instant reduced-fat dairy creamer was produced by two-stage drying (i.e. spray drying followed by fluidized-bed drying). In this study, control (0% inulin and 0% maltodextrin) and two commercial regular and instant coffee creamers (A and B) were also considered for comparison purposes. The results showed that the regular creamer containing 25% maltodextrin and 7.5% inulin had the largest particle size, highest viscosity and most desirable wettability among all formulated regular creamers. The yield of reduced-fat coffee creamer was significantly increased from 43.55 to 94.60% by increasing the amount of fat replacers to the maximum level (25% maltodextrin and 7.5% inulin). The current study revealed that the application of fluidized-bed drying for agglomeration led to significantly improve the wettability and instant properties of the instant creamer. In this study, the formulated instant creamer containing 25% maltodextrin and 7.5% inulin was the most desirable product as compared to all creamers.

Keywords: reduced-fat dairy creamer, inulin, maltodextrin, spray drying, fluidized-bed drying

1. Introduction

Coffee is one of the most vastly consumed beverages. Coffee drink is usually consumed in black or white form, depending on the taste of the consumer. Coffee creamers, also known as "coffee whitener" or "coffee sweetener", are liquid or granular substances intended to substitute for

milk or cream as an additive to coffee or other beverages [18, 28]. As stated by Tuot et al. [51], a desired coffee creamer should have specific physicochemical and functional characteristics particularly in terms of solubility, viscosity and stability. In addition, it should provide a good whitening effect after adding to hot coffee or similar hot beverages [41]. During the past few years, the demand of consumers for healthier products has significantly increased, lowering the tendency for consumption of high-fat foods. Hence, one of the main health issues for coffee drinkers is the presence of high percentage of fat in creamer formulation. As reported by Lambert [33], coffee creamer mainly contains high amount of vegetable fat (37–51%) and corn syrup (41–46%). Sudha et al. [47] suggested that the replacement of fat with various fat replacers led to the reduction of fat content and calories in food products.

There are some difficulties for the drying of coffee creamer due to the low glass transition temperatures (T_g) of the components such as high sugar and caseinate that leads to stickiness problems. In order to prevent stickiness and caking issue during storage, specific drying aids or wall materials (such as gum arabic, xanthan gum, maltodextrin, inulin, etc.) with high glass transition temperature (T_g) are used [21]. Maltodextrin is a carbohydrate polymer made up of D-glucose units with dextrose-equivalent (DE) of under 20 [40, 52]. Maltodextrin is used as a dispersing aid, flavor carrier, bulking agent, fat replacer, volume enhancer, texture modifier, encapsulating agent and wall material [12]. It has many advantages such as highly soluble, relatively low cost, neutral aroma, taste, mouth feel and good protection of flavors against oxidation as compared to other drying aids [50].

Inulin is a non-digestible prebiotic soluble carbohydrate with very low energy value [17]. It is used as a sweetener component, especially in combination with high-intensity sweeteners, texture modifier and fat replacer [23]. Dietary fibers such as inulin are functional ingredients which are commonly used in different food products in order to modify physical and structural properties of hydration, viscosity, texture, sensory characteristics and oil holding capacity and also prolong the shelf life of products [30, 37].

The final characteristics of the dried products are broadly affected by the drying type and condition. The spray drying techniques are one of the most commonly applied techniques for manufacturing creamer [4]. Spray drying involves the transformation of feed from a liquid or slurry form to dry powder [34]. Spray-dried powders may have small particles with low bulk density, leading to inadequate flowability and poor reconstitution properties, thus causing difficulties in handling, transportation and storage. Manufacturers require free-flowing powders without any dust, and these requirements are met just by applying agglomeration process [42]. Agglomeration is a combination of wetting and nucleation, consolidation and growth and attrition and breakage [25]. Fluidization is a promising alternative technology, which allows the simultaneous drying, encapsulation and agglomeration in a single stream, reducing operation costs, saving time, simultaneously reducing the caking issue and improving the physicochemical properties (i.e. flowability, density, dissolution and dispersion characteristics) of the powder [2, 5, 11, 39].

The present study was conducted to investigate the effect of inulin (0, 2.5, 5 and 7.5%, w/w) and maltodextrin (0, 15, 20 and 25%, w/w) and fluidized-bed drying on the characteristics of

the reduced-fat creamers. Inulin and maltodextrin have been used as a proper drying agent, fat replacer and wall material in powder technology and processing. It was hypothesized that there is a possibility to produce the reduced-fat coffee creamer with more nutritional benefit by partial replacement of its fat with proper fat replacer (like inulin and maltodextrin). In this study, water activity (a_w), wettability, apparent viscosity, solubility, particle size and color of differently formulated regular and instant reduced-fat creamers were examined. The one-stage drying (i.e. spray drying) was applied to produce the regular reduced-fat dairy creamer (RRDC), while two-stage drying (i.e. spray drying followed by fluidized-bed drying) was employed to manufacture the instant reduced-fat dairy creamer (IRDC). All formulated creamers were compared with the properties of control (0% inulin and 0% maltodextrin) and commercial creamers (A and B). To the best of our knowledge, non-data of the different drying process and components were reported about reduced-fat dairy creamer.

2. Materials and methods

2.1. Materials

The following components were used in creamer formulation: Maltodextrin (DE = 10, Roquette Freres Co, Lestrem, France), long-chain inulin (Fibruline Xl, Warcoing, Warcoing, Belgium), silicon dioxide (Sigma Aldrich, St. Louis, MO, USA), dipotassium hydrogen phosphate (Nacalai Tesque Co, Kyoto, Japan) and soy lecithin (Kordel's Co, CA, USA). Other ingredients such as commercial skim milk powder, an instant coffee (Brazilian freeze-dried Gold Bon CAFÉ), regular commercial coffee creamer (A) and instant commercial coffee creamer (B), hydrogenated palm kernel oil, sugar and vanilla were purchased from the supermarket (Kuala Lumpur, Malaysia). **Table** shows the composition of regular and instant commercial creamers applied for comparison purposes.

2.2. Creamer preparation

Reduced-fat creamer emulsions were produced according to a method described by Hedayatnia et al. [22] with minor modification (**Figure**). Initially, the dispersed phase (**A**) containing the hydrogenated palm kernel oil (8% w/w) and soy lecithin (emulsifier, 0.5% w/w) was mixed in a 100-mL beaker and kept in a thermo-controlled water bath (70°C and rotated at 100 rpm for 20 min). The aqueous phase (**B**) which consists of sodium caseinate (2.5% w/w),

Composition	Regular commercial creamer A	Instant commercial creamer B
Fat (%)	34.0	34.6
Carbohydrate (%)	57.1	56.9
Protein (%)	2.0	1.3

Table. The composition of regular and instant commercial creamers applied for comparison purposes.

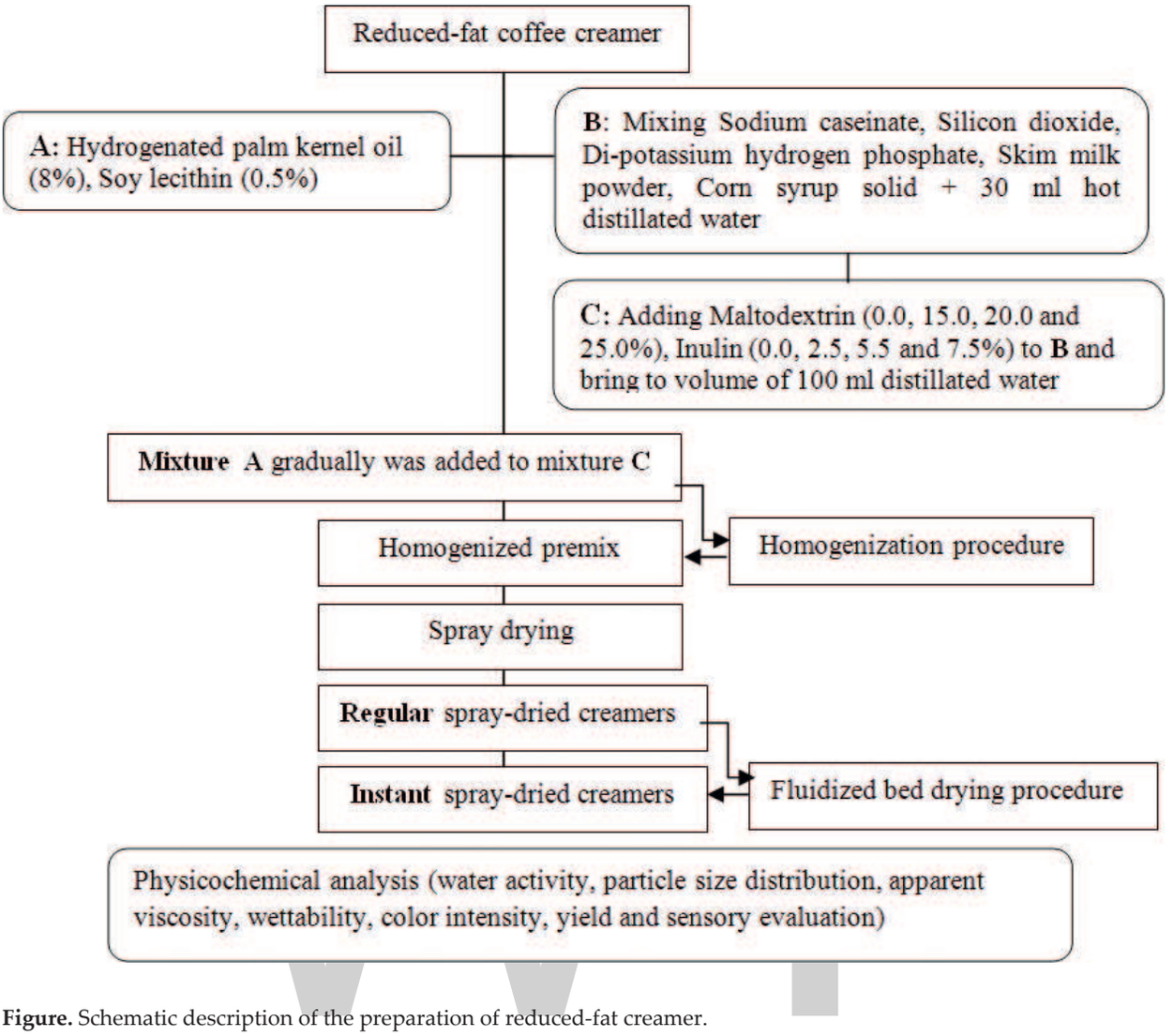

Figure. Schematic description of the preparation of reduced-fat creamer.

silicon dioxide (1.0% w/w), dipotassium hydrogen phosphate (2.5% w/w), skim milk powder 7% (w/w) and corn syrup solid (15% w/w) was prepared by gradually dispersing them into 100-mL hot distilled water (70 ± 5°C) and stirred consequently with a magnetic at 100 rpm for 5 min. Subsequently, different concentrations of inulin (0.0, 2.5, 5.0 and 7.5% w/w) and maltodextrin (0, 15, 20 and 25%) (**C**) were gradually added to the aqueous phase (**B**) to prepare the emulsion continuous phase. Hot distilled water (70 ± 5°C) was added to each creamer formulation up to 100%. In the final stage, upon mixing the ingredients, dispersed phase (**A**) was gradually added to the premix and gently stirred for 10 min. Subsequently, the coarse creamer emulsion was homogenized by a high-pressure homogenizer (at 200- and 180-MPa pressure for 2 cycles) prior to the drying process. All formulated reduced-fat creamers and control were prepared under the same drying condition depending on the regular or instant case.

2.3. Spray drying procedure

After homogenization, the creamer emulsion was fed into a lab scale mini spray dryer (BÜCHI model B-290, Flawil, Switzerland). The samples were atomized with a rotary atomizer into the

drying chamber. In the present study, spray drying procedure was set at the following condition: inlet temperature, 180 ± 5°C; outlet temperature, 80 ± 5°C; pressure, 552 kPa; and feed rate, 10 mL/min.

2.4. Fluidized-bed drying

In this study, a laboratory scale fluidized-bed dryer (Aeromatic-Fielder AG, GEA Co, Copenhagen, Denmark) was used for agglomeration process under the following experimental condition: 50°C (inlet fluidizing air temperature), 5 mL/min (solution feed rate) and 1.5 m/s (atomizing air pressure) for 30 min. In this study, the creamer powder (150 g) was placed in a container. Then, 30-mL aqueous solution of lecithin concentration (2%, w/w) was fed by a peristaltic pump and sprayed from a spray nozzle, which was located at the top of the chamber. The lecithin solution acts as a binder during the drying process as recommended for fluidization process by Dhanalakshmi et al. [13]. The solution droplets fell down on the creamer powders, while the filtrated hot air from the bottom of the chamber flowed throughout the chamber to reduce the moisture content and dustiness of particles. The atomization of the feed solution was stopped for 5 min every 10 min during fluidization, and the gas flow rate was increased steadily to ensure the proper flow pattern of the solids, and the balance between the coating and agglomeration mechanisms (layering and particle coalescence) could be reached. This helps to compensate the moisture and prevent further stickiness in the drying chamber. Vanilla (5% w/w) was added at the final drying stage because of thermal sensitivity of aromatic compounds. Additional flavors could be added to enhance the overall flavor of the reduced-fat products [53].

3. Analytical tests

3.1. Water activity (a_w)

Water activity (a_w) of all regular and instant creamers was measured in triplicate by using an AquaLab water activity metre (Series 3TE, Decagon Devices Inc., Pullman, WA, USA) with ±0.001 sensitivity at 21°C.

3.2. Average particle size

Average particle size ($D[_{4,3}]$) was determined by measuring the volume-weighted mean diameter (de Brouckere mean diameter, $D_{4,3}$) in triplicate for each sample. The experiments was performed by means of a particle size analyzer with powder feeder unit (Model 2000 hydro S, Malvern Instrument, Worcestershire, UK) equipped with a Mastersizer software 2000 (Version 5.13). The volume-weighted mean diameter is estimated by the following equation:

$$D\,[4,3] = \Sigma n_i D_i^4 / \Sigma n_i D_i^3$$

where n_i is the number of particles with diameter D_i[15].

3.3. Wettability determination

The wettability of creamers was determined according to the method described by Gong et al. [19] with minor modification. In this experiment, 100-mL hot distilled water (70 ± 5°C) was poured into a 250-mL glass beaker; then 10 g of creamer powder was poured into the beaker. The time required for the powder to completely become wet was recorded as wetting time. This measurement was carried out in triplicate for each sample.

3.4. Apparent viscosity measurement

The apparent viscosity of all creamers was measured with a rheometer (RheolabQC Rheometer, Anton Paar Co, Österreich, Austria) at room temperature (25 ± 1°C). The experiment was conducted by reconstituting 20-g creamer with100-mL hot distilled water (70 ± 5°C). Then, 25 ml of prepared solution (20%, w/w) was shaken prior to analysis. Prior to shearing test, all samples were left 5 min to reach the equilibrium condition. Apparent viscosity was measured in triplicate for each sample.

3.5. Color evaluation

The color intensity of all creamers was measured by a Hunter Lab colorimeter (Model A60–1012-402, Fairfax County, VA, USA). The color intensity was expressed in the CIELAB space as L* (lightness; 0 = black, 100 = white) and b* (+b = yellowness, −b = blueness) values [24]. For color measurement, 10 mg of sample was placed in a transparent polypropylene bag for analysis. The color measurement was done in triplicate for each sample.

3.6. Yield determination

The drying yield was measured according to the method described by Koocheki et al. [31]. The averages of three individual measurements were considered for each sample:

$$Y = 100 \times (M_1/M_2)$$

M_1 = mass of initial ingredients (g); M2 = mass of final powders (g).

3.7. Experimental design and statistical analysis

A full factorial design technique was considered to prepare different samples (**Table**). One-way analysis of variance (ANOVA) and Fisher's multiple comparison tests were used to find out the significant ($p < 0.05$) or insignificant ($p > 0.05$) difference among all samples. Then, the data were subjected to two-way analysis of variance (ANOVA) to determine the main and interaction effects of inulin and maltodextrin on the creamer characteristics. The degree of significance of all independent variables could be determined with F-ratio. The factor or independent variable with higher F-ratio represents the factor with more significant powerful effect and vice versa [38]. Also, the t-test was applied to analyze the significant ($p < 0.05$) difference among mean values of samples before and after agglomeration process. Minitab

Components	Formulations															
	CS	A	B	C	D	E	F	G	H	I	J	K	L	M	N	O
Maltodextrin (%)	0	0	0	0	15	15	15	15	20	20	20	20	25	25	25	25
Inulin (%)	0	2.5	5.0	7.5	0	2.5	5.0	7.5	0	2.5	5.0	7.5	0	2.5	5.0	7.5
HPKO (%)	8.0	8.0	8.0	8.0	8.0	8.0	8.0	8.0	8.0	8.0	8.0	8.0	8.0	8.0	8.0	8.0
Sodium caseinate (%)	2.5	2.5	2.5	2.5	2.5	2.5	2.5	2.5	2.5	2.5	2.5	2.5	2.5	2.5	2.5	2.5
Skim milk powder (%)	7.0	7.0	7.0	7.0	7.0	7.0	7.0	7.0	7.0	7.0	7.0	7.0	7.0	7.0	7.0	7.0
DPHP (%)	2.5	2.5	2.5	2.5	2.5	2.5	2.5	2.5	2.5	2.5	2.5	2.5	2.5	2.5	2.5	2.5
Silicon dioxide (%)	1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0
Lecithin (%)	2.5	2.5	2.5	2.5	2.5	2.5	2.5	2.5	2.5	2.5	2.5	2.5	2.5	2.5	2.5	2.5
Solid corn (%)	15.0	15.0	15.0	15.0	15.0	15.0	15.0	15.0	15.0	15.0	15.0	15.0	15.0	15.0	15.0	15.0
Vanilla (%)	5.0	5.0	5.0	5.0	5.0	5.0	5.0	5.0	5.0	5.0	5.0	5.0	5.0	5.0	5.0	5.0

HPKO, hydrogenated palm kernel oil; DPHP, dipotassium hydrogen phosphate; final volume was adjusted up to 100 ml by distilled water.

Table. The compositions of different reduced-fat creamer slurries.

version 16 (Minitab Inc., State College, PA, USA) was used to run the statistical analysis. All formulated creamers were compared with control and two commercial creamers (A and B) to investigate the impact of different fat replacers and drying techniques.

4. Results and dissociation

4.1. Effect of different fat replacers and drying techniques on water activity

Figure shows that the water activity (a_w) of the formulated creamers was significantly ($p < 0.05$) affected by the type and content of fat replacer as well as drying technique. As shown in **Figure** a, water activity of regular creamers significantly decreased from 0.36 to

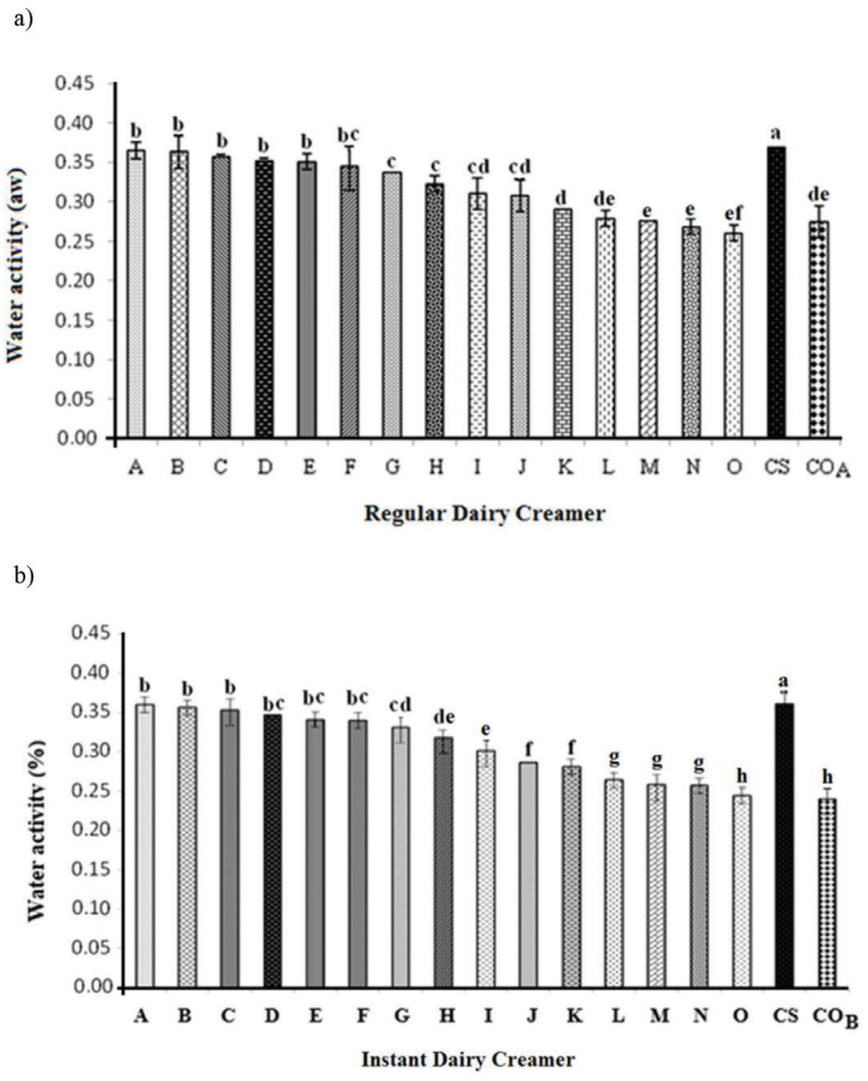

Figure. Water activity of differently formulated regular creamers (a) and instant creamers (b) as compared to the control (CS) and two regular and instant commercial creamers (CO) (a and B). [a–m] Significant differences at the confidence level of $p \leq 0.05$ (mean ± SD, n = 3); A–C: Creamers with 0% maltodextrin and 2.5, 5.0 and 7.5% inulin, respectively. D–G: Creamers with 15% maltodextrin and 0, 2.5, 5.0 and 7.5% inulin, respectively. H–K: Creamers with 20% maltodextrin and 0, 2.5, 5.0 and 7.5% inulin, respectively. L–O: Creamers with 25% maltodextrin and 0, 2.5, 5.0 and 7.5% inulin, respectively.

0.26 with increase in maltodextrin and inulin contents. The samples containing higher maltodextrin and inulin contents had lower water activity, while control sample (CS) had the high-est water activity among all formulated creamers (**Figure** a). The control sample is probably more hygroscopic than the other formulated creamers. The result showed that the control creamer adsorbed more water than other formulated creamers. It had the highest stickiness and very poor reconstitution properties as well. In this case, Kumar and Mishra [32] explained that the proper water activity of powder should be between 0.20 and 0.25. Water activity (a_w) of instant spray-dried samples also varied from 0.36 to 0.24 (**Figure** b). It was concluded that the single and interaction effects of inulin and maltodextrin significantly ($p < 0.05$) affected the water activity of regular and instant spray-dried creamers (**Table**). Maltodextrin has highly hygroscopic effect with the extensive ability to capture the free moisture, so aw reduction can be due to such maltodextrin function.

Table also shows that the water activity of the creamer was significantly influenced by fluidized-bed drying. This difference was analyzed by comparing the water activity of the regular and instant creamer before and after fluidized-bed drying, respectively. Maltodextrin showed more significant ($p < 0.05$) effects than inulin as indicated by its higher F-ratio. It was observed that the agglomeration induced by fluidized-bed drying significantly ($p < 0.05$) decreased the water activity of instant creamers. This might be attributed to long residence time (about '30 min) and hot air (50°C) injected throughout fluidized-bed the dryer. The hot air caused

Creamer	Creamer characteristics	Linear effect				Interaction effect		R^2
		Inulin		Maltodextrin		Inulin* Maltodextrin		
		p-value	F-ratio	p-value	F-ratio	p-value	F-ratio	
Regular	Water activity	0.000	52	0.000	793	0.000	1	0.971
	particle size	0.000	14	0.000	1744	0.000	74	0.994
	Apparent viscosity	0.000	48	0.000	1442	0.278[a]	1	0.990
	Wettability	0.000	22	0.000	832	0.006	4	0.988
	Lightness (L*)	0.000	158	0.000	5181	0.000	40	0.997
	Yellowness (b*)	0.000	134	0.000	8691	0.000	22	0.999
	Yield	0.367[a]	1	0.002	8	0.477[a]	1	0.690
Instant	Water activity	0.000	585	0.000	1389	0.000	12	0.998
	particle size	0.000	108	0.000	2259	0.001	7	0.995
	Apparent viscosity	0.000	859	0.000	38,957	0.000	95	0.999
	Wettability	0.000	550	0.000	800	0.000	5	0.988
	Lightness (L*)	0.000	7609	0.000	265,995	0.000	206	0.999
	Yellowness (b*)	0.000	474	0.000	15,137	0.000	32	0.997
	Yield	0.000	23	0.000	4009	0.000	12	0.998

[a]Non-significant ($p > 0.05$).

Table. Two-way ANOVA showing the single main effect and interaction effect of inulin and maltodextrin on characteristics of regular and instant reduced-fat dairy creamers.

Creamers	Mean	p-value	T-value	Test
RSRC	0.33	0.000	5.87	Water activity (a_w)
ISRC	0.29			
RSRC	58.68	0.000	29.55	Wettability
ISRC	37.97			
RSRC	5.66	0.000	−12.84	Apparent viscosity
ISRC	6.30			
RSRC	66.05	0.000	−9.96	Particle size
ISRC	120.15			
RSRC	84.33	0.000	12.61	Lightness (L*)
ISRC	81.01			
RSRC	13.79	0.000	−14.99	Yellowness (b*)
ISRC	15.70			
RSRC	64.40	0.360[a]	−0.92	Yield
ISRC	66.59			

RSRC: Regular spray-dried reduced-fat creamer.
ISRC: Instant spray-dried reduced-fat creamer.
[a]Insignificant at $p > 0.05$.

Table. Student ***t***-test for significant comparison of the regular and instant reduced-fat dairy creamers.

more water evaporation from the surface of the particles, thus providing less stickiness (wall deposition). The results showed that the regular creamers had higher water activity than the instant creamers, indicating a higher amount of freely available water for the undesirable biological reactions and consequently inducing shorter shelf life.

4.2. Effect of different fat replacers and drying techniques on wettability and apparent viscosity

Table shows the wettability and apparent viscosity of different reduced-fat dairy creamer as compared to two commercial creamers and control. The wettability and viscosity of the regu-lar and instant dairy creamer were significantly ($p < 0.05$) affected by the different composi-tions and drying techniques. Wettability is an important instant property of powder, and it is defined as the ability of particles to overcome the surface tension between the liquid (solvent) and themselves [14]. It is also directly affected by the interactions between two phases [9]. In general, wettability is considered to be the rate-controlling step of the reconstitution process [29] with the surface content strongly affecting the wettability [16]. In the current study, the regular (75 s) and instant (63 s) control creamers showed the longest wetting time among all formulated creamers (**Table**). This might be explained by the effects of fat replacer and total solid contents on the wettability of the reduced-fat creamer (**Table**). The presence of free fat on the particle surface could reduce wettability of control sample due to its hydrophobicity, making it difficult for water to penetrate into the powder.

Sample	Regular creamers			Instant creamers		
	Wettability (s)	Apparent viscosity (mPa.s)	Yield (%)	Wettability (s)	Apparent viscosity (mPa.s)	Yield (%)
Control*	75.00 ± 0.82^{a}	4.75 ± 0.19^{a}	43.00 ± 1.83^{l}	63.00 ± 0.00^{a}	4.85 ± 0.01^{n}	43.30 ± 1.20^{l}
MA0%, IN2.5%	75.50 ± 0.70^{a}	4.88 ± 0.02^{ab}	43.55 ± 0.62^{l}	61.00 ± 1.4^{a}	4.84 ± 0.01^{n}	43.55 ± 0.62^{l}
MA 0%, IN 5%	75.50 ± 0.70^{a}	5.04 ± 0.06^{b}	47.00 ± 0.00^{k}	56.50 ± 0.70^{b}	5.21 ± 0.01^{m}	47.00 ± 0.31^{k}
MA0%, IN7.5%	76.00 ± 0.93^{a}	5.06 ± 0.05^{b}	47.77 ± 0.31^{k}	55.50 ± 0.70^{b}	5.29 ± 0.01^{l}	47.77 ± 0.31^{k}
MA15%, IN0%	69.00 ± 0.97^{b}	5.32 ± 0.02^{b}	53.35 ± 0.50 ^{j}	54.00 ± 1.41^{b}	6.03 ± 0.02^{k}	53.35 ± 0.50 ^{j}
MA15%, IN2.5%	66.50 ± 0.70^{bc}	5.37 ± 0.02^{b}	55.03 ± 0.04^{i}	44.00 ± 2.12^{c}	6.01 ± 0.01^{k}	55.03 ± 0.04^{i}
MA15%, IN 5%	64.00 ± 1.06^{cd}	5.46 ± 0.02^{b}	60.55 ± 0.78^{h}	40.00 ± 0.00^{d}	6.21 ± 0.01^{i}	60.55 ± 0.78^{h}
MA15%, IN7.5%	61.50 ± 0.83^{d}	5.54 ± 0.01^{bc}	61.45 ± 0.63^{h}	39.50 ± 3.53^{d}	6.18 ± 0.01^{j}	61.45 ± 0.63^{h}
MA20%, IN0%	58.50 ± 0.70^{e}	5.76 ± 0.02^{cd}	65.80 ± 1.13^{g}	35.00 ± 3.53^{ef}	6.49 ± 0.01^{h}	65.80 ± 1.13^{g}
MA20%, IN2.5%	58.50 ± 0.75^{e}	5.81 ± 0.02^{cd}	66.00 ± 1.41^{g}	36.00 ± 1.41^{e}	6.56 ± 0.02^{f}	66.00 ± 1.41^{g}
MA20%, IN 5%	54.00 ± 1.21^{f}	5.89 ± 0.01^{cd}	70.60 ± 0.55^{f}	32.50 ± 0.70^{fg}	6.52 ± 0.00^{g}	70.60 ± 0.55^{f}
MA20%, IN7.5%	50.50 ± 0.65^{g}	6.04 ± 0.03^{d}	75.66 ± 0.48^{e}	30.00 ± 0.00^{g}	6.75 ± 0.02^{e}	75.66 ± 0.48^{e}
MA25%, IN0%	45.50 ± 2.05^{h}	6.29 ± 0.00^{d}	79.77 ± 0.48^{d}	24.00 ± 1.41^{h}	7.09 ± 0.01^{d}	79.77 ± 0.48^{d}
MA25%, IN2.5%	44.00 ± 1.40^{h}	6.35 ± 0.01^{d}	85.06 ± 0.09^{c}	24.00 ± 0.73^{h}	7.12 ± 0.01^{c}	85.06 ± 0.09^{c}
MA25%, IN 5%	40.00 ± 0.09^{ij}	6.41 ± 0.01^{d}	89.45 ± 0.77^{b}	19.00 ± 1.40^{i}	7.35 ± 0.01^{a}	89.45 ± 0.77^{b}
MA25%, IN7.5%	40.50 ± 0.77^{ij}	6.53 ± 0.03^{d}	94.60 ± 0.56^{a}	13.00 ± 1.40^{j}	7.32 ± 0.01^{b}	94.60 ± 0.56^{a}
Commercial creamers	43.00 ± 1.41^{hi}	5.82 ± 0.02^{d}	—	17.00 ± 0.00^{i}	7.29 ± 0.00^{b}	—

*Control (0% inulin and maltodextrin); mean values ± standard deviation with different lowercase letters in the same column indicating significant difference ($P < 0.05$); control (0%MA & 0%IN); MA, maltodextrin; IN, inulin.

Table. Significant differences ($p < 0.05$) among different regular and instant reduced-fat dairy creamers as compared to two regular and instant commercial creamers in terms of wettability, apparent viscosity and yield.

As shown in **Table**, the regular spray-dried creamers exhibited different levels of wettability (40–75.50 s), while the instant spray-dried creamers lower faster wettability (13–61 s) than regular creamers with similar formulations. This was comparable with the wettability of the regular commercial creamer A (43 s) and instant commercial creamer B (17 s), respectively. The results showed that the wetting time of regular and instant creamers was decreased by increasing the particle size and decreasing the water activity of creamers. Jakubczyk et al. [26] reported that the wettability of the apple puree powder was improved from '45 to '33 (s) by increasing maltodextrin from 6 to 15% (w/w). The amount of wall materials significantly affected the wettability of the final powder. The results showed that there was a reverse relationship between wetting time and the content of wall materials (i.e. inulin and maltodextrin).

Characteristics		Inulin	Maltodextrin	Total solid content	Wettability	Viscosity
Viscosity	r-correlation	0.180	0.938	0.950	−0.988	—
	p-value	0.506	0.000*	0.000*	0.000*	
Wettability	r-correlation	−0.156	−0.925	−0.931	—	—
	p-value	0.564	0.000*	0.000*		
Particle size	r-correlation	0.656	0.907	0.924	−0.903	0.941
	p-value	0.045*	0.000*	0.000*	0.001*	0.000*

r = r Pearson correlation; significant at $p < 0.05$; r > 0.9 represents positive strong correlation, while and r > −0.9 represents negative strong correlation.

Table. Correlation analysis between total solid content, inulin and maltodextrin contents and creamer characteristics.

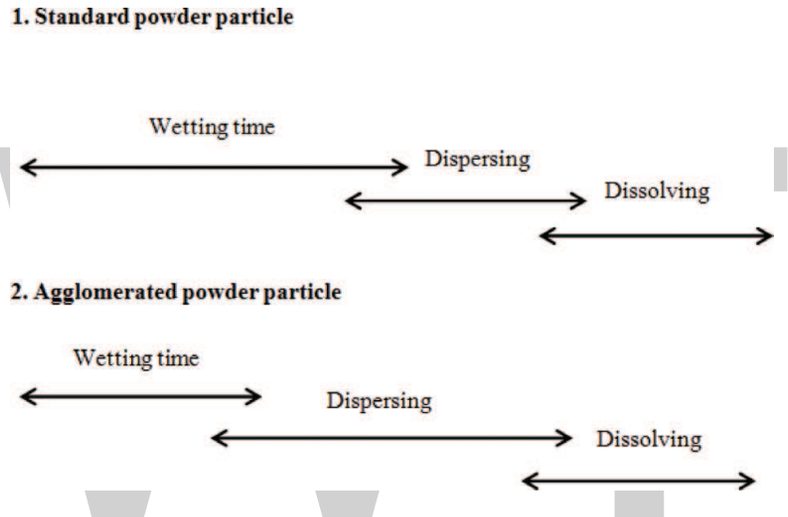

Figure. Dissolution timeline for regular and agglomerated (instant) powder showing the overlaps between different phases with time [14].

The creamer containing the highest maltodextrin (25%) and inulin (7.5%) showed the shortest wetting time among all formulated creamers (**Table**).

The instant creamer exhibited significantly higher wettability (shorter wettability) than the regular creamer (**Table**) due to large particles exhibiting more empty spaces among themselves, resulting in easier penetration by the liquid (i.e. water) [15]. Lecithin can modify the flowability and wettability of dried powders due to its potential surface active properties with higher porosity and better wettability [13]. **Figure** clearly shows the schematic of dissolution timeline for standard and agglomerated powder [14] by correlation between the wettability, dispersibility and solubility in the regular (non-agglomerated) and instant creamers.

As shown in **Table**, the apparent viscosity of regular spray-dried creamers varied from 4.88 to 6.53 (mPa.s) as compared to the control (4.75 mPa.s) and regular commercial creamer A (5.82 mPa.s). In addition, the viscosity of the instant spray-dried creamers varied from 4.84 to 7.35 (mPa.s) compared to the commercial instant creamer B (7.29 mPa.s) and control sample (4.85 mPa.s) (**Table**). The result showed that the apparent viscosity of regular and instant

creamers was significantly ($p < 0.05$) increased by increasing the concentration of maltodextrin and inulin in the creamer formulation. This could be due to increasing the total solids of samples and positive effect of inulin and maltodextrin on the formation of a stable particle gel with tridimensional network in the present of aqueous phase which led to enhance the viscosity of creamer. Debon et al. [10] also reported the similar observation. They reported that the addition of 5% inulin to the formulation significantly increased the apparent viscosity of low-fat fermented milk. It might be due to several different factors such as (i) the interaction between inulin and milk protein (i.e. sodium caseinate) leading to enhance the viscosity, (ii) the high inulin capacity to retain water [46] and (iii) the capacity to retain water by formation of small aggregates of inulin microcrystal [20]. This might be due to the increase in the amount of soluble materials and reduction in the moisture content.

The apparent viscosity of the spray-dried creamers was greatly enhanced after agglomeration via fluidized-bed drying (**Table**). This could be explained by the significant ($p < 0.05$) effects of the agglomeration on the intermolecular interactions among creamer particles which resulted in higher viscosity. In addition, the lower moisture content and higher total solid content can be also responsible for the higher viscosity of the instant creamers. Water acts as a mobility enhancer, resulting in a larger free volume and a reduction in the viscosity [48].

4.3. Effect of different fat replacers and drying techniques on average particle size

In the current study, the average particle size of different creamers was determined by measuring the volume-weighted mean. The particle size of the powder can significantly affect its appearance, flowability, wettability and dispensability [43]. **Figure** showed a significantly increase in the particle size of different formulated creamers. The results showed that the creamer O containing 25% maltodextrin and 7.5% inulin exhibited the largest particle size (101.45 μm), while the control sample had the smallest particle size among all regular spray-dried dairy cream-ers, respectively (**Figure a**). As stated by Master [36], the particle size is highly influenced by the viscosity of the feed. Similar observations were previously reported by Jinapong et al. [27] wherein increasing the solids content of instant spray-dried soymilk powders from 5.2 to 13.0% significantly resulted in enlargement of the particle size from 14.54 to 23.59 (μm).

Figure b shows that agglomeration process by fluidized-bed drying technique significantly ($p < 0.05$) increased the volume-weighted mean ($D_{4,3}$) or particle size of the instant spray creamers compared to the regular creamers. As stated by Chen and Özkan [8], agglomeration results in larger particles, bigger particle clusters and better flow characteristics than one-stage drying. The instant spray-dried creamers exhibited different particle size, ranging from 46.45 to 193.26 μm compared to control creamer (47.35 μm) and the commercial instant creamer B (190.94 μm) (**Figure b**). As stated by Carić [7], the most suitable particle size for rapid dispersion is around 150–200 μm. Binder in the wet agglomeration process leads to the enlargement of particle size, thereby improving the flowability of the final powder [3, 44]. Jinapong et al. [27] reported that the particle size of the instant spray-dried soymilk was increased from 25 to 260 μm after subjecting the sample to fluidized-bed drying. This finding was also reported also by Machado et al. [35] wherein agglomerated soy protein had much larger particles than non-agglomerated regular creamer. In addition, it was found that the agglomeration through fluidized-bed drying also caused stickiness reduction and flowability

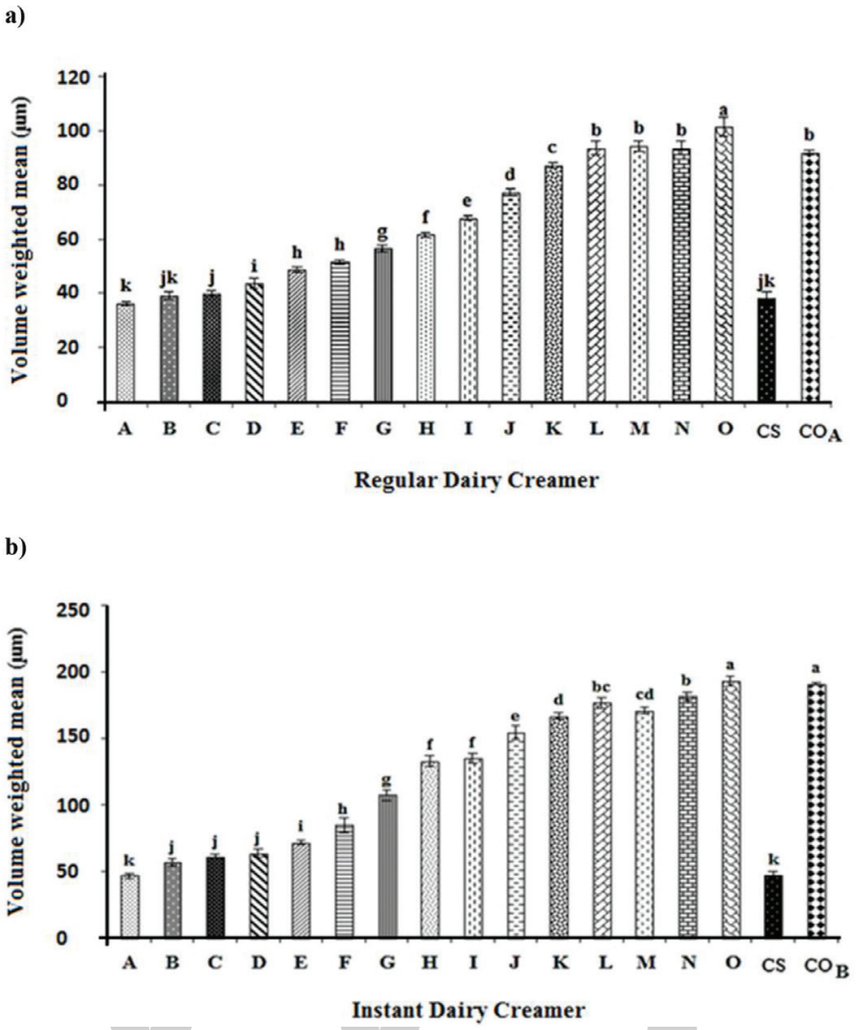

Figure. Volume-weighted mean (or average particle size) of differently formulated regular creamers (a) and instant creamers (b) as compared to the control (CS) and two regular and instant commercial creamers (CO) (a and B). [a–m] Significant differences at the confidence level of $p \leq 0.05$ (mean ± SD, n = 3); A–C: Creamers with 0% maltodextrin and 2.5, 5.0 and 7.5% inulin, respectively. D–G: Creamers with 15% maltodextrin and 0, 2.5, 5.0 and 7.5% inulin, respectively. H–K: Creamers with 20% maltodextrin and 0, 2.5, 5.0 and 7.5% inulin, respectively. L–O: Creamers with 25% maltodextrin and 0, 2.5, 5.0 and 7.5% inulin, respectively.

improvement in the creamer. As the particle size increases, the adhesive forces decrease due to the attractive forces (i.e. van der Waals) among creamer particles [54]. However, the application of the spray drying process followed by the agglomeration process resulted in a significant ($p < 0.05$) better flowability and reconstitution properties than the spray drying alone. Dhanalakshmi et al. [13] indicated that the powder flowability and reconstitution are highly affected by the particle size, shape and distribution, the particle surface properties as well as the geometry of the system. As shown in **Table**, the linear and interaction effects of the inulin and maltodextrin had significant ($p < 0.05$) effect on the volume-weighed mean (or particle size) of spray-dried creamers.

4.4. Effect of different fat replacers and drying techniques on yield

Table shows the yield of regular and instant dairy creamers compared to the control sample. The control sample had remarkably lower yield (43%) than other regular spray-dried creamers.

The low yield was observed for the control. This could be due to the stickiness of this sample to the spray drying chamber and cyclone wall. This might be because the control did not contain inulin and very low percentage of maltodextrin as a drying aid. Stickiness is one of the main technological issues in the production of powders such as coffee creamer because it results in a reduction of the yield and stability. The result showed significant improvement in the production yield by increasing the maltodextrin and inulin content in the formulation (**Table**). This was in agreement with the previous finding reported by Shrestha et al. [45] for spray-dried tomato pulp. In fact, the addition of drying aids such as maltodextrin with high glass transition temperature (>145°C) to the premix is one of the most suitable ways to increase the stability, decrease the stickiness and improve the yield [21, 45]. In addition, maltodextrin can help to shorten the drying time, thus reducing the input energy required for spray drying process. According to Adhikari et al. [1], the improvement of yield (recovery) might be due to the formation of a thin protein-rich membrane at the particle-air interface. The high glass transition temperature of this surface layer causes the conversion of this thin membrane into a glassy state, which prevents particles from sticking to each other and to the walls of the dryer which resulted in the decrease of the wall deposition during drying and increase of the yield. As shown in **Table**, maltodextrin with higher F-ratio had higher significant effects than inulin on the yield. There was no significant ($p > 0.05$) difference between the yields of single- or double-step drying processes for regular and instant creamer, respectively.

Figure. The appearance of regular commercial creamer (a) as compared to the control creamer (b) and regular formulated-reduced-fat creamer containing 25% maltodextrin and 7.5% inulin.

It means that there was not any material loss or stickiness to the wall during fluidized-bed dying process. This could be attributed to the high efficiency of such drying technique.

4.5. Effect of different fat replacers and drying techniques on color

Figure shows the appearance of the regular creamer as compared to the control and com-mercial creamer. The results indicated that differently formulated creamers, control and com-mercial creamer had significant ($p < 0.05$) different color intensity in terms of L* and b* (**Table**). In general, the lightness (L*) of all formulated regular and instant creamers was enhanced by increasing maltodextrin and inulin concentrations in the creamer formulation. The result indi-cated that the addition of maltodextrin and inulin to the creamer formulation led to decrease the stickiness and increase the lightness of samples. Similar observation was reported by Shrestha et al. [45] on spray-dried orange juice. The regular and instant creamer with 25% maltodextrin and 7.5% inulin had the highest L*, while the control showed the lowest L*among all samples due to the caramelization reaction (**Table**). The regular creamer containing the highest maltodextrin content (25%) exhibited almost similar color to the regular commercial

	Regular creamers		**Instant creamers**	
Sample	L*	b*	L*	b*
Control*	71.07 ± 0.05^{l}	22.49 ± 0.21^{a}	70.73 ± 0.21^{o}	24.57 ± 0.05^{a}
MA0%, IN2.5%	73.28 ± 0.33^{k}	22.04 ± 0.06^{b}	71.20 ± 0.11^{n}	23.71 ± 0.21^{b}
MA0%, IN5%	72.67 ± 0.27^{k}	21.09 ± 0.13^{c}	72.23 ± 0.00^{m}	23.62 ± 0.11^{b}
MA0%, IN7.5%	71.48 ± 0.55^{l}	20.14 ± 0.04^{d}	70.46 ± 0.02^{p}	22.50 ± 0.47^{c}
MA15%, IN0%	78.91 ± 0.07^{j}	18.37 ± 0.36^{e}	74.05 ± 0.00^{l}	20.69 ± 0.09^{d}
MA15%, IN2.5%	82.30 ± 1.47^{i}	18.09 ± 0.07^{e}	76.38 ± 0.03^{k}	20.42 ± 0.17^{d}
MA15%, IN 5%	84.63 ± 0.21^{h}	17.46 ± 0.53^{f}	79.13 ± 0.02^{j}	17.47 ± 0.00^{e}
MA15%, IN7.5%	85.57 ± 0.19^{g}	15.15 ± 0.00^{g}	80.48 ± 0.01^{i}	17.44 ± 0.00^{e}
MA20%, IN0%	87.46 ± 0.24^{f}	11.63 ± 0.02^{h}	82.11 ± 0.00^{h}	13.92 ± 0.00^{f}
MA20%, IN2.5%	87.12 ± 0.01^{f}	11.03 ± 0.02^{i}	84.05 ± 0.11^{g}	13.32 ± 0.00^{g}
MA20%, IN 5%	89.28 ± 0.17^{e}	10.28 ± 0.01^{j}	84.30 ± 0.02^{f}	12.61 ± 0.02^{h}
MA20%, IN7.5%	89.80 ± 0.00^{de}	10.83 ± 0.08^{i}	86.73 ± 0.02^{e}	11.76 ± 0.03^{i}
MA25%, IN0%	90.90 ± 0.50^{c}	7.73 ± 0.00^{k}	88.73 ± 0.02^{c}	10.79 ± 0.11^{j}
MA25%, IN2.5%	90.51 ± 0.00^{cd}	7.22 ± 0.30^{l}	88.71 ± 0.01^{c}	9.21 ± 0.00^{k}
MA25%, IN 5%	92.56 ± 0.57^{b}	6.99 ± 0.00^{l}	89.17 ± 0.19^{b}	8.56 ± 0.01^{l}
MA25%, IN7.5%	95.32 ± 0.00^{a}	6.85 ± 0.07^{l}	90.15 ± 0.01^{a}	8.08 ± 0.07^{m}
Commercial creamers (Regular and instant)	95.44 ± 0.40^{a}	7.00 ± 0.07^{l}	88.59 ± 0.00^{d}	8.20 ± 0.02^{m}

*Control (0% inulin and maltodextrin); mean values ± standard deviations with different lowercase letters in the same column indicating significant difference ($P < 0.05$); MA, maltodextrin; IN, inulin.

Table. Significant different ($p < 0.05$) color intensity of different regular and instant reduced-fat dairy creamers as compared to two regular and instant commercial creamers.

creamer A (**Table**). According to Roland et al. (1999), reduced-fat ice cream made by only maltodextrin exhibited similar whiteness to 10% fat ice cream. Moreover, the control and creamer containing 0% maltodextrin and 2.5% inulin had the highest b*among all samples. On the other hand, the regular and instant samples containing 25% maltodextrin exhibited similar yellowness (b*) as compared to regular and instant commercial samples (A and B). The instant commercial creamer B and the formulated creamer containing 25% maltodextrin and 7.5% inulin had the lowest b* among all instant creamers (**Table**). The lightness (L*) and yellowness (b*) of the instant creamers were slightly decreased after applying the agglomeration process. This might be attributed to the effects of binder solution and Maillard reaction on the lightness of the instant creamer. Szulc and Lenart [49] also reported similar findings for the agglomerated dairy powders. They explained that Maillard reaction was responsible for the color changes during agglomeration (i.e. fluidize-bed drying). Color can change during the drying process due to several chemical reactions. Most of the time, enzymatic activity is not desirable, because it affects the amount of nutrients in food (e.g. hydrolysis of lecithin by phospholipase) or the color of the products. The yellowish color of regular and instant creamer is mainly due to the non-enzymatic reaction, either by caramelization or by Maillard reactions. The caramelization process is a complex series of chemical reactions promoted by the direct heating of sugars [6].

5. Conclusions

The present work describes the possibility of producing regular and instant reduced-fat dairy creamers by spray and fluid-bed drying and the changes in some of the physical, chemical and powder properties of the creamer powders depending on the maltodextrin and inulin levels. A significant effect of the type and concentration of the fat replacers (wall materials) on the process yield, wettability, viscosity, solubility, color, water activity and particle size was found. The results showed that the process has some difficulties for drying of control samples. The use of wall materials (maltodextrin and inulin) significantly improved the drying process and leads to improve the physicochemical properties of reduced-fat dairy creamer functionality. The highest wettability, viscosity, solubility, yield and lightness and lowest water activities were obtained from the samples containing the highest contents of maltodextrin (25% w/w) and inulin (7.5%). As a result, the current study also revealed that the instant formulated-reduced-fat creamers from two-stage drying (spray drying followed by fluidized-bed drying) showed significantly ($p < 0.05$) better quality than regular creamer from one-stage drying (spray dying only). The current study suggests optimizing the fluidized-bed drying condition for preparation and commercialization of instant reduced-fat dairy creamer.

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References

[1] Adhikari B, Howes T, Bhandari BR, Langrish TAG. Effect of addition of proteins on the production of amorphous sucrose powder through spray drying. Journal of Food Engineering. 2009;**94**:144-153

[2] American Dairy Products Institute. Standards for Grades of Dry Milk Including Method of Analysis. Bull. Chicago: ADPI; 1992. p. 916

[3] Barbosa-Canovas GV, Juliano P, Yan H. Physical and chemical properties of food powders. In: Onwulata C, editor. Encapsulated and Powdered Foods. Boca Roton: CRC Press; 2005. pp. 40-71

[4] Beeson CA, Erickson L. U.S. Patent No. 6,287,616. Washington, DC: U.S. Patent and Trademark Office. 2001

[5] Benelli L, Cortés-Rojas DF, Souza CRF, Oliveira WP. Fluid bed drying and agglomeration of phytopharmaceutical compositions. Powder Technology. 2015;**273**:145-153

[6] Bonazzi C, Dumoulin E. Quality changes in food materials as influenced by drying processes. In: Tsotsas E, Mujumdar AS, editors. Modern Drying Technology. Weinheim: Wiley-VCH Verlag; 2011. pp. 1-20

[7] Carić M. Milk powders: Types and manufacture and physical and functional properties of milk powders. In: Roginski H, Fuquay JW, Fox PF, editors. Encyclopedia of Dairy Sciences. New York: Academic Press; 2003. pp. 1869-1880

[8] Chen XD, Özkan N. Stickiness, functionality, and microstructure of food powders. Drying Technology. 2007;**25**:959-969

[9] Cuq B, Rondet E, Abecassis J. Food powders engineering, between knowhow and science: Constraints, stakes and opportunities. Powder Technology. 2011;**208**:244-251

[10] Debon J, Prudêncio ES, Cunha Petrus JC. Rheological and physico-chemical characterization of prebiotic microfiltered fermented milk. Journal of Food Engineering. 2010;**99**:128-135

[11] Desai KG, Park HG. Solubility studies of valdecoxib in presence of carriers, co-solvent and surfactants. Drug Development Research. 2004;**62**:41-48

[12] Descamps N, Palzer S, Roos YH, Fitzpatrick JJ. Glass transition and flowability/caking behaviour of maltodextrin DE 21. Journal of Food Engineering. 2013;**119**:809-813

[13] Dhanalakshmi K, Ghosal S, Bhattacharya S. Agglomeration of food powder and applications. Critical Reviews in Food Science and Nutrition. 2011;**51**:432-441

[14] Fang Y, Selomulya C, Chen XD. On measurement of food powder reconstitution properties. Drying Technology. 2007;**26**:3-14

[15] Ferrari CC, SPM G, De Aguirre JM. Effect of spray-drying conditions on the physicochemical properties of blackberry powder. Drying Technology. 2012;**30**:154-163

[16] Gaiani C, Ehrhardt JJ, Scher J, Hardy J, Desobry S, Banon S. Surface composition of dairy powders observed by X-ray photoelectron spectroscopy and effects on their rehydration properties. Colloids and Surfaces. B, Biointerfaces. 2006;**49**:71-78

[17] Glibowski P, Bukowska A. The effect of pH, temperature and heating time on inulin chemical stability. Acta Scientiarum Polonorum. Seria: Technologia Alimentaria. 2011;**10**:189-196

[18] Golde AE, Schmidt KA. Quality of coffee creamers as a function of protein source. Journal of Food Quality. 2005;**28**:46-61

[19] Gong Z, Zhang M, Mujumdar AS, Sun J. Spray drying and agglomeration of instant bayberry powder. Drying Technology. 2007;**26**:116-121

[20] Gonzalez-Tomás L, Coll-Marqués J, Costell E. Viscoelasticity of inulin–starch-based dairy systems. Influence of inulin average chain length. Food Hydrocolloids. 2008;**22**:1372-1380

[21] Goula AM, Adamopoulos KG. Effect of maltodextrin addition during spray drying of tomato pulp in dehumidified air: II. Powder properties. Drying Technology. 2008;**26**:726-737

[22] Hedayatnia S, Mirhosseini H, Amid BT, Sarker ZI, Veličkovska SK, Karim R. Effect of different fat replacers and drying methods on thermal behaviour, morphology and sensory attributes of reduced-fat coffee creamer. LWT- Food Science and Technology. 2016;**72**:330-342

[23] Hursh H, Martin J. Low-carb and beyond: The health benefits of inulin. Cereal Foods World. 2005;**50**:57-60

[24] Islas-Rubio AR, de la Barca AMC, Cabrera-Ch avez F, Cota-Gast elum AG, Beta T. Effect of semolina replacement with a raw popped amaranth flour blend on cooking quality and texture of pasta. LWT- Food Science and Technology. 2014;57:217-222

[25] Iveson SM, Litster JD, Hapgood K, Ennis BJ. Nucleation, growth and breakage phenomena in agitated wet granulation processes: A review. Powder Technology. 2001;**117**:3-39

[26] Jakubczyk E, Ostrowska-Ligeza E, Gondek E. Moisture sorption characteristics and glass transition temperature of apple puree powder. International Journal of Food Science and Technology. 2010;**45**:2515-2523

[27] Jinapong N, Suphantharika M, Jamnong J. Production of instant soymilk powders by. 2008

[28] Kelly PM, Oldfield DJ, O'Kennedy BT. The thermostability of spray dried imitation coffee whiteners. International Journal of Dairy Technology. 1999;**52**:107-113

[29] Kim EHJ, Chen XD, Pearce D. Surface characterization of four industrial spray-dried dairy powders in relation to chemical composition, structure and wetting property. Colloids and Surfaces. B, Biointerfaces. 2002;**26**:197-212

[30] Kim Y, Faqih MN, Wang SS. Factors affecting gel formation of inulin. Carbohydrate Polymers. 2001;**46**:135-145

[31] Koocheki A, Ghandi A, Razavi SM, Mortazavi SA, Vasiljevic T. The rheological properties of ketchup as a function of different hydrocolloids and temperature. Food Science and Technology. 2009;**44**:596-602

[32] Kumar P, Mishra HN. Yoghurt powder-a review of process technology, storage and utilization. Food and Bioproducts Processing. 2004;**82**:133-142

[33] Lambert LM. Modern Dairy Products. New York: Chemical publishing Co. Inc.; 1970. pp. 397-408

[34] Maa YF, Nguyen PA, Sit K, Hsu CC. Spray-drying performance of a bench-top spray dryer for protein aerosol powder preparation. Biotechnology and Bioengineering. 1998;**60**:301-309

[35] Machado VG, Hirata TAM, Menegalli FC. Agglomeration of soy protein isolate in a pulsed fluidized bed: Experimental study and process optimization. Powder Technology. 2014;**254**:248-255

[36] Masters K. Spray Drying Handbook. 5th ed. Essex: Longman Scientific and Technical; 1992

[37] Meyer D, Bayarri S, Tárrega A, Costell E. Inulin as texture modifier in dairy products. Food Hydrocolloids. 2011;**25**:1881-1890

[38] Mirhosseini H, Tan CP, Hamid NS, Yusof S, Chern BH. Characterization of the influence of main emulsion components on the physicochemical properties of orange beverage emulsion using response surface methodology. Food Hydrocolloids. 2009;**23**:271-280

[39] Mukherjee S, Bhattacharya S. Characterization of agglomeration process as a function of moisture content using a model food powder. Journal of Texture Studies. 2006;**37**:35-48

[40] Nep EI, Conway BR. Physicochemical characterization of grewia polysaccharide gum: Effect of drying method. Carbohydrate Polymers. 2011;**84**:446-453

[41] Oldfield D, Singh H. Functional properties of milk powders. Food Science and Technology-New York-Marcel Dekker. 2005;**146**:365

[42] Pietsch W. Readily engineer agglomerates with special properties from micro-and nano-sized particles. Chemical Engineering Progress. 1999;**95**:67-81

[43] Reineccius GA. The spray drying of food flavors. Drying Technology. 2004;**22**:1289-1324

[44] Shittu TA, Lawal MO. Factors affecting instant properties of powdered cocoa beverages. Food Chemistry. 2007;**100**:91-98

[45] Shrestha AK, Howes T, Adhikari BP, Bhandari BR. Water sorption and glass transition properties of spray dried lactose hydrolysed skim milk powder. LWT- Food Science and Technology. 2007;**40**:1593-1600

[46] Soukoulis C, Lebesi D, Tzia C. Enrichment of ice cream with dietary fibre: Effects on rheological properties, ice crystallisation and glass transition phenomena. Food Chemistry. 2009;**115**:665-671

[47] Sudha ML, Srivastava AK, Vetrimani R, Leelavathi K. Fat replacement in soft dough biscuits: Its implications on dough rheology and biscuit quality. Journal of Food Engineering. 2007;**80**:922-930

[48] Sunooj KV, Radhakrishna K, George J, Bawa AS. Factors influencing the calorimetric determination of glass transition temperature in foods: A case study using chicken and mutton. Journal of Food Engineering. 2009;**91**:347-352

[49] Szulc K, Lenart A. Surface modification of dairy powders: Effects of fluid-bed agglomeration and coating. International Dairy Journal. 2013;**33**:55-61

[50] Takeiti CY, Kieckbusch TG, Collares-Queiroz FP. Morphological and physicochemical characterization of commercial maltodextrins with different degrees of dextrose-equivalent. International Journal of Food Properties. 2010;**13**:411-425

[51] Tuot J, Octavia W, Sher AA. U.S. Patent No. 8,658,230. Washington, DC: U.S. Patent and Trademark Office; 2014

[52] Uthumporn U, Zaidul IS, Karim AA. Hydrolysis of granular starch at sub-gelatinization temperature using a mixture of amylolytic enzymes. Food and Bioproducts Processing. 2010;**88**:47-54

[53] Yackinous C, Guinard JX. Flavor manipulation can enhance the impression of fat in some foods. Journal of Food Science. 2000;**65**:909-914

[54] Zimon AD. Adhesion of Dust and Powder. 2nd ed. New York: Consultants Bureau; 1982