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Research Article

Fatty acid profile and related properties of some olive oil samples from local market place of Kahramanmaraş, Türkiye

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Abstract

Fatty acids are the largest component group of olive oils. Fatty acid composition is one of the most frequently used criteria in the characterization of olive oils. Therefore, in this research, the fatty acid composition of 6 olive oil samples taken from local markets in Kahramanmaraş city (Türkiye) was analyzed. Using the fatty acid composition, unsaturated fatty acids, monounsaturated fatty acids, polyunsaturated fatty acids, saturated fatty acids, iodine value and quality index were calculated. It was determined that the oleic acid and linoleic acid contents of the samples (in fatty acids) were between 71.40-73.08 and 6.96-9.16 % in fatty acids. With future studies, more detailed and comprehensive information can be obtained about the olive oils offered for sale in local markets. In this way, it is thought that useful information can be provided to consumers and manufacturers who want to produce quality products.

Keywords: Oleic Acid, MUFA, PUFA, Quality Index

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INTRODUCTION

Fatty acid composition is an original data that can be used to determine the origin of olive oils. Olive oils can be characterized regionally by creating data banks with a large number of oil composition analyses [10]. 98-99% of olive oil consists of free fatty acids and triacylglycerols, defined as the saponifiable fraction. Triacylglycerols consist of three fatty acids and one glycerol [7]. Oleic acid is the highest fatty acid in olive oil, followed by palmitic acid and linoleic acid. Fatty acid compositions vary according to region and varieties [9]. Olive oil authentication and characterization have great importance not only to consumers but also to suppliers, producers, retailers, traders and legal regulators [3, 10]. Olive oils sold in national market chains, like other products, are subjected to many control and analyzes are routinely carried out by both the state and market representatives [3]. However, olive oils sold in local markets may sometimes be subject to less control. Therefore, in this research, olive oils purchased from local markets were analyzed. Fatty acids constitute the largest composition of olive oils. They are also used for the characterization and legal control of olive oils. The aim of the research was to determine the fatty acid composition of olive oils, both their compliance with legal limits and the similarities with the characteristics of the region.

MATERIAL AND METHOD

Two bottles each of six different olive oil samples were purchased from local markets in Kahramanmaraş city center (Türkiye). The labels of the samples were checked and it was seen that all of them had production permission from the Ministry of Agriculture and Forestry and their shelf life had not expired. The samples were analyzed without waiting. The standard method was used to determine the fatty acid composition of the samples [6]. According to the method, fatty acids are first subjected to methylation to obtain fatty acid methyl esters. It was then analyzed using a gas chromatography device (Perkin Elmer - Clarus 580, USA).

Saturated fatty acids (SFA) were calculated by adding myristic acid (C14:0), palmitic acid (C16:0), heptadecanoic acid (C17:0), stearic acid (C18:0), arachidic acid (C20:0) and behenic acid (C22:0). Monounsaturated fatty acids (MUFA); calculated by adding palmitoleic acid (C16:1), heptodecanoic acid (C17:1), oleic acid (C18:1) and eicosenic acid (C20:1). Polysaturated fatty acids (PUFA); calculated by linoleic acid (C18:2) and linolenic acid (C18:3). Iodine value (IV) was calculated as following formula IV = (0.93*MUFA) + (1.35* linoleic acid) + (2.62*linolenic acid) [8]. Quality index (Iq) was calculated as follows; oleic acid / (palmitic acid + linoleic acid) [4]. Statistical analyzes was done JMP computer program.

RESULTS AND DISCUSSION

In this study major fatty acids palmitic acid, palmitoleic acid, stearic acid, oleic acid and linoleic acid were determined between 12.80-14.44, 0.80-1.06, 3.30-3.79, 71.40-73.08 and 6.96-9.16. Major fatty acids of olive oil samples were given in Table 1. [9]. reported the palmitic acid, palmitoleic acid, stearic acid, oleic acid and linoleic acid of olive oils of five olive variety (‘Nizip yaglık’, ‘Ayvalık’, ‘Kilis yağlık’, ‘Halhalı’ and ‘Karamani’) from Southeastern Anatolia between13.60-16.31, 1.06-1.57, 1.55-3.82, 60.83-71.44 and 7,90-13.37 % in fatty acids. [2]. reported palmitic acid, palmitoleic acid, stearic acid, oleic acid and linoleic acid of eleven olive oil sample from Southeastern Anatolia between 9.05-16.40, 0.8-2.2, 1.3-4.2, 63.4-75.4 and 5.6-15.3 % in fatty acids. Similar results were determined for palmitic acid with thats reported by [9] and [2]. While oleic acid was within the values determined by [2], it was determined to be higher than the values specified by [9]. All major fatty acid were determined between the limits of Turkish Food Codex for extra virgin olive oil [13].

Minor fatty acids of olive oil samples were given in Table 2. Myristic acid content was determined as 0.01 for five of six samples. Heptadecanoic acid, cis 10 heptodecanoic acid, linolenic acid, arachidic acid, eicosenic acid and behenic acid of samples were determined between 0.14-0.17, 0.21-0.30, 0.18-0.26, 0.42-.050, 0.12-0.56 and 0.08-0.14 % in fatty acids. Heptadecanoic acid, Cis 10 Heptodecanoic acid, linolenic acid and arachidic acid of oils of olive cultivar from Southeastern Anatolia were reported between 0.00-0.23, 0.22-0.38, 0.77-0.88 and 0.13-0.27 in fatty acids [9]. Palmitic acid, stearic acid, oleic acid, linoleic acid and linolenic acid of oils of 34 olive genotypes from Şırnak city (southeastern anatolia region) were reported between 10.34-20.92, 2.25-3.91, 49.33-67.96, 7.52-31.51 and 0.63-2.72 % in fatty acids [12].

[2], reported the heptadecanoic acid, heptodesenoic acid, arachidic acid, eicosenic acid and behenic acid of olive oils from Southeastern Anatolia between 0.06-0.15, 0.05-0.23, 0.17-0.69, 0.20-0.26 and 0.05-0.50 % in fatty acids. In a study conducted on different olive varieties in the Southeastern Anatolia region, a decrease in palmitic, stearic and oleic acid levels and an increase in linoleic acid were reported in the oils of late harvested olives [1]. Eicosenic acid content of one sample was slightly higher than permitted limit of Turkish Food Codex for extra virgin olive oil. All of the other minor fatty acids were detected between legal limits of extra virgin olive oil [13]. MUFA, PUFA, SFA, MUFA/SFA, IV and Iq of olive oil samples were given in Table 3. These values were calculated from analysis results fatty acids. MUFA, PUFA, SFA, MUFA/SFA, IV and Iq 77.41-78.25, 7.22-9.34, 17.00-18.46, 8.28-10.76, 81.33-84.78 and 3.07-3.59 % in fatty acids respectively. MUFA, PUFA and SFA, content oils of seven olive varieties from Turkey were reported between 53.88-67.04, 8.84-23.61 and 12.48-18.94 % in fatty acids [5]. [11] reported the quality index of oils from newly developed olive genotypes in the range of 3.0-4.5 (field average). In this study higher MUFA and lower PUFA content were determined than that's of reported by [5] and similar Iq was determined with [11].

CONCLUSION

It was observed that the olive oils analyzed in this research were similar to some of the fatty acid compositions reported for olive oils from the southeastern Anatolia region. However, some results from the same region were found to be different. It is thought that the observed differences may be due to effects such as olive variety, climate, harvest maturity and harvest year. The research showed that all fatty acids, except eicosenoic acid, were within legal limits of extra virgin olive oil. It was observed that eicosenoic acid was very close to the permissible limit, but slightly higher. It is thought that it would be beneficial to conduct more studies to regionally characterize the olive oils produced.

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Table 1. Major Fatty Acids of Olive Oil Samples (% in Fatty Acids)

Sample	Palmitic Acid (C16:0)	Palmitoleic Acid (C16:1)	Stearic Acid (C18:0)	Oleic Acid (C18:1)	Linoleic Acid (C18:2)
A	14.16±0.04a	1.00±0.01ab	3.34±0.02d	72.41±0.66a	6.96±0.06b
B	14.39±0.23a	1.06±0.05a	3.30±0.01d	71.40±0.21bc	7.34±0.08b
C	13.28±0.11b	1.03±0.04a	3.79±0.01a	72.98±0.18a	7.06±0.08b
D	12.80±0.35c	0.80±0.01c	3.41±0.01c	72.38±0.45ab	9.16±0.21a
E	13.44±0.03b	1.02±0.02a	3.39±0.03c	73.08±0.18a	7.27±0.33b
F	14.16±0.03a	0.94±0.01b	3.52±0.03b	70.83±0.47c	8.93±0.10a
CV (%)	1.32	1.56	1.18	0.55	2.18

Values expressed as the mean ± standard deviation. Different letters indicate statistical difference for each colon. CV: coefficients of variation.

Table 2. Minor Fatty Acids of Olive Oil Samples (% in Fatty Acids)

Samples	Myristic Acid (C14:0)	Heptadecanoic Acid (C17:0)	Heptodesenoic Acid (C17:1)	Linolenic Acid (C18:3)	Arachidic Acid (C20:0)	Eicosenic Acid (C20:1)	Behenic Acid (C22:0)
A	0.01±0.001a	0.14±0.001d	0.21±0.001d	0.26±0.001a	0.47±0.02b	0.46±0.007c	0.08±0.001c
B	0.01±0.001a	0.15±0.001c	0.23±0.001c	0.25±0.007b	0.50±0.01a	0.12±0.001e	0.08±0.001c
C	0.01±0.001a	0.14±0.001d	0.23±0.001c	0.25±0.007b	0.42±0.01c	0.56±0.007a	0.12±0.007b
D	0.01±0.001a	0.17±0.001a	0.30±0.01a	0.18±0.001d	0.48±0.01ab	0.49±0.01b	0.14±0.007a
E	0.02±0.001b	0.17±0.001a	0.27±0.01b	0.20±0.001c	0.45±0.01b	0.50±0.01b	0.11±0.001b
F	0.01±0.001a	0.16±0.001b	0.23±0.001c	0.24±0.007b	0.50±0.01a	0.36±0.01d	0.12±0.007b
CV (%)	-	0.15	1.16	1.90	2.47	2.66	4.73

Values expressed as the mean ± standard deviation. Different letters indicate statistical difference for each colon. CV: coefficients of variation.

Table 3. MUFA, PUFA, SFA, MUFA/SFA, IV and Iq of Olive Oil Samples.

Samples	MUFA	PUFA	SFA	MUFA/SFA	IV	Iq
A	77.41	7.22	18.16	10.72	82.07	3.43
B	76.11	7.59	18.42	10.03	81.33	3.29
C	78.58	7.31	17.75	10.76	83.25	3.59
D	77.37	9.34	17.00	8.29	84.78	3.30
E	78.25	7.47	17.58	10.48	83.11	3.53
F	75.88	9.17	18.46	8.28	83.24	3.07

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Research Article

The Use of Pumpkin and Melon Seeds Milk in The Field of Gastronomy

[1]Ayşe Aslı KARLI, [2]Banu KOÇ**

Abstract

Consumers today want food that is not only natural, nutritious, and tasty but also produced with respect for the environment. Today, researchers are focusing on the development of alternative milk and dairy products from plant-based sources, as there are not enough milk sources, consumers have different dietary preferences (vegan/vegetarian diets) and allergens and sensitivities to dairy products (lactose intolerance). Vegetable milk products are products obtained by extracting fruit, vegetable, cereal or seeds with water. These products are rich in vitamins, minerals and fiber and are also known as functional foods. The seeds are a source of vitamins, minerals and proteins, which can make them a good substitute for milk, especially in areas with food shortages. In this study, it was aimed at obtaining milk from freeze-dried melon and pumpkin seeds, producing pudding with the obtained milk, and determining the sensory quality characteristics of the pudding. The total phenolic content and antioxidant activity values of the powder, milk and pudding samples were also determined. The highest antioxidant activity (45.11%) and the highest total phenolic content (1232 mg GAE/100 g) were found in melon seed powder. The pudding samples prepared from melon and pumpkin seed milk were rated as moderately/good acceptable products according to the score scale.

Keywords: Vegetable Milk, Melon Seed Powder, Pumpkin Seed Powder, Pudding

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Introduction

Population growth and environmental pollution have begun to limit access to food. Studies on the utilization of waste in food production have gained importance today. Food waste, which is primarily used as fertilizer or animal feed, has recently been increasingly used to produce food. The most economical and beneficial solution to avoid environmental damage, especially by reducing the carbon footprint, is to use food waste for food fortification [26]. With their highly effective nutrient composition, seeds contain sources such as protein, iron, fiber, vitamins, and omega 3, which are necessary for maintaining a healthy life. The nutritional properties of seeds have made them an important food in the diet [14].

In our country, pumpkin seeds are generally preferred to be consumed as salty nuts and are also used for variety in breakfast bars, baked goods, salads, and cakes. The oil extracted from pumpkin seeds is preferred as edible oil as well as nutraceuticals (nutrients and nutritional components prepared in the form of medicines and used for therapeutic purposes) [26]. Pumpkin seeds are rich in proteins, phytosterols, polyunsaturated fatty acids, antioxidants, vitamins (especially carotenoids and tocopherol), and minerals (potassium and magnesium) [17, 22, 28].

The melon, a member of the genus Cucumis from the Cucurbitaceae family, is a round or oval, fragrant, richly juicy fruit, usually yellow or orange in color. The first production of melons dates back 5000 years [16]. In the studies on the origin of the melon, some researchers refer to the cultivation areas as those of Central Asian Turks [13]. It is stated that melon seeds contain 30–40% fat, 15–25% protein, 15% fiber and the minerals potassium, calcium, magnesium, iron, copper, zinc and phosphorus [2]. In West Africa and Nigeria, it is consumed as a thickener, egusi soup, melon ball snacks and ogiri, a fermented condiment. In our country, melon seed milk, called sübye (pepitada), is traditionally consumed in Izmir [27]. It is also known that this plant milk is consumed as "melon milk" in Nigeria and Iran and as a "melon seed drink" in South America [24].

Plant-based milk production from legumes and seeds with a high oil content has been used since the 13th century. Today, with the realization of its nutritional richness, plant-based milk production has been developed and offered to consumers as a substitute for milk and dairy products. Vegetable milk, also called vegan mil, is a definition for products derived from plants that resemble milk but do not contain milk fat or important components of milk. Although these products do not have the content of dairy products, they are defined as an alternative product with similar sensory and functional properties, especially for people with lactose intolerance. These products are described as healthy alternative products with healthy fatty acids and carbohydrates as well as vitamins and antioxidants [23, 11].

There is no study in the literature on the use of powdered products obtained by freeze-drying pumpkin and melon seeds as a substitute for milk in a traditional product, the pudding dessert. In this study, it was aimed at obtaining milk from freeze-dried melon and pumpkin seeds, producing pudding with the obtained milk, and determining the sensory quality characteristics of the pudding.

Materials and Methods

Materials

Melon (*C. melo* subsp. *melo* cv. Kırkağaç) and pumpkin (*Cucurbita Moschata*) were obtained from a supermarket in Adana, Turkey. The seeds of the products obtained were sorted. Pumpkin seeds and melon seeds were stored at -24˚C. All drying experiments were completed within the 3 days. All solvents and chemicals were obtained from Sigma Aldrich (St. Louis, MO, USA).

Freeze Drying

Freeze drying of seeds was carried out at −55 °C for 48 h using a freeze dryer (FreeZone 6, Labconco, USA). After drying, the samples were grinded, packaged and stored.

Preparation of the seed milk

Melon and pumpkin seed milk was prepared according to the method described by [5], with some modifications to obtain a higher dry matter content, which was desirable for the drying process. 50 g of seeds were crushed in an electric blender (Sinbo, SCM2934, Türkiye) for one minute without adding water. Then 150 ml of tap water was added to the crushed seeds to mix them a second time. After adding water, the powder product was kept for 2 hours, and the mixture was filtered into a glass container using a sieve (212 µm). Then the residue was removed by adding 75 ml of tap water for mixing. The slurry was filtered again through a 212 µm sieve. After the fifth mixing and the third filtration process, the milk was finally obtained.

Production of Pudding

The pudding (muhallebi in Turkish) was made using traditional methods. The control pudding consisted of 200 mL of cow's milk, 34 g sugar, 5 g wheat starch, 8 g flour, and 2 g butter. Cow's milk was used for the formulation of the control pudding. Instead of cow's milk, 200 mL of melon seed milk was used for melon seed milk pudding and 200 mL of pumpkin seed milk for pumpkin seed milk pudding. The cooking time was set at 20 minutes.

Analysis

Moisture contents of freeze-dried powders and vegetable milks were determined by infrared moisture analyzer at 105˚C. A colorimeter (3NH colorimeter, China) was used for color measurements of (CIE L^*, a^* and b^* values). The bulk density (ρ_b) and tapped density (ρ_t) and porosity (ε) of samples were determined using the method reported by [15]. In addition, the particle density (ρ_p) of the powders was analyzed by a pycnometric technique with 2-propanol as previously reported by [4]. The flow characteristics of the powder samples were described as Carr index (CI) and Hausner ratio (HR) using equations (1) and (2), respectively [9,12]. The wettability and dispersibility of the powders were determined as reported by [15]. The solubility of the powders was performed as specified by [8]. Solubility (%) was calculated using the mass difference (after drying/initial weight).

$$CI = \frac{(\rho_t - \rho_b)}{\rho_t} \times 100. \quad (1)$$

$$HR = \frac{\rho_t}{\rho_b} \quad (2)$$

Samples (1 g) were extracted with 80% methanol (10 mL) in a shaking incubator (Mikrotest, MSC-30, Turkey) at 37 °C for 1 h. The mixtures were then centrifuged (PCE Instruments, CFE100, Germany) at 6000 rpm for 10 min, and the supernatants were collected. The total phenolic content (TPC) in the methanol extract of the samples was determined by the Folin–Ciocalteau method and the procedure reported by [1]. All spectrometric measurements were performed in triplicate. The calibration curve was prepared using gallic acid, and the results were expressed as gallic acid equivalents (mg GAE/ g dry weight). The antioxidant activity of the samples was measured using the DPPH (1,1-diphenyl-2-picrylhydrazyl) radical-scavenging method according to [7] with some modifications. The extracts (0.1 mL) were added to a 2.9 mL DPPH solution (100 ppm). The mixture was shaken vigorously and left in the dark at room temperature for 30 min. Then, the absorbance was measured at 517 nm. Percent inhibition of DPPH radical was calculated as follows:

$$Percent\ Inhibition\ (I\%) = \frac{A_{control} - A_{sample}}{A_{control}} \times 100 \quad (3)$$

A panel of 10 semi-trained panelists, consisting of staff and students, evaluated the sensory characteristics of the pudding samples. The sensory evaluation of the samples gave grades using a 5-point hedonic scale according to appearance, taste and flavor, consistency, and overall liking. The degree of preference was determined using an acceptance test on a 5-point hedonic scale (5 = like extremely; 3 = neither like nor dislike; 1 = dislike extremely).

Statistical Analysis

The presented results are an average of triplicate observations. The statistical analysis was carried out using SPSS Statics 24.0 (SPSS Inc., Chicago, USA). One-way ANOVA was conducted to define the effect of drying methods on quality parameters of avocado powder. Duncan multiple range test was used at 95 % significance level to see the significant differences.

Results and Discussion

Physical Properties of Powder

The physical properties of freeze-dried melon seed powder (MSP) and freeze-dried pumpkin seed powder (PSP) are shown in Table 1. Bulk density and tapped density were important characteristics for the classification of the final product obtained by freeze-drying [4]. Low bulk density of food powder was not desirable due to the possibility of product oxidation [25]. The bulk densities of melon seed powder and pumpkin seed powder were 406.5 kg/m^3 and 330 kg/m^3, respectively. The high moisture content of powder products can affect factors such as flowability and stickiness. Good flowability and non-stickiness of the products are particularly important for the processing, storage and packaging of powder products [6]. The flowability indicator is the Carr index and the stickiness ratio is the Hausner ratio. [9] classified the flowability of powders as very poor, poor, fair, good and very good for Carr Index values above 45, 35–45, 20–35, 15–20 and below 15 respectively. In addition, flowability is described as good if the Hausner ratio is below 1.2, medium if it is between 1.2 and

1.4 and poor if it is above 1.4 [12]. In addition, if the Hausner ratio is less than 1.25, it is interpreted as free-flowing, and if it is greater, it is interpreted as an indicator of poor flowability [10]. In this study, both products were found to have poor flow properties with Hausner Ratio and Carr Index values.

The reconstitution properties of powders are also listed in Table 1. These properties were very important quality parameters for ready-to-dilute beverages. The wettability of the powders was a critical step of the redispersing process; the wetting time was used to study the instantaneous behavior of the product [18]. The wettability time was 2.96 s and 5.63 s for MSP and PSP powders, respectively. According to these results, MSP and PSP powders exhibited a low degree of solubility and dispersibility in water.

Color

The values L^*, a^*, and b^* of freeze-dried melon seed powder (MSP), freeze-dried pumpkin seed powder (PSP), melon seed milk (MSM), and pumpkin seed milk (PSM) are given in Table 2.

Among the powdered products, melon seed powder had the highest lightness value. The a* and b* values of melon seed powder are higher than those of pumpkin seed powder. The b* values of melon seed powder and pumpkin seed powder were close to each other. In the melon milk and pumpkin milk samples, the pumpkin milk was found to have the highest lightness value, while the lightness values of these products were close to each other. When the a^* and b^* values of melon and pumpkin seed milk were analyzed, it was found that the a^* values of pumpkin and melon seed milk were close to each other, but there was a difference between the b^* values, and the pumpkin seed milk was more yellowish. In a study on the use of melon seed powder in the production of gluten-free tulumba dessert, the results of the color analysis of melon seed powder L^*, a^*, and b^* values were found to be 56.76, 1.73, and 15.25, respectively [20]. In the study conducted by [2] on the quality characteristics and shelf life of melon seed milk, L^*, a^*, and b^* values of 75.56, 1.24, and 2.44, respectively, were determined. It is assumed that the main reason why the studies in the literature differ from each other and from this study is due to the different drying methods. In addition, the differences in the raw materials (melon and pumpkin) and the different methods used in the production of milk are responsible for these deviations.

Total Phenolic Content and Antioxidant Activity

The total phenolic content and antioxidant activity of melon seed powder (MSP), pumpkin seed powder (PSP), melon seed milk (MSM), pumpkin seed milk (PSM), cow's milk, MSM pudding, PSM pudding, and cow's milk pudding are given in Table 3. The highest antioxidant activity and the highest total phenolic content were found in melon seed powder. The values for total phenolic content and antioxidant activity of the control cow's milk were higher than those of the melon and pumpkin seed milks. It was found that the values of antioxidant activity and total phenolic content of the milk obtained from melon seeds and pumpkin seeds were close to each other. The total phenolic values of the pudding made with plant milk were close to the total phenolic values of the plant milk, but the antioxidant activity values

decreased by half. In addition, the levels of total phenolic content and antioxidant activity of pudding made from plant milk are significantly higher than those obtained from cow's milk. [19] determined that the total phenolic content of plant milk produced from pumpkin seeds was between 212.6-423.2 mg GAE/L, and [21] determined that the total phenolic content of melon seeds was 304.10 mg/100 g. The levels of total phenolic content determined in this study were consistent with the studies in the literature.

Sensory Quality Characteristics of Pudding

Pudding is a very well-known dairy dessert characterized by its semi-solid form, creamy texture, and the possibility of adding various flavored products [3]. The results of the sensory evaluation of the MSM pudding, PSM pudding, and cow's milk pudding are shown in Table 4. In terms of appearance characteristics, the pudding made with melon seed milk was liked more than the pudding made with pumpkin seed milk, with no significant difference found between them, but a significant difference was found compared to the pudding made with cow's milk. Although no undesirable foreign or sour odor or taste was detected in any of the samples, the PSM pudding, with its flavor characteristics, was more liked than the MSM pudding. There was no statistically significant difference between pudding made from MSM and pudding made from PSM in terms of the flavor criterion, while there was a statistically significant difference with pudding made from cow's milk. It was found that the melon flavor was intense in the MSM pudding sample, but the pumpkin seed flavor was less intense in the PSM pudding. While there was a statistically significant difference between the overall liking scores of the samples. The pudding samples prepared from melon and pumpkin seed milk were rated as moderately/good acceptable products according to the score scale

Conclusion

In this study, pumpkin seed milk and melon seed milk were obtained from freeze-dried melon and pumpkin seed powders. The resulting plant milk was used to produce pudding and compared with pudding made from cow's milk in terms of sensory quality characteristics. The sensory analysis showed that the sensory quality of the pudding made with melon seed milk was higher than that of the pudding made with pumpkin seed milk, but the scores were close to each other. The pudding made with cow's milk achieved the highest score. When comparing the total phenolic values and antioxidant activity of the pudding samples, pudding samples obtained from melon and pumpkin seed milk were higher than those of the pudding made with cow's milk. In this study, it was found that the milk obtained from melon and pumpkin seeds can be used making desserts.

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Table 1. Physical Properties of Powder

Properties/Sample	MSP	PSP
Moisture Content	8,63±0.37	7.49±0.58
Bulk density (kg/m^3)	406.5±4.85	330.22±11.10
Tapped density (kg/m^3)	526.2±5.17	483.97±13.35
Particle density (kg/m^3)	1352.64±8.99	817.20±90.66
Carr Indeks	22.75±1.29	24.72±3.39
Hausner Ratio	1.29±0.02	1.33±0.06
Wettability (s)	2.96±0.57	5.63±0.11
Dispersability (%)	31.83±3.04	34.25±3.06
Solubility (%)	16.00±5.9	17.91±0.65

* MSP, Melon seed powder, PSP, pumpkin seed powder

Table 2. Color Values of Melon Seed Powder (MSP), Pumpkin Seed Powder (PSP), Melon Seed Milk (MSM) and Pumpkin Seed Milk (PSM)

Sample	*L*	*a*	*b*
MSP	71.33±0.69	11.71 ±0.15	34.53 ±0.38
PSP	66.33 ±0.35	6.57 ±0.33	33.99 ±0.20
MSM	55.46 ±0.18	-1.94 ±0.14	1.63 ±0.05
PSM	56.56 ±0.16	-2.78±0.30	11.77 ±0.34

* MSP, Melon seed powder, PSP, pumpkin seed powder, MSM, melon seed milk and PSM, pumpkin seed milk

Table 3. Antioxidant Activity and Total Phenolic Content of Samples

Sample	Total Phenolic Content (mg GAE/100 g)	Antioxidant Activity (Inhibition %)
MSP	1232.78±28.48	45.11 0.08
PSP	1100.26±3.99	28.85±1.16
MSM	459.96±14.23	10.43 1.35
PSM	442.21±6.85	12.85 0.16
Milk	655.23±10.65	14.82 0.25
MSM Pudding	464.50±15.36	6.45 0.15
PSM Pudding	398.04±14.90	7.04 0.83
Milk Pudding	37.63±9.56	2.66 0.43

* MSP, Melon seed powder, PSP, pumpkin seed powder, MSM, melon seed milk and PSM, pumpkin seed milk

Table 4. Results of Sensory Analysis of Pudding

Sensory Characteristics	MSM Pudding	PSM Pudding	Milk Pudding
Appearance	3.55 ^{b}±1.15	3.10 ^{b}±1.55	4.50 ^{a}±0.89
Taste and Flavor	3.16 ^{a}±1.21	3.37 ^{a}±1.46	4.47 ^{b}±0.61
Consistency	3.70 ^{a}±1.30	3.40 ^{a}±1.14	4.10 ^{a}±1.02
Overall Liking	3.47 ^{b}±0.84	2.84 ^{a}±1.07	4.89 ^{c}±0.32

* MSP, Melon seed powder, PSP, pumpkin seed powder, MSM, melon seed milk and PSM, pumpkin seed milk

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Research Article

Determinatiom of Germinated Wheat Flour Addition on the Sensory and Texture Properties of White Bread and Whole Wheat Bread

[1]Selin ILGINLI, [2]İlkay GÖK**

Abstract

The aim of this study is to compare and identify addition of germinated wheat flour on the sensory and texture properties of white bread and whole wheat bread. Germinated wheat flour was mixed with white flour and whole-wheat flour at the ratio of 50% (w/w). Sensory analysis was conducted on the bread samples to evaluate their appearance, texture, taste, aroma, and overall satisfaction. Results showed that bread prepared with germinated wheat flour mix was more fragile, rough and moisty internal structure. However in terms of taste, no significant differences among the samples ($p>0.05$) were found. The aroma analysis revealed a noticeable grain and wheat scent. Overall, bread made from germinated wheat flour provided more palate satisfaction compared to the other types of bread. Texture analysis also yielded similar results, indicating that bread prepared with germinated wheat flour was thicker, less elastic, and less sticky than the others. According to the statistical analysis, germinated wheat flour bread was generally considered as successful, and can bu used for bread preparation by mixing with wheat flour.

Keywords: Germination, Germinated Wheat, Functional Antioxidant Food, Wheat Germ, Bread, Wheat

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Introduction

In societies, grains and grain products are considered fundamental sources of nutrition. Bread, in particular, is seen as the most crucial grain product. However, in the world we live in today, bread is no longer perceived as the sole staple food source. It has evolved into diverse products that cater to changing consumer preferences, securing its place in the market. This diversification has prompted the need to redefine the product mix. Today, bread has transcended its fundamental utility and ascended to a level where it is not only a staple but also a genuine and differentiated product [13].

Germination is an economical and simple method to enhance nutrient quality. Nutrient levels in sprouts have been shown to be higher compared to non-germinated seeds, and several studies have demonstrated elevated levels of anti-nutrients in sprouts [47]

Edible grain and seed sprouts, when used as a food source, provide the human body with numerous antioxidants. These types of foods are considered particularly rich in antioxidant compounds. The main antioxidants found in such foods include:

- Chlorophyll
- Vitamins A, E, and C
- Phenolic compounds
- Zinc
- Selenium

Additionally, broccoli, radish, Brussels sprouts, mustard, and cabbage sprouts contain a variety of glucosinolates and isothiocyanates [24].

Seed germination/sprouting has been practiced since ancient times. However, its importance has increased in recent times, particularly in the context of healthy nutrition. Sprouts contain numerous components that are essential for human health, contributing to the prevention of various diseases [36]. Edible germinated grains are obtained through the process of soaking seeds in water for a specific period, followed by washing and draining, and then allowing them to germinate under suitable humidity and temperature conditions. The germinating process brings about significant changes in the nutritional components of edible seeds. These changes are influenced by environmental conditions such as temperature, duration, lighting, as well as the type of seed and cultural diversity [24].

Today, some consumers incorporate the shoots of seeds germinated from legumes, grains, brassicas, and root vegetables into their diet by consuming them as salads. Additionally, germinated seeds and the functional foods derived from them are beginning to find their place in the food industry. Various germinated grains are used in a range of food products in the food industry, including breakfast items, salads, soups, pasta, and baked goods [27].

The increasing awareness of conscious consumption has elevated the value of germinated grains and vegetables in food consumption. Particularly in recent years, germinated food products have become highly popular in the gastronomy sector. The habit of germinating is spreading globally, not only among those who engage in sports and prioritize their nutrition but also among a broader audience [19].

Materials and Methods

Material Production

Germinated wheat flour and other wheat flours were obtained from the Kappadokia brand in Kırşehir. Yeast and salt were sourced from local markets.

White Flour: Kappadokia, Kırşehir/Turkey
Whole Wheat Flour: Kappadokia, Kırşehir/Turkey
Germinated Wheat Flour: Kappadokia, Kırşehir/Turkey
Dry Yeast: Dr. Oetker, Turkey
Water: Şahinbey/Gaziantep Tap Water, Gaziantep/Turkey
Instant Yeast: Dr. Oetker, Turkey
Salt: Billur, Turkey

For each type of flour, the same brand of salt and yeast has been used. The amount of water was determined based on the water absorption capacity of the flour.

Sensory Analysis

The initial phase of the sensory analysis of the bread used in this study was conducted at the Gaziantep Gastronomy Academy, involving gastronomy undergraduate and graduate students, as well as chefs from Bulla, the academy's practice restaurant. The panelists participating in the sensory analysis comprised 48 individuals, ranging in age from 20 to 53 (28 males, 20 females). As the bread samples were intended for general consumer preference, panelists did not receive specific sensory analysis training.

Additionally, a sensory analysis was carried out at the İzmir/Torbalı Public Education Center (Torbalı HEM). In this sensory analysis, 23 untrained panelists (15 males, 8 females) were involved. The overall sensory analysis, conducted with a total of 71 untrained panelists [29], has been completed through this process.

The produced bread samples, sized uniformly (4 cm^2 surface area, 6 cm height) and including both crust parts (bottom crust and top crust), were coded differently (e.g., 07, 04, 93, etc.). They were presented to untrained panelists, consisting of both men and women, along with drinking water, a saliva cup, green apple, and plain crackers. Panelists were instructed to conduct a comprehensive sensory analysis for each bread sample, answering questions related to color, taste, appearance, texture, overall liking, and likelihood of repurchase on the sensory analysis form provided to them [22, 29, 32]

For the evaluation of the samples in the sensory analysis, a 7-point hedonic scale was utilized, and panelists were asked to rate each bread sample on a scale from one to seven [22, 23]. To provide a more detailed view of the obtained sensory analysis results, a radar chart method was preferred. Random sample selection was employed in the sample selection process. The universe in the consumer test conducted within the scope of sensory analysis consisted of Gaziantep Gastronomy Academy employees, Bulla Applied Restaurant chefs, Torbalı Public Education Center (HEM) employees, and participants in basic culinary training programs.

Texture Analysis

Texture analysis was conducted using the Texture Analysis Machine (TA.XT PlusC, Stable Micro Systems, UK) at the Central Laboratory of Çukurova University (ÇÜMERLAB). Bread samples, each cut to a size of 36mm on every side, were tested with a 25mm aluminum cylindrical probe. Each sample was compressed up to 50%. Texture Profile Analysis (TPA) was performed with a force resolution of 0.1g and a speed range of 0.01-40mm/s. Five types of bread samples (white bread (WHE), whole wheat bread (WWHE), bread made from germinated wheat flour (GWHE), bread made from a mixture of white flour and germinated wheat flour (MGWHE), and bread made from a mixture of whole wheat flour and germinated wheat flour (MWWHE)) were produced in triplicate, totaling 15 bread samples. Four samples were taken from each bread, cut to a diameter of 36mm, and flattened at the top for better results. This test measured the hardness, adhesiveness, springiness,

gumminess, chewiness, and resilience degrees of the bread.

The bread subjected to this test was baked on the same day as the test and cooled for at least 2 hours. After cooling, the packaging process was carried out, and the samples were processed 3 hours after packaging.

Statistical Analysis

The sensory analysis of five bread samples (WHE, WWHE, GWHE, MGWHE, MWWHE) was conducted with 71 untrained panelists. They were asked to evaluate and score the appearance features, texture characteristics, taste attributes, aroma qualities, and overall satisfaction. Descriptive statistics, including mean scores, standard deviations (SD), variances, and medians, were calculated. For multiple comparisons, the data were subjected to statistical analysis using Analysis of Variance (ANOVA) with IBM SPSS software (version 26.0; SPSS Inc., Chicago, IL, USA). Sensory analysis results were analyzed using Friedman tests, and LSD ranking tests were employed to determine if there were any significant differences between the samples ($p < 0.05$).

Volume and Mass Determination

The masses of the prepared bread doughs were measured with precision scales before baking. All bread samples were baked at the same temperature (190°C). Approximately 60 minutes after baking (once cooled), each bread sample underwent a second measurement. The difference in mass between the dough stage and after baking was recorded. The percentage mass loss was determined according to the formula in equation(2.1). Mass loss results are shown in *figure 2.*

$$\text{Mass Loss } (\%)=(A1-A2/A1)\times 100 \quad (2.1)$$

Where:

$A1$: Weight of raw dough (g)

$A2$: Weight of the baked bread after cooling (g)

Volume determination based on the displacement principle with flaxseed was carried out in accordance with the AACC 10-05 method [1]. The flaxseed method involves adding a known volume of flaxseed to a food sample. After the sample is filled with flaxseeds, the volume filled by the seeds is measured. This measurement determines the volume of the sample filled with seeds. The specific volume value for the bread samples was determined by relating the volume value to the weight value. The volume was determined according to the formula in equation (2.2). Volume loss results are shown in *figure 3*.

$$\text{Volume}=(A\times C/A\times B)*\text{H}=FV/HFC \quad (2.2)$$

Where:

A: Cap Width

B: Cap Height

C: Cap Volume

H: Height Difference

FV: Flaxseed Volume

HFC: Height of Flaxseed in the Cap

Study Results and Discussion

In this study, wheat that had been germinated, dried, and ground into flour was used, in contrast to its more commonly used form of ungerminated wheat. For ease of comparison, bread was produced using white flour (WHE) and whole wheat flour (WWHE). Additionally, bread was made

by combining germinated wheat flour with other flours (white flour and whole wheat flour) in a mixture (50% each). These five bread samples (WHE, WWHE, GWHE, MGWHE, MWWHE) underwent both sensory and texture analyses.

According to the sensory analysis, the bread made from germinated wheat flour (GWHE), the main subject of the study, was found to be firmer compared to the other types of bread used in the study. The bread made from germinated wheat flour (GWHE) exhibited fragility in texture characteristics, which correlated with its firmness. In the breads produced in a mixture (MGWHE and MWWHE), it was observed that the addition of germinated wheat flour resulted in an increase in fragility and firmness levels *(Table 3).*

In terms of aroma characteristics, the bread made from germinated wheat flour had a distinct aroma and taste, with a pronounced intensity of grain and wheat aromas. Looking at the overall satisfaction results, GWHE lagged behind WHE in terms of general liking, but it showed similar results to the other bread types (WWHE, GWHE, MGWHE) used in the study*(Figure 5).*

Texture analysis results confirmed that, in line with sensory analysis findings, GWHE exhibited a higher level of firmness compared to the others. The spreadability level of GWHE was lower, consistent with the fragility level observed in sensory analysis*(Table 2, Table 3).*

In conclusion, despite having an unusual taste and texture, GWHE received positive feedback in all conducted analyses. It was determined that germinated wheat flour can be successfully used in breadmaking, either alone or in a mixture.

According to the analysis and observations:

1. **General Liking:** The bread made from germinated wheat flour (GWHE), while not a conventional bread type, was well-received*(Figure 5).*
2. **Purchase Intention:** Consumers were able to discern the difference in bread made from germinated wheat flour (GWHE)*(Figure 5).*
3. **Consumability Decision and Intent to Re-Try:** White bread (WHE) was more liked compared to bread made from germianted wheat flour (GWHE)*(Figure 5).*
4. **Flavor Perception:** Bread made with a mixture of germinated wheat flour (MGWHE and MWWHE) was closely associated with whole wheat bread (WWHE)*(Figure 5).*
5. **General Satisfaction:** Bread made from germinated wheat flour (GWHE) was compared to rye bread in terms of color and texture*(Figure 5).*
6. **Texture Analysis:** While white bread (WHE) distinctly differed in texture, other bread samples (WWHE, GWHE, MGWHE, MWWHE) showed similar results *(Table 2).*

Additionally, it is noted that all bread samples, regardless of the inclusion of germinated wheat flour (GWHE), underwent the same stages and tests in the simple bread-making logic. The bread made from germinated wheat flour (GWHE) did not progress to the product development stage due to this reason. In future studies, it is believed that if bread made from germinated wheat flour (GWHE) is prepared using different techniques and yeast types, the results could be significantly different. Factors such as fermentation time, degree of

fermentation, and type of yeast strongly influence the bread's form. Therefore, selecting the right bread-making stages and ingredients according to germinated wheat flour becomes crucial for facilitating the general population's preference for white bread in the next stage.

Conclusion

In studies by Ünsal et al. (2020) [43], observations were made in sensory analysis results of flatbreads made with germinated flour. An increase in the proportion of germinated wheat flour led to a decrease in symmetry and shape, affecting all sensory characteristics significantly. In particular, a noticeable decrease in pore structure was observed in flatbreads, dependent on the amount of germinated wheat flour used.

Kömürcü (2021)[26], compared bread samples with the addition of germinated esperia wheat flour with a control sample. It was determined that as the amount of germinated flour increased, the liking ratio decreased. Evaluation of this bread in terms of taste, aroma, and general liking suggested that using less germinated wheat flour would result in a bread closer to general liking. In this study, GWHE received less preference compared to the familiar WHE. Despite its unfamiliar taste and texture, GWHE's liking situation remained average in sensory analyses*(Figure 5)*. Therefore, GWHE represents a healthy bread alternative.

In conclusion, the bread made from germinated wheat flour has been identified as an alternative bread type based on sensory analysis and texture analysis results. It was well-received in sensory analysis, showing aromatic qualities, and was repeatedly desired to be tried by the panelists. Structurally, it exhibited similarities to commonly consumed bread types today (white bread, whole wheat bread) and was found to be consumable. The mixed bread types produced in the study for diversification purposes (MGWHE and MWWHE) also received positive feedback in sensory analysis. White bread made from common wheat flour (WHE) was generally preferred as a familiar taste. Other bread types, excluding WHE, showed similar results in terms of preference. Whole wheat bread (WWHE), a well-known variety, showed similar results to bread made with germinated wheat flour. These bread varieties were found to be functional alternatives for daily use, indicating ease of use and potential for regular consumption*(Table 2, Table 3)*.

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Table 1. Standard Bread Recipes

Standard White Bread Recipe	250 g white flour, 163 ml water, 2 g instant yeast, 3 g salt
Standard Whole Wheat Bread Recipe	250 g whole wheat flour, 167 ml water, 2 g instant yeast, 3 g salt
Standard Germinated Wheat Bread Recipe	250 g germinated wheat flour, 168 ml water, 2 g instant yeast, 3 g salt
Standard Germinated Wheat and White flour, 165 Bread Recipe	125 g germinated wheat flour, 125 g white ml water, 2 g instant yeast, 3 g salt
Standard Germinated Wheat and Whole wheat Wheat Bread Recipe	125 g germinated wheat flour, 125 g whole flour, 167 ml water, 2 g instant yeast, 3 g salt

Fig1 Flow Chart for Bread Preparation

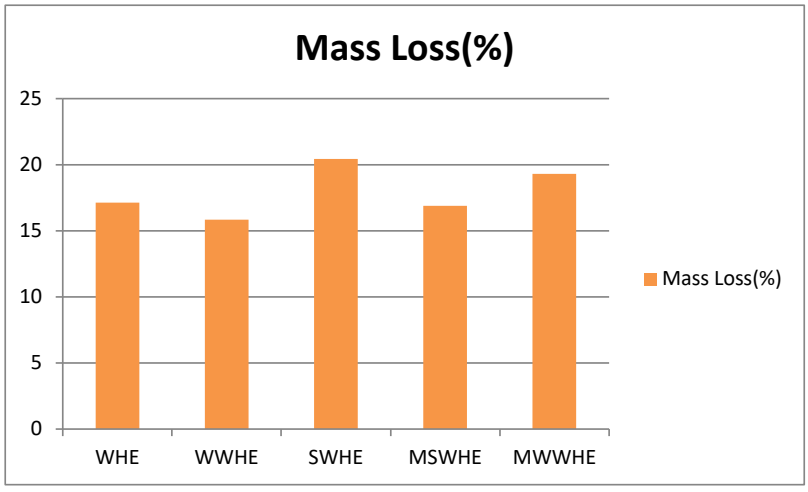


Fig 2 Specific Mass Graphic

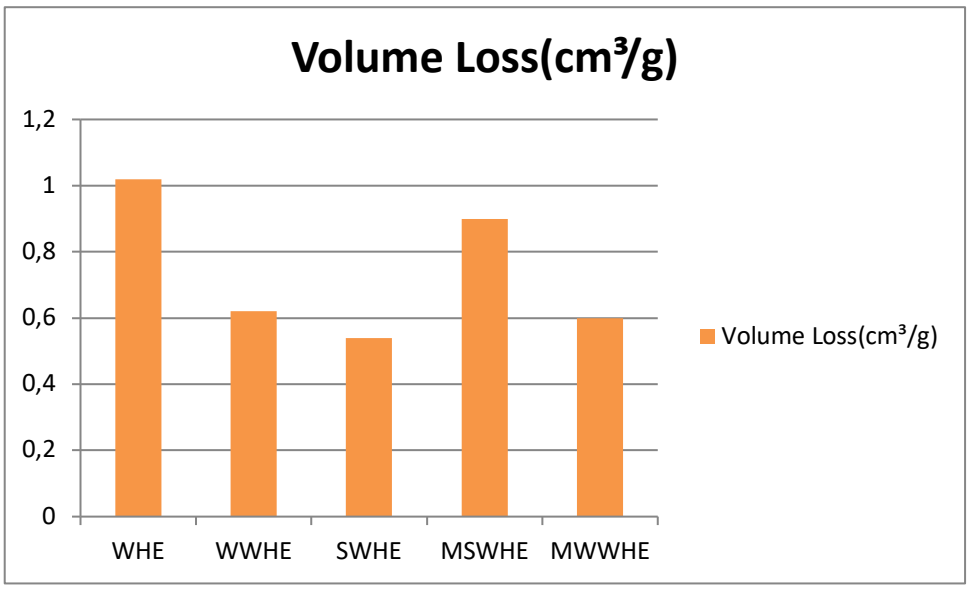


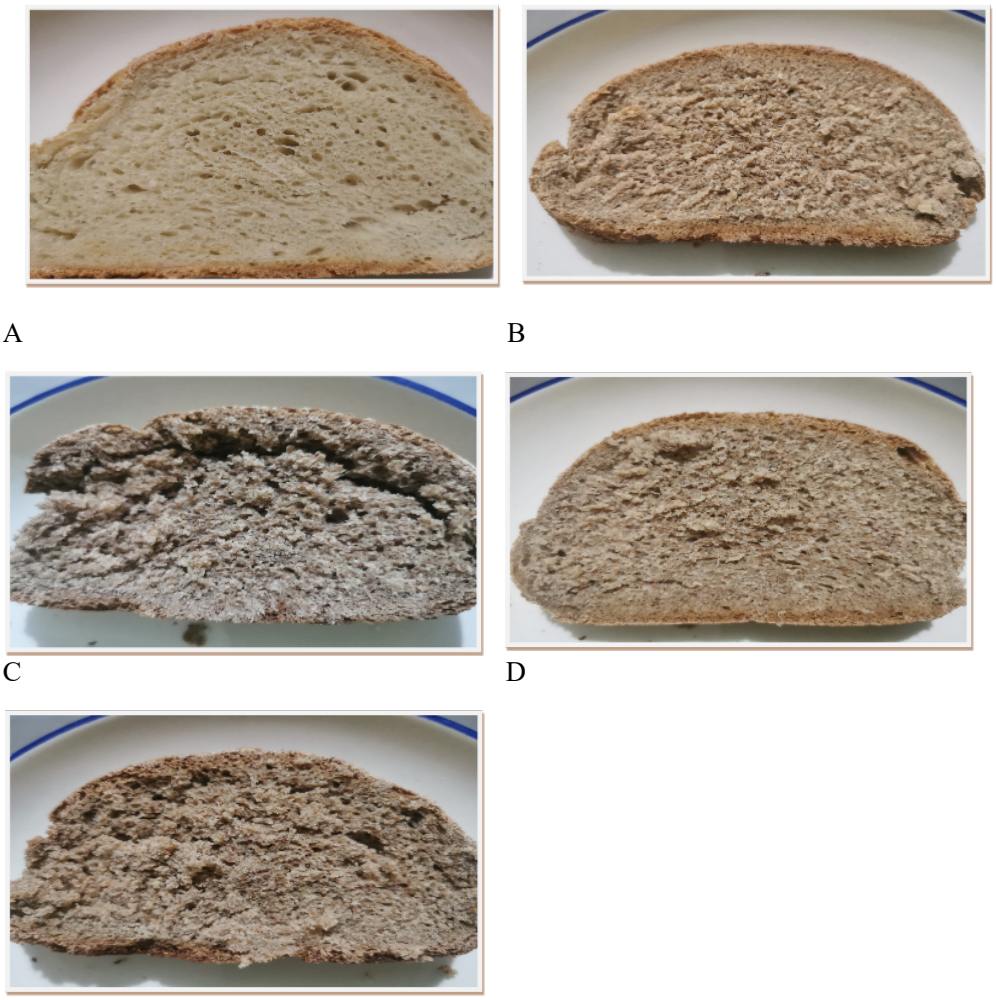
Fig 3 Specific Volume Graphic

Table 2 Texture Analysis of Bread Samples

Types of flour used	Firmness (g)	Springiness (mm)	Gumminess (g)	Chewiness (Nmm)	Durability (N)	Stickiness (mm)
WHE	517,6 ± 40,52^{e}	0,25 ± 0,01^{a}	376,50 ± 28,97^{e}	382,52 ± 35,39^{d}	23,49 ± 0,001^{a}	0,18 ± 0,001^{a}
WWHE	663,29 ± 36,36^{d}	0,18 ± 0,004^{b}	384,85 ± 19,35^{d}	292,23 ± 18,51^{e}	0,05 ± 0,0007^{cd}	0,14 ± 0,0008^{cd}
GWHE	1768,86 ± 140,01^{a}	0,18 ± 0,18^{d}	989,66 ± 111,99^{b}	744,74 ± 88,20^{b}	0,05 ± 0,001^{c}	0,13 ± 0,005^{d}
MGWHE	1621,47 ± 330,72^{b}	0,18 ± 0,005^{b}	1061,03 ± 56,41^{a}	803,21 ± 42,73^{a}	0,05 ± 0,0008^{c}	0,15 ± 0,001^{bc}
MWWHE	1019,24 ± 26,51^{c}	0,21 ± 0,004^{c}	682,46 ± 18,42^{c}	600,20 ± 23,49^{c}	0,07 ± 0,0005^{b}	0,16 ± 0,0005^{ab}

Table 3 Consumer Test Sensory Analysis Data of Bread Varieties

Types of Flour Used	Appearance Features	Texture Features	Flavor Attributes	Flavor Profile	Overall Satisfaction
WHE	5,43 ± 0,21^{d}	3,08 ± 0,27^{e}	2,90 ± 0,26^{e}	2,51 ± 0,09^{e}	5,72 ± 0,35^{a}
WWHE	5,13 ± 0,44^{e}	3,75 ± 0,20^{c}	3,36 ± 0,51^{b}	4,35 ± 0,27^{d}	4,75 ± 0,31^{b}
GWHE	6,71 ± 0,35^{a}	4,16 ± 0,18^{a}	3,35 ± 0,12^{c}	4,69 ± 0,26^{a}	3,90 ± 0,32^{d}
MGWHE	5,76 ± 0,31^{b}	4,00 ± 0,21^{b}	3,23 ± 0,03^{d}	4,46 ± 0,18^{c}	3,10 ± 0,10^{e}
MWWHE	5,58 ± 0,11^{c}	3,58 ± 0,26^{d}	3,55 ± 0,21^{a}	4,50 ± 0,19^{b}	3,90 ± 0,30^{c}



A B C D E

Fig4 White Bread Pore Appearance (A), Whole Wheat Bread Pore Appearance (B), Germinated Wheat Bread Pore Appearance (C), MGWHE Pore Appearance (D), MWWHE Pore Appearance (E)

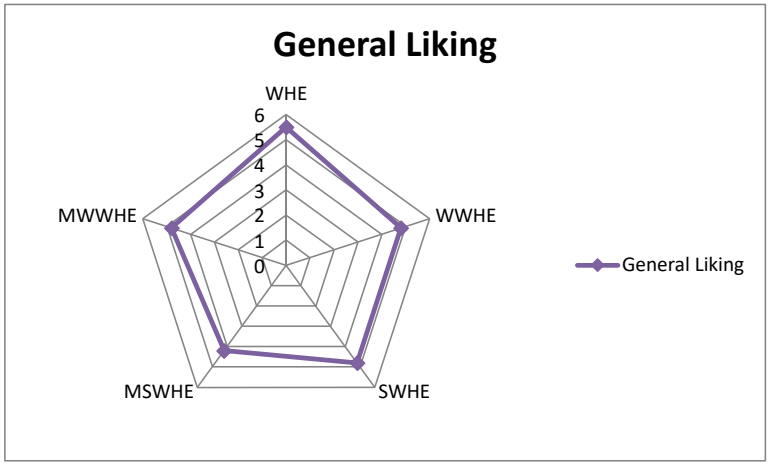


Fig5 General Liking Graphic

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Research Article

High Hydrostatic Pressure (HHP) Processing on Food Bioactives

Ozlem TOKUSOGLU [1,2]

Abstract

High Hydrostatic Pressure (HHP) is an excellent food processing technology that has the potential to retain the bioactive constituents with health properties in fruits, cereals, and other foods. HPP-treated foods retain more of their fresh-like features and can be marketed at a premium over their thermally processed counterparts. HPP can have an effect on food yield and on sensory qualities such as food color and texture. High pressures can also be used to enhance extraction of compounds from foods. Recent studies have shown that high pressure extraction (HPE) can shorten processing times, and provide higher extraction yields while having less negative effects on the structure and antioxidant activity of bioactive constituents. The use of HPE enhances mass transfer rates, increases cell permeability, and increases diffusion of secondary metabolites. Also, HPP increased the capacity to extract phenolic constituents, and HPP-treated samples retain higher levels of bioactive compounds and bioactive phenolics.

Keywords: High Hydrostatic Pressure (HHP), Processing, Bioactives, Foods

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Introduction

In recent years, there has been increasing interest in moving from conventional methods of processing for food preservation toward the use of novel and emerging nonthermal food processing technologies, to control or eliminate microbes, enzymes, or chemical reactions and deliver more fresh-like, nutritious, value- added, and safe high-quality food products to satisfy consumer demand for less processed foods with an extended shelf-life and that are free from additives. High pressure processing (HPP), irradiation, pulsed electric field (PEF), ultraviolet light (UVL), and other nonthermal processing methods are becoming increasingly popular to treat foods, capable of eliminating harmful microorganisms in foods, while *minimizing* thermal degradation reactions in foods compared to thermal processing [2, 3, 16]. More information on various nonther- mal processing technologies is available from the Nonthermal Processing Division of the Institute of Food Technologists (www.ift.org/divisions/nonthermal/). The major aims of utilizing these methods are to improve food safety and food quality concomitantly, and thereby facilitate the development of innovative high value products and the creation of new opportunities for expanding markets.

High hydrostatic pressure or ultrahigh-pressure processing or HPP is one technology that has begun to fulfill its potential to satisfy both consumer and scientific requirements, and it is a leading alternative in replacing thermal processing in some food applications in the drive to meet increasing consumer demand for foods featuring improved organoleptic qualities and higher acceptance [24]. The technology is especially beneficial for heat sensitive products [3]. HPP can be conducted at ambient or moderate temperatures, thereby eliminating thermally induced cooked off- flavors. Compared to thermal processing, the HPP of foods results in products with a fresher taste, better appearance, and texture.

In the 1990s, the HPP technology began gaining prominence in the food industry because of its advantages for inactivating microorganisms and enzymes at ambient or relatively low temperatures with less adverse affect on the flavor, color, and nutritional constituents of foods compared to conventional thermal processes [19, 20, 4].

Companies began marketing commercial HPP-treated products, such as jam, fruit juice, sauces, rice wine, and rice cake [17]. In recent years, HPP has been successfully implemented in food industries worldwide (United States, Europe, and Japan) to extend shelf-life or improve safety of fruit products (avocado, guacamole, salsa, applesauce, fruits juices, etc.), ready-to-eat (RTE) meats, and fresh oysters. Among the most successfully commercialized HPP-treated food products are sliced, cooked ham and a range of tapas products in Spain [15]. Tapas products are convenient heat-and serve mini-pork sausages made with Spanish paprika and marinated diced pork. The benefits of HPP for increasing the retention of food organoleptic attributes and other more fresh-like characteristics combined with increased convenience and extended shelf-life will no doubt continue to increase for the market [8].

The HPP provides an alternative means of killing vegetative bacteria, spoilage organisms (yeasts and molds), viruses, and bacterial spores that can cause food spoilage or food-borne diseases without compromising food sensory quality attributes or food nutrients. In many cases with vegetative pathogens and bacterial spores, the survival curves for organisms subjected to HPP exhibit nonlinear inactivation kinetics “shoulders” [10, 14] or “tailing” [11]. Predictive microbiol-ogy models provide convenient tools to assess whether a process will ensure the safe preservation of foods. Two examples are the quasi-chemical model [30, 29] and the Weibull distribution model, both of which are nonlinear models that can accurately describe the nonlinear inactivation kinetic models of vegetative pathogens (*Escherichia coli*, *Listeria monocytogenes*) in foods treated with HPP [11, 12]. An enhanced version of the

quasi-chemical model is being developed to account for unique features of the inactivation kinetics of bacterial spores of *Bacillus amyloliquefaciens* by HPP, including the presence of a subpopulation of increased baro resistance.

As indicated above, the HPP pasteurization safely inactivates vegetative cells, and some enzymes, while retaining nutritive content, sensory attributes, and a fresh-like character of foods. HPP tends to affect cell membranes, enzymes, and large molecules. Macromolecules such as proteins and starches can undergo changes in their native structure during HPP treatments (and during thermal treatments) that can be used to influence texture. Doona et al. [9] studied the retrogradation kinetics, water dynam- ics, and thermometric characteristics of HPP-treated wheat starch [1,12,14,24]. In contradistinction, HPP generally has little effect on the primary structure of low molecular weight food individual components such as flavors, vitamins, pigments, peptides, lipids, and saccharides. In general, HPP tends not to destroy the covalent bonds between atoms of the constituent molecules. The energy used during HPP treatment is relatively low and covalent bonds tend to have low compressibility below 2000 MPa, whereas the process affects hydrogen bonds and ionic and hydrophobic interactions in macromolecules. HPP protects nutraceuticals, functional food ingredients, and so on, whose functionality can be compromised by the use of heat. HPP is an innovative, emerging technology with potential for optimizing intake of nutrient and nonnutrient phytochemicals in foods [8].

High Pressure Processing (HPP)

HPP conditions in the range of 300–700 MPa at moderate initial temperatures (around ambient) are generally sufficient to inactivate vegetative pathogens for pasteurization processes, some enzymes, or spoilage organisms to extend shelf-life. For example, HPP is used to inactivate spoilage organisms and extend shelf-life (and provide extra assurance against pathogens) at conditions of 400 MPa and 15°C (in a 320 L unit) for sliced cooked ham, and 600 MPa and 15°C (in a 218 L unit) for dry-cured ham and tapas products [15]. To inactivate bacterial spores such as *Clostridum botulinum* for the production of ambient shelf-stable, low-acid foods requires high pressure and high temperature combina- tions. Such processes typically involve high pressures in the range of 600–800 MPa and higher initial temperatures around 80–90°C. During pressurization, rapid adiabating heating generates temperatures above 121°C. This process, achieving commercial sterility in low-acid foods, is called "pressure-assisted thermal sterilization" and has several technical advantages over conventional thermal sterilization methods (shorter processing times, improved food quality, and increased energy efficiency).

HPP: Principles of Operation

A typical HPP process (Ohio State University 2009) involves treating packaged food products (usually in flexible plastic pouch material or plastic bottles) by loading them into a high pressure vessel filled with an incompressible transmission fluid (usually water) then closing the vessel. Using one or more pumps, fluid is pumped into the vessel to increase the pressure to the intended end-level and then the pumping is stopped. The packaged food products are subjected to these combinations of high hydrostatic pressure and temperature for a sufficient time to induce inactivation of the target organisms or enzymes and then the pressure is released. Since pressure is transmitted uniformly throughout the package and product, the food retains its original shape. This works particularly well for unstructured foods containing water, whereas foods with internal air pockets (strawberries, marshmallows, some bakery items) tend to col- lapse, and dry solids tend not to have enough moisture to allow efficient microbial destruction. When the product is removed from the high pressure vessel, the package is covered with water. In the case of RTE meats, for example, some companies use cold drying equipment to remove the water and prepare the package for labeling and packing. Cold drying helps

maintain product quality by reducing the potential effects of using heat.

Large-Scale and research HPP equipment

There are a number of high pressure equipment manufacturers worldwide making HPP equipment for food preservation (Ohio State University 2009). Systems cost in the range of $0.5–2.5 million, depend- ing on the size of the vessel, extent of automation, and other design features. Units can range in sizes of 420 L, 350 L, or 150 L, and systems run in batch or semicontinuous modes of operation for food industry purposes. Figure 1 demonstrates large-scale HPP equipment, including a 420 liter unit (Figure 1a) and a 350 liter unit (Figure 1b). Both units are in a horizontal configuration. The illustration in Figure 1c depicts the semicontinuous mode of operation, in which carriers full of packaged food products enter on a conveyor belt from the left and are loaded into the pressure vessel. After the HPP treatment, the carriers are removed from the pressure vessel, and exit the area to the right on the conveyor belt. The treated products are removed from the carrier, dried, labeled, and packed for shipping and distribution. Figure 1d shows a smaller vertical configuration HPP unit used to process oysters. A 215 L batch system has the capacity to produce about 10 million pounds of food per year and products may cost about $0.03–0.10 more per pound than thermally processed counterparts.

Significantly smaller laboratory-scale units are also manufactured to operate on the same general basic principles but for research purposes, and they are available at research facilities and universities worldwide. Figure 2 depicts one such unit that operates at pressures of 100,000 psi with sample sizes of 10–30 mL. Figure 2a shows the front view of the high pressure research unit with the accompany- ing workstation to the left and the bath cover emanating from the top. In addition to containing heat in the bath where the high pressure chamber vessel cylinder is located, the bath cover also acts as a potential safety shield. Figure 2b details the arrangement of the pressure unit's components, with the chamber vessel cylinder located in the covered bath and connected to the pump and an assortment of valves for regulating and releasing high pressures. Figure 3a shows the actual interior of the high pressure unit, with the components labeled in accordance with the schematic of the interior side view in Figure 3b. Figure 4a shows the top view of the bath with the bath cover removed and the bath fluid drained to reveal the chamber vessel cylinder (with the cap removed). The corresponding cross-sectional side view of the bath (Figure 4b) shows the chamber vessel cylinder with the cap in place and a thermocouple inserted into the sample chamber.

HPP on Bioactive Components

Consumer perception of food quality depends not only on microbial quality, but also on other food factors such as biochemical and enzymatic reactions and structural changes [4, 23]. In this context, HPP can have an effect on food yield and on sensory qualities such as food color and texture [18]. High pressures can also be used to enhance extraction of compounds from foods. Recent studies have shown that high pressure extraction (HPE) can shorten processing times, and provide higher extraction yields while having less negative effects on the structure and antioxidant activity of bioactive constituents. The use of HPE enhances mass transfer rates, increases cell perme- ability, and increases diffusion of secondary metabolites [13, 28]. Also, HPP increased the capacity to extract phenolic constituents, and HPP-treated samples retain higher levels of bioactive compounds [1, 31, 32, 33, 34, 36, 37].

HPP effects on antioxidant Phenolics and antioxidant activity

The study of Patras et al. (2009) was undertaken to assess the effect of HPP treatments and conventional thermal processing on antioxidant activity, levels of bioactive antioxidant compounds (polyphenols, ascorbic acid, and anthocyanins), and the color of strawberry and blackberry purées [21]. It was reported that key antioxidants (cyanidin-3-glycoside, pelargonidin-3-glucoside, and ascorbic acid)

in strawberry and blackberry purées and the antioxidant activity of these purées were quantified after various HPP treatments (400, 500, 600 MPa/15 min/10–30°C) and thermal treatments (70°C/2 min). Table 1 shows the antioxidant indices of HPP-treated and thermally processed strawberry and blackberry purées [21]. The three different pressure treatments did not cause any significant changes in ascorbic acid levels. Following thermal processing ($P_{70} \geq 2$ min), the ascorbic acid content degraded by 21% compared to the unprocessed purée. Similarly, no significant changes in anthocy- anin compounds were observed in HPP-treated and unprocessed purées, while conventional thermal treatments significantly reduced the anthocyanin levels (Patras et al. 2009). Patras et al. [21] reported that antioxidant activity of HPP-treated strawberry and blackberry purées were significantly higher than in thermally processed purées [21].

Qui et al. (2006) studied the stability and isomerization of lycopene by HPP. Standard lycopene and tomato purée were pressurized at 100, 200, 300, 400, 500, or 600 MPa for 12 minutes and at controlled temperature (20 ± 1°C), then stored at refrigerator temperature (4 ± 1°C) and ambient laboratory tem- perature (24 ± 1°C) under lightproof conditions. Afterward, HPP-treated and controlled lycopene and its *cis*-isomers in tomato purée samples were measured [27] by HPLC and IR spectral analysis after 2, 4, 8, and 16 days of storage (Table 2). It was found that 500 and 600 MPa led to the highest reduction of lycopene, while 400 MPa could retain the maximal stability of lycopene [27]. The highest stability of lycopene in tomato purée was found when pressurized at 500 MPa and stored at 4 ± 1°C in the study of Qui et al. [27] which retained most of the total lycopene content in tomato purée (6.25 ± 0.23 mg/100 g; see Table 2). It was established that HHP is an alternative preservation method for producing ambient-stable tomato products in terms of lycopene conservation [27].

Prasad et al. [25]. determined that HPE has tremendous potential for use in flavonoid extraction. After 30 minutes of HPE of Litchi (*Litchi chinensis* Sonn.) fruit pericarp (LFP), the extract yield, total phenolic level, 1,1-diphenyl-2-picrylhydrazyl radical scavenging activity (DPPH), and superoxide anion scavenging ability were determined by Prasad et al. [25]. The extraction yield by treatments of 400 MPa HPE for 30 minutes was 30%, while that by conventional extraction (CE, control) was 1.83%. There was no significant difference in the total phenolic content (as mg/g DW [dry weight]) among the two extraction methods (HPE and CE). It was found that the DPPH radical scavenging activity obtained by HPE (400 MPa) was the highest (74%), while that of ascorbic acid was the lowest (44%), when using a 10 mg/mL concentration. Additionally, HPE could provide a more effective alternative than CE, because HPE requires less organic solvents and a shorter extraction time [25]. Table 3 describes the quantification of the individual flavonoids epicatechin (EC), epicatechin gallate (ECG), catechin (C), and procyanidin B_2 and total flavonoids from LFP tissues by conventional extrac- tion (CE), ultrasonic extraction (UE), and HPE. Both EC and ECG were identified and quantified as the major flavonoids, while C and procyanidin B_2 were identified as the minor compounds [25]. The total flavonoid content detected was 0.65, 0.75, 0.29, and 0.07 mg/g dry weight by HPE at 200 and 400 MPs, UE, and CE, respectively. The HPE increased the flavonoid extraction yield up to 2.6 times in comparison with UE, and up to 10 times compared with CE.

Patras et al. (2009) reported of the effect of thermal and HPP on antioxidant activity and the color sta- bility of tomato and carrot purées. High pressure processed purées had significantly higher antioxidant capacities when compared to thermally treated samples. High pressure treatments at 600 MPa retained more than 93% of ascorbic acid (vitamin C) as compared to thermally processed tomato purées (Table 4; see [21].

Yen and Lin [35] reported that the level of retention of ascorbic acid in guava purée proceeded according to the following decreasing order: (400 MPa for 15 min) > (88–90°C for 24 s) > (600 MPa for 15 min). In the

study given by Patras et al. (2009), ascorbic acid levels were in the order (600 MPa) > (water immersed purées) > (400 MPa) > (500 MPa).

Zhang et al. [37] reported a higher extractability of flavonoids from propolis by HPE. Similar results were reported in the extraction of anthocyanins from grape by-products (Corrales et al. 2008), and flavones and salidroside from *Rhodiola sachalinensis* using HPE [37].

Prasad et al. [26] indicated that effects of HPE on the extraction yield, total phenolic content, and the antioxidant activity of longan fruit (*Dimpcarpus longan* Lour.) pericarp. The different solvent effects, solvent concentration (25–100%, v/v), solid-to-liquid ratio (1:25–1:100, w/v) were individually determined using these optimum extraction conditions. With utilizing the various pressures of HPP (200–500 MPa), durations (2.5–30 min), and temperatures (30–70°C), the extraction yield, total pheno- lics, and scavenging activities of superoxide anion radical and 1,1-dipheny l-2-picrylhydrazyl (DPPH) radical by HPE were determined and compared with those from a conventional extraction. The HPE pro- vided a higher extraction yield and required a shorter extraction time compared to CE. In addition, the total phenolics and the antioxidant activities of HPE were higher than those produced by CE. Table 4 shows the effect of thermal (TP) and high pressure treatments on anti-radical power, total phenols, total carotenoid content in tomato purées.

Tokuşoğlu et al. [31] reported that the total phenolics of table olives increased (2.1–2.5)-fold after HPP (as mg gallic acid equivalent/100 g). Phenolic hydroxytyrosol in olives increased on aver- age (0.8–2.0)-fold, whereas oleuropein decreased on average (1–1.2)-fold after HPP (as mg/kg dwt). Antioxidant activity values varied from 17.238–29.344 mmol Fe^{2+}/100 g for control samples, and 18.579–32.998 mmol Fe^{2+}/100 g for HPP-treated samples. In the HPP application of olives, total mold was reduced 90% at 25°C, and it was reduced 100% at 4°C based on the use of the Rose- Bengal Chloramphenicol Agar (RBCA). Total aerobic-mesofilic bacteria load was reduced 35–76% at 35 ± 2°C based on the use of plate count agar (PCA). Citrinin load was reduced 64–100% at 35 ± 2°C. Citrinin contamination (CITcont) at concentrations of 2.5 ppb and less in table olives degraded by 56%, whereas concentrations of 1 ppb CITcont in table olives degraded 100% [31].

Corrales et al. [7] examined the extraction capacity of anthocyanins from grape by-products (Figure 5) assisted by HPP and other techniques. The HPP at 600 MPa showed feasibility and selec- tivity for extraction purposes. After 1 hour of extraction, the total phenolic levels of grape by-product samples subjected to this novel HHP technology was 50% higher than in the control samples [7].

From a nutritional prospective, HPP is an excellent food processing technology that has the potential to retain the bioactive constituents with health properties in fruits, cereals, and other foods. HPP-treated foods retain more of their fresh-like features and can be marketed at a premium over their thermally processed counterparts.

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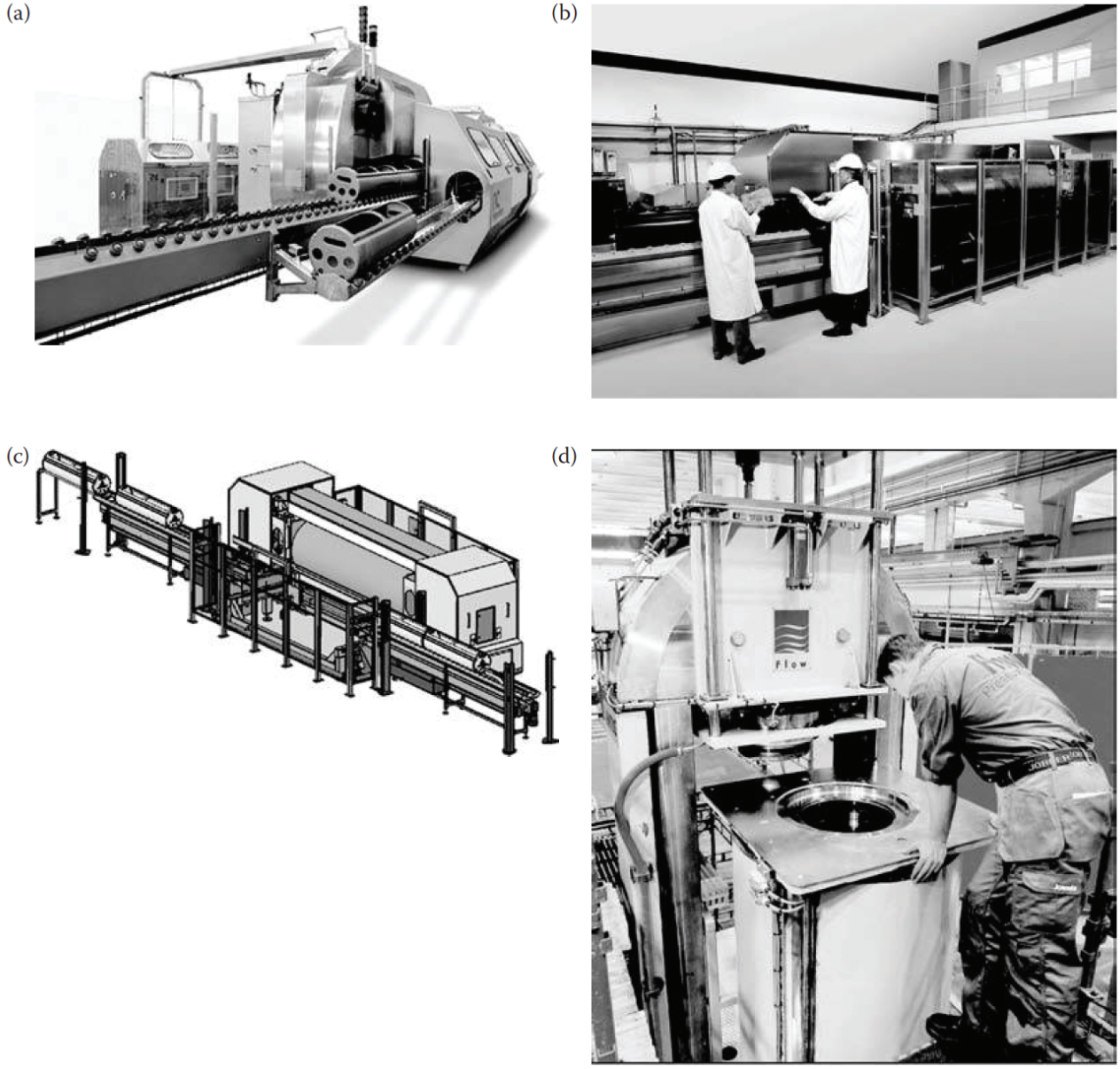

FIGURE 1 Large-scale HPP equipment: horizontal configuration units, including (a) a 420 liter unit (Courtesy of NC Hyperbaric.), (b) a 350 liter unit (Courtesy of Avure Technologies Inc.), and (c) an illustration depicting the semicontinuous mode (Courtesy of Avure Technologies Inc.), in which carriers enter the pressure vessel area on a conveyor belt from the left. After HPP treatment, the carrier is removed from the vessel and exits to the right on the conveyor belt. For comparison, the vertical configuration unit shown in (d) is used to process oysters (Courtesy of Avure Technologies Inc.)

TABLE 1
The Antioxidant Indices of HPP-Treated and Thermally Processed Strawberry and Blackberry Purées

	Antiradical Power $(g/L)^{-1}$		Total Phenols, mg GAE/100g DW[e]		Anthocyanin, mg/100g DW		Ascorbic Acid, mg/100g DW	
Treatment	**Strawberry**	**Blackberry**	**Strawberry**	**Blackberry**	**Strawberry[f]**	**Blackberry[g]**	**Strawberry**	**Blackberry**
Unprocessed	1.55 ± 0.07^{a}	2.86 ± 0.23^{a}	855.02 ± 6.52^{a}	1694.19 ± 3.0^{a}	202.27 ± 0.50^{a}	1004.90 ± 8.60^{a}	633.10 ± 9.31^{a}	nd
Thermal	1.16 ± 0.01^{b}	2.78 ± 0.26^{a}	817.01 ± 5.26^{b}	1633.62 ± 8.4^{a}	145.82 ± 6.40^{b}	975.28 ± 7.90^{b}	496.11 ± 0.04^{b}	nd
HPP (400 MPa)	1.25 ± 0.05^{b}	3.87 ± 1.11^{a}	859.03 ± 6.56^{a}	1546.26 ± 8.0^{a}	173.34 ± 6.51^{ab}	1039.21 ± 4.51^{a}	574.30 ± 3.93^{c}	nd
HPP (500 MPa)	1.30 ± 0.02^{ab}	3.70 ± 0.57^{a}	926.00 ± 5.93^{a}	1724.65 ± 0.7^{b}	202.53 ± 5.40^{a}	1014.21 ± 0.10^{a}	577.10 ± 6.52^{c}	nd
HPP (600 MPa)	1.33 ± 0.02^{a}	4.80 ± 1.79^{b}	939.01 ± 0.99^{c}	1778.44 ± 6.0^{b}	204.30 ± 1.60^{a}	1014.47 ± 1.00^{a}	599.11 ± 0.60^{c}	nd

Source: Adapted from Patras, A., Brunton, N. P., Pieve, S. D., and Butler, F., *Innov. Food Sci. Emerg. Technol.,* 10, 308–13, 2009b.
Notes: Values are mean ± standar deviation, $n = 3$, mean values in a column with different letters are significantly different at $p < .05$; nd = not detected.
[a] Dry weight.
[b] Expressed as mg/100g DW pelargonidin-3-glucoside.
[c] Expressed as mg/100g DW cyanidin-3-glucoside.

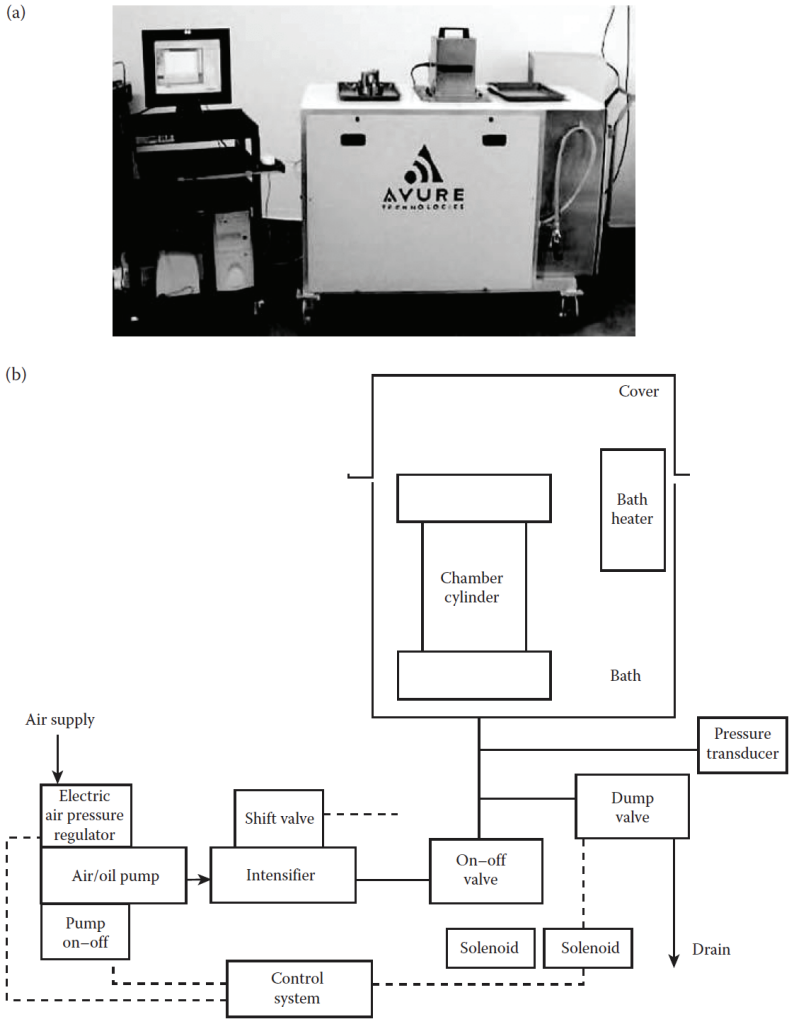

FIGURE 2 A smaller HPP unit for laboratory research that operates up to 100,000 psi with sample sizes of 10–30 mL: (a) front view of the HPP unit with the bath cover emanating from the top (Courtesy of Avure Technologies Inc.) and (b) schematic of the HPP unit locating the bath cover and HPP chamber inside the controlled temperature bath.

TABLE 2

Total Lycopene Losses in Lycopene Standard (as Percentage) and Total Lycopene Content in Tomato Puree (as mg/100g) as a Function of Storage Time at 4 ± 1°C, at Six Different HHP Conditions

Storage Time (Days)	Untreated (0MPa)	Pressure Applied (MPa)					
		100	200	300	400	500	600
LYCOPENE							
0	2.10 ± 0.02	2.10 ± 0.02	2.11 ± 0.02	2.11 ± 0.02	2.13 ± 0.02	20.8 ± 1.12	56.3 ± 3.02
2	3.05 ± 0.23	2.10 ± 0.02	2.11 ± 0.02	2.11 ± 0.02	2.13 ± 0.02	20.8 ± 1.12	56.3 ± 3.02
4	5.22 ± 0.34	2.40 ± 0.05	2.52 ± 0.09	2.34 ± 0.07	2.29 ± 0.09	21.7 ± 1.19	57.4 ± 3.34
8	6.13 ± 0.40	2.49 ± 0.07	2.63 ± 0.09	2.45 ± 0.09	2.39 ± 0.11	22.7 ± 1.21	57.4 ± 3.34
16	7.89 ± 0.44	4.21 ± 0.23	3.29 ± 0.28	3.78 ± 0.22	2.70 ± 0.28	25.7 ± 1.41	60.4 ± 3.76
TOMATO PUREE							
0	5.16 ± 0.12	5.33 ± 0.13	5.39 ± 0.11	5.48 ± 0.12	5.55 ± 0.12	6.25 ± 0.23	5.10 ± 0.10
2	5.18 ± 0.13	5.39 ± 0.12	5.42 ± 0.12	5.50 ± 0.13	5.50 ± 0.13	6.20 ± 0.21	5.11 ± 0.11
4	5.18 ± 0.13	5.37 ± 0.12	5.43 ± 0.12	5.51 ± 0.13	5.50 ± 0.13	6.21 ± 0.20	5.10 ± 0.12
8	5.17 ± 0.13	5.37 ± 0.13	5.40 ± 0.15	5.51 ± 0.13	5.48 ± 0.14	6.19 ± 0.22	5.08 ± 0.10
16	4.37 ± 0.10	5.17± 0.12	5.22 ± 0.16	5.26 ± 0.12	5.18 ± 0.13	6.11 ± 0.23	4.88 ± 0.12

Source: Adapted from Qiu, W., Jiang, H., Wang, H., and Gao, Y., *Food Chem.,* 97, 516–23, 2006.

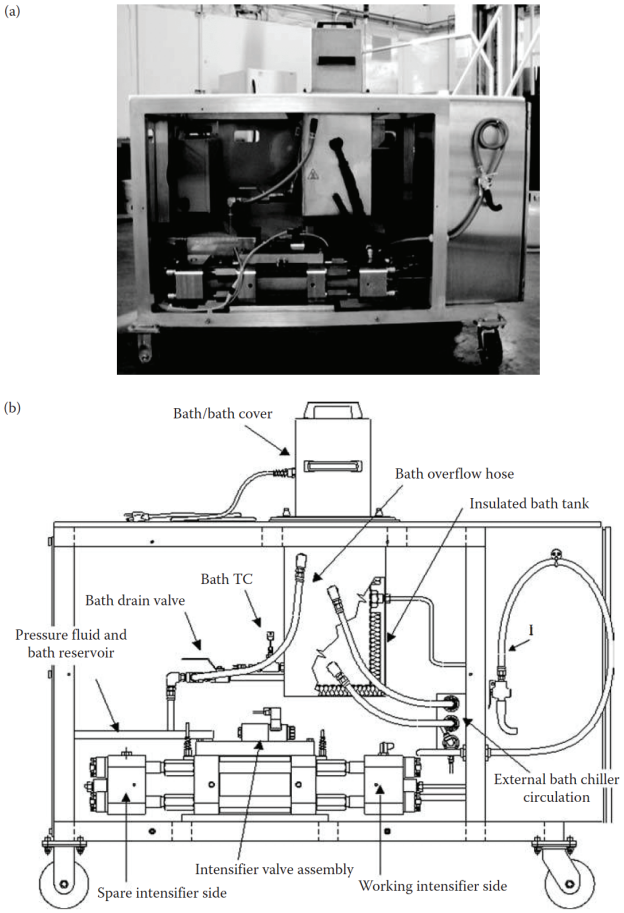

FIGURE 3 (a) Actual and (b) schematic diagram of the interior of the HPP unit viewed from the side.

TABLE 3

The Quantification of Individual Flavonoids from Litchi Fruit Pericarp Tissues by Conventional Extraction, Ultrasonic Extraction and High Pressured-Extraction

Flavonoids (mg/g DW)*	Extraction Methods			
	CE	UE	HPE at 200 MPa	HPE at 400 MPa
Epicatechin	0.0414 ± 0.001	0.16 ± 0.04	0.32 ± 0.002	0.348 ± 0.06
Epicatechin gallate	0.0121 ± 0.003	0.06 ± 0.01	0.019 ± 0.04	0.2527 ± 0.04
Catechin	0.0002 ± 0.0	0.0020 ± 0.0005	0.0016 ± 0.001	0.0160 ± 0.07
Procyanidin B_2	0.0175 ± 0.0003	0.0731 ± 0.0011	0.14 ± 0.03	0.1346 ± 0.03
Total flavonoids	0.0712 ± 0.004	0.2951 ± 0.051	0.6516 ± 0.07	0.7513 ± 0.2

Source: Adapted from Prasad, K. N., Yang, B., Zhao, M., Ruenroengklin, N., and Jiang, Y., *Journal of Food Process Engineering*, 32, 828–43, 2009a.

Notes: Values reported are means of triplicate determinations ($n = 3$) ± SD.
DW* = dry weight; CE = conventional extraction; UE = ultrasonic extraction; HPE = high-pressure extraction.

TABLE 4

Effect of Thermal (TP) and High Pressure Treatments on Antiradical Power, Total Phenols, Ascorbic Acid and Total Carotenoid Content in Tomato Purées

Samples	Antiradical Power $(g/l)^{-1}$	Total Phenols (mg GAE/100g)	Total Carotenoids (mg/100g βCE)	Ascorbic Acid (mg/100g)
Unprocessed	0.37 ± 0.04	360.56 ± 9.89	37.02 ± 3.07	204.83 ± 4.88
TP	0.34 ± 0.03	341.13 ± 4.83	33.40 ± 1.55	125.14 ± 5.174
HPT400 MPa	0.43 ± 0.01	337.36 ± 15.31	28.42 ± 2.65	115.25 ± 5.54
HPT500 MPa	0.40 ± 0.02	367.50 ± 17.58	30.25 ± 7.17	95.67 ± 3.71
HPT600 MPa	0.47 ± 0.03	371.73 ± 15.15	100.85 ± 0.11	192.13 ± 4.83
LSD*	0.04	24.35	8.44	9.05

Source: Adapted from Patras, A., Brunton, N., Da Pieve, S., Butler, F., and Downey, G., *Innov. Food Sci. Emerg. Technol.*, 10, 16–22, 2009a.

Notes: Values reported are means of triplicate determinations ($n = 3$) ± SD; expressed on dry weight basis.
*Least significant difference ($p = 5\%$).

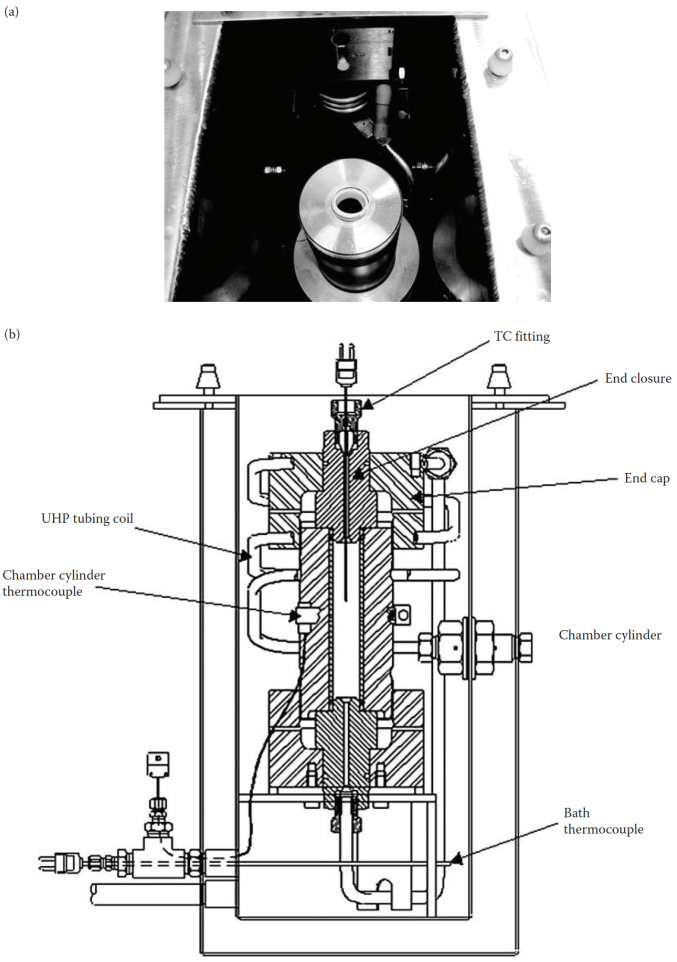

FIGURE 4 Close-up of the top view reveals (a) the actual chamber vessel cylinder (with the cap removed) inside the thermal bath (with bath cover removed and the bath fluid drained) and (b) the corresponding cross-sectional side view of the bath shows the chamber vessel cylinder with the cap in place.

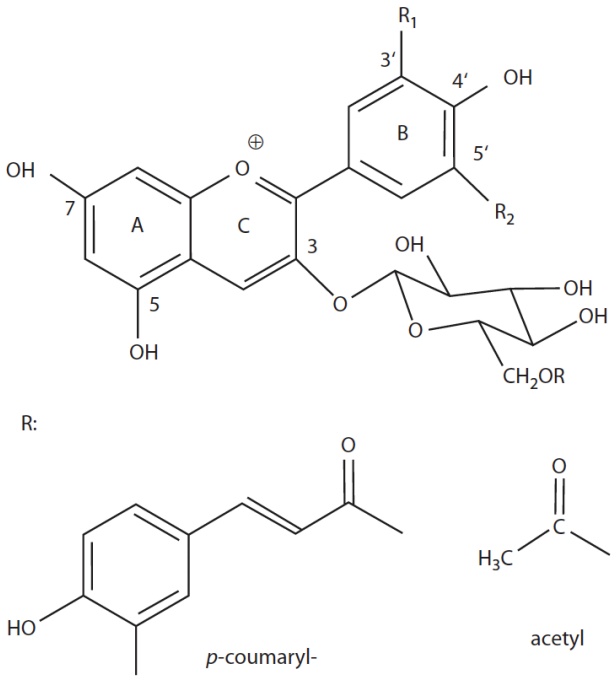

FIGURE 5 Anthocyanins in grape by-products. (Adapted from Corrales, M., Toepfl, S., Butz, P., Knorr, D., and Tauscher, B., *Innov. Food Sci. Emerg. Technol.*, 9, 85–91, 2008.)

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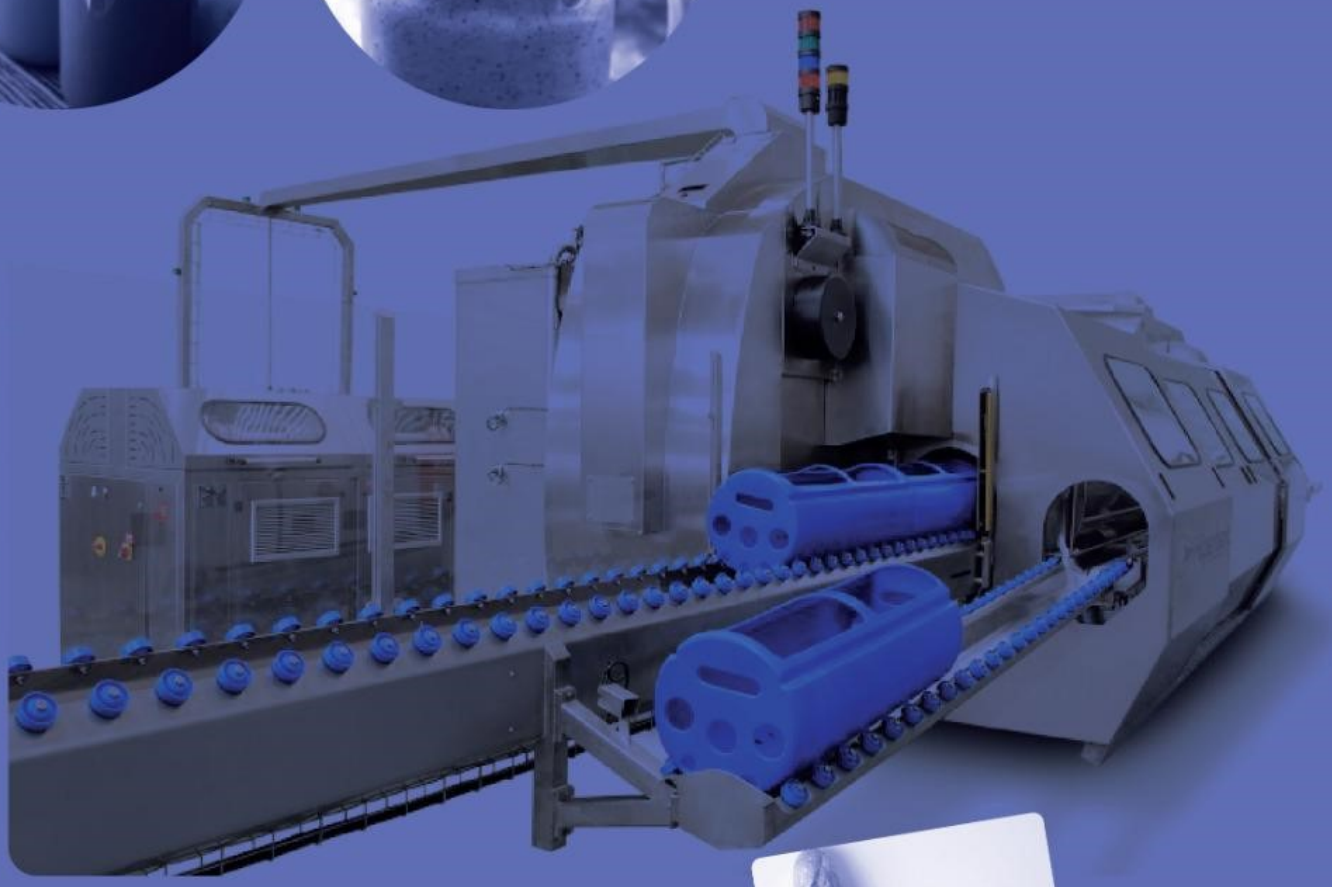

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