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Research Article

Influence of Moderate High-Pressure Homogenization on Quality of Bioactive Compounds of Functional Food Supplements

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Current interest in health has led to an increase in demand for functional food supplements as well as in industry concern for maintaining the bioactive compounds of such foods via the application of new technologies. In this study, we evaluated the effect of moderate high-pressure homogenization (HPH) treatments (80 and 120 MPa) versus thermal treatment (80°C, atmospheric pressure) on the functional bioactive compounds from four different functional supplements stored under accelerated conditions (40°C ± 2°C and 75% ± 5% relative humidity) for 6 months. HPH proved to be a better alternative than thermal treatment for functional supplements containing heat-sensitive compounds such as vitamin C, vitamin A, and unsaturated fatty acids (10-hydroxy-2-decenoic acid). The proanthocyanidin, cynarin, chlorogenic, and iron contents, however, were not initially affected by HPH treatments. The storage time caused important reductions in the majority of the compounds studied (mainly in vitamins C, B_{12}, and A), although the lowest decrease was found in the HPH samples. The food matrix had an important effect on the final functional composition and required the optimization of HPH treatments for each functional food supplement. HPH is a recommended alternative to thermal treatment for functional food supplements, in particular when they are rich in thermolabile bioactive compounds.

1. Introduction

The current trend in developed societies towards an unhealthy lifestyle results in factors such as stress, unbalanced diets and unhealthy eating habits, a lack of physical exercise, and many other factors that have a negative impact on our health. In addition, a number of diseases and physiological conditions (colon cancer, gastrointestinal conditions, and pregnancy) can produce iron deficiency, which is the most common and widespread nutritional deficiency worldwide that can lead to anemia [1]. Given this context, certain food supplements such as nutraceuticals can therefore play an important role in the improvement of health. A *nutraceutical* is a product that is isolated or purified from foods which provides medical or health benefits, including the prevention and treatment of diseases [2]. Some bioactive compounds found in fruits and vegetables (vitamins, phenolic compounds, unsaturated fatty acids, etc.) have exhibited good health-related properties when used for treating physiological disorders or chronic diseases. For example, the consumption of cranberries has been recommended for the prevention of urinary tract infections, as the proanthocyanidins found in the cranberries can inhibit the adherence of *Escherichia coli* to uroepithelial cells [3]. Phenolic acids, such as cynarin (1,3-*O*-dicaffeoylquinic acid) from artichoke, and other caffeoylquinic derivatives, such as chlorogenic acid (5-*O*-caffeoylquinic acid), have hepatoprotective activity [4]. Other important compounds include the unsaturated fatty acids, such as 10-hydroxy-2-decenoic acid (10-HDA), which is only available in royal jelly and has been shown to have pharmacological activities such as anticancer, anti-inflammatory, and antiallergic effects [5].

Nevertheless, functional food supplements have to be manufactured and then stored in proper conditions in order to be maximally effective. Along these lines, the use of novel technologies such as high pressure could help reduce the degradation of such bioactive compounds. There are two different high-pressure technologies depending on the application technique, although both have some common features. The first one, known as high hydrostatic pressure (HHP), uses water as the pressure transmitting medium and subjects liquid and solid foods to 100–1000 MPa at room or mild processing temperatures. This technique instantaneously transmits isostatic pressure to the product, independent of its size, shape, and food composition, yielding highly homogeneous products [6]. The second technique, high-pressure homogenization (HPH), is used as a continuous treatment, contrary to HHP. HPH is based on the application of high pressure to nonpacked fluid foods. In this case, a high-pressure pump is used in the homogenizer to force the liquid through a small orifice between a valve and its valve seat [7]. The homogenization pressures normally used in the industry range between 20 and 60 MPa, but more recently developed homogenizers can reach much higher pressures of up to 350–400 MPa. This magnitude of pressure leads to microbial and enzyme inactivation, which enhances the safety and shelf life of perishable foods while reducing the thermal effects on the foods' functional and sensory qualities [8]. The inactivation of microorganisms through HPH treatment has been described previously by Guan et al. (2016), where the application of HPH treatment (190 MPa/60°C/1 and 3 passes) to mango juice decreased counts of aerobic mesophilic bacteria (2 $\log_{10}$ CFU/mL), molds, and yeasts (under the detection limit, <1 CFU/mL) and increased shelf life (60 days stored at 4°C) [9]. Additionally, HPH treatment (150 MPa/55°C/3 passes) in saline solution, inoculated (5 log CFU/mL) with *Lactobacillus plantarum* and *Lactobacillus brevis*, decreased the viable count to 1.1 and 0.85 log CFU/mL, respectively [10]. On the other hand, HPH treatment (150 MPa/40°C/3 passes) to banana juice completely inactivated the pectate lyase activity, one log cycle reduction in mesophilic bacteria was obtained, and luminosity was significantly higher than when treated at higher pressures (200, 300, and 400 MPa). Also, the banana juice resulted to be brighter and less viscous than the untreated one [11].

There are numerous reports describing the effect of HHP on the bioactive content of fruit juice and purée [6, 8, 12]. However, to the best of our knowledge, there are a few studies related to the functional quality of nutraceutical products after using the HPH treatment. The nutraceutical industry uses microbiologically controlled ingredients [13] and does not require high doses of pressure for microbial inactivation but does need to maintain the bioactive compounds in the products. Indeed, the microbial, sensorial, and physicochemical quality of functional products have been previously reported by Martínez-Sánchez et al. [14]. The objective of this work was therefore to focus on the effects of moderate HPH pressures (80 MPa and 120 MPa) and a conventional heat treatment (0.1 MPa, atmospheric pressure) on the bioactive compounds of four functional supplements, which were stored for up to 6 months under accelerated conditions (40°C ± 2°C and 75% ± 5% RH). These moderate HPH pressures were selected as the nutraceutical industry uses functional ingredients that are microbiologically controlled [15] and the pathogenic reduction does not have to be guaranteed as these functional ingredients are material certified free of this kind of pathogenic bacteria.

2. Materials and Methods

2.1. Chemicals. To prepare the functional supplements, the following ingredients were used: dry cranberry (*Vaccinium macrocarpon* L.) extract with a guaranteed minimum proanthocyanidin content of 5% from Proprietary Nutritionals Inc. (Pharmachem Laboratories, Inc., NY, USA); dry artichoke extract from Naturex (Avignon, France) with a guaranteed minimum cynarin content of 1%; royal jelly with a minimum of 1.5% 10-HDA from Zhejiang Bee Products Co., Ltd. (Changtai, China); vitamins C and B_{12} from Sanofi Chimie (Paris, France); vitamins A and E from DSM Nutritional Products Ltd. (Basel, Switzerland); and ferrous gluconate as an iron source (Indukern, Barcelona, Spain). For analytical determinations, trifluoroacetic acid, acetic acid, sodium hydroxide, acetonitrile, chloroform, and methanol (high-performance liquid chromatography (HPLC) grade) were obtained from Sigma-Aldrich Química S.A. (Madrid, Spain). For vitamin B_{12} detection, the Easy-Extract® immunoaffinity column from R-Biopharm AG (Glasgow, Scotland) was used. Ultrapure water was obtained using a Milli-Q system (Academic Gradient A10, Millipak™ 40, Millipore, Paris, France). Moreover, the following standards obtained from Sigma-Aldrich Química S.A. (Madrid, Spain) were used: L-ascorbic acid, chlorogenic acid, vitamin B_{12} in the form of cyanocobalamin (V2876), vitamin A in the form of retinol acetate (469589), vitamin E in the form of α-tocopherol acetate (47786), and cyanidin-3-O-glucoside chloride. Cynarin (1,3-dicaffeoylquinic acid) was obtained from Apin Chemicals Limited (Abingdon, UK), and 10-hydroxy-2-decenoic acid (10-HDA) was obtained from Alfresa Pharma Corporation (Osaka, Japan). A standard solution was made of 1000 mg L^{-1} of iron in a matrix with 2% HNO_3 from High Purity Standards (Charleston, USA).

2.2. Functional Food Supplements. Four functional supplements with different functional purposes were made under Clean Room conditions (Class 10,000). One product made from cranberry juice extract rich in proanthocyanidins was targeted for cystitis treatment. The second functional product made from dry artichoke extracts was targeted for liver diseases. This product contained cynarin and other caffeoylquinic derivatives such as chlorogenic acid. The third product was made from royal jelly with a guaranteed minimum of 1.5% 10-HDA. The final functional product was an iron supplement for the prevention of iron deficiency anemia. The composition of these four supplements (cranberry, artichoke, royal jelly, and iron) with the addition of different vitamins is shown in Table 1. These compositions were made according to the common functional ingredients present in nutraceutical products in the current market.

TABLE 1: Composition of the functional supplements studied.

Product	Main compounds	Concentration per mL
Cranberry supplement	Cranberry dry extract juice* (*Vaccinium macrocarpon* L.)	720 mg
	Vitamin C	7.5 mg
Artichoke supplement	Artichoke dry extract** (*Cynara scolymus*)	15 mg
	Lemon juice	0.07 mg
	Vitamin E	2 mg
	Vitamin B_{12}	0.25 μg
Royal jelly supplement	Royal jelly***	15 mg
	Vitamin C	9 mg
	Vitamin B_{12}	0.25 μg
	Vitamin A	190 μg
	Vitamin E	1.6 mg
Iron supplement	Iron	1.5 mg
	Vitamin C	7.5 mg
	Vitamin B_{12}	0.03 μg
	Vitamin A	58 μg
	Vitamin E	0.54 mg

*Initial proanthocyanidin content in cranberry dry extract juice was 5.5 mg mL^{-1}. **Initial cynarin content was 0.12 mg mL^{-1} and chlorogenic acid was 0.22 mg mL^{-1} in the artichoke dry extract. ***Initial 10-HDA content in royal jelly was 2.5 μg mL^{-1}.

2.3. Manufacturing Procedure and Storage Conditions. The control pasteurization process for each functional food supplement was prepared as described below. In a heat exchanger (Inoxpaser, RP-13/006 model, Molina de Segura 129, Spain) at 0.1 MPa (atmospheric pressure), deionized hot water (110°C) was stirred at 20 rpm in a tank. When the temperature decreased to 80°C, plant extracts were added, with this process taking 15 min. After that, at 60°C, the liposoluble compounds were added and the temperature was maintained for 5 min, and at 40°C, nonliposoluble compounds and potassium sorbate were added (1.8 mg mL^{-1}, pH = 4.5 and 5.5). This last step took 5 more minutes. Samples were cooled until they reached 40°C and directly packed. For the HPH treatments, the same ingredients used in the control pasteurisation process were added in the same order to a tank at room temperature (22°C). In this case, and depending on the ingredient's solubility, it was not necessary to wait until the total dilution of each one. The pressure used in the HPH treatment works by helping to emulsify the mixture between lipo- and nonliposoluble compounds, thereby reducing the processing time from the 25 min needed for the pasteurization process to less than 10 min. The mixture was kept under continuous stirring. When each food supplement had all the functional ingredients, the mixture was processed using an instantaneous high-pressure homogenizer (GEA Niro Soavi, Ariete NS3006 Model, Parma, Italy) at a flow rate of 60 L h^{-1}. Two HPH doses were studied, 80 MPa with a final temperature after HPH treatment of 33°C and 120 MPa

where samples reached 43°C. The inlet temperature and pressure for both HPH treatments were room temperature and atmospheric pressure (0.1 MPa). The treatment time was 5 seconds for each treatment. After HPH treatments, samples were collected into a tank and the homogenized mixtures of each food supplement were directly packed.

Samples from all treatments were then bottled in sterile brown glass flasks (250 mL) under nitrogen gas and sealed with polyethylene caps. The full experiment was independently conducted three times, each time constituting a repetition. All samples were stored for up to 6 months under accelerated conditions (40°C ± 2°C and 75 ± 5% RH conditions) [13]. According to the European Medicines Agency, under such accelerated conditions, each month of storage is equivalent to 6 months at room temperature [15]. Bioactive compounds were analyzed in triplicate in all treatments at 0, 1, 2, 4, and 6 months of storage.

2.4. Water Soluble Vitamins

2.4.1. Ascorbic Acid. The detection of ascorbic acid in the samples was monitored by HPLC. A volume of 15 mL of sample was added to 10 mL of ultrapure water and shaken for 1 min on a vortex shaker (Reax, Heidolph, Schwabach, Germany). The sample was filtered through 0.45 μm filters (Micron Analitica S.A., Madrid, Spain) and directly analyzed by HPLC (Waters 2695 system, Alliance, Singapore). The separation of ascorbic acid was performed using a Luna C18 stainless-steel column (5 μ 110 A, 4.6 mm × 250 cm, Micron Analitica S.A., Madrid, Spain). A 0.1 mL L^{-1} solution of trifluoroacetic acid was used as the mobile phase. The flow rate was 1.4 mL min^{-1} with an analysis time of 4 min. The chromatograms were recorded at 260 nm using external standards of L-ascorbic acid.

2.4.2. Vitamin B_{12} (cyanocobalamin). Determination of vitamin B_{12} was performed according to Campos-Giménez et al. [16] using immunoaffinity columns (R-Biopharm, Easy-Extract). Once the cyanocobalamin was retained in those columns, it was eluted with 3 mL of methanol into a glass test tube. Each tube was concentrated to dryness with nitrogen gas at 25°C. Then, each sample was reconstituted for HPLC analysis (Agilent 1100 System, Agilent Technologies, Waldbronn, Germany). Vitamin B_{12} was analyzed using a C18 stainless-steel column (Kinetex 2.6 μ, 100 A, 150 × 4.60 mm, Phenomenex, Madrid, Spain). A 0.025% solution of trifluoroacetic acid, pH 2.6, and acetonitrile was used as the mobile phase. The flow rate was 0.25 mL min^{-1}. Chromatograms were recorded at 361 nm. The amount of vitamin B_{12} was quantified by comparison with external standards of cyanocobalamin.

2.5. Fat Soluble Vitamins

2.5.1. Vitamin A (Retinol Acetate) and Vitamin E (α-Tocopherol Acetate). Both fat soluble vitamins were analyzed according to Klejdus et al. [17] using solid phase extraction via polypropylene cartridges (Strata™ C-18-E; 200 mg 3 mL^{-1}, Phenomenex, Madrid, Spain). Samples were placed in

a cartridge and connected to a vacuum pump. Then, 2 mL of a methanol : water (5 : 95) solution was added to wash out impurities, and 2 mL of methanol and 2 mL of chloroform were added to elute the analytes. The sample was filtered through $0.22 \, \mu m$ filters (Micron Analitica S.A., Madrid, Spain) and placed in an HPLC vial. The column and HPLC were the same as those used for the vitamin B_{12} analyses. A 0.010% solution of trifluoroacetic acid and methanol was used as the mobile phase. The flow rate was $0.7 \, mL \, min^{-1}$, and chromatograms were recorded at 325 nm for vitamin A and at 294 nm for vitamin E. The fat soluble vitamins were quantified by comparing them with external standards of retinol acetate for vitamin A and α-tocopherol acetate for vitamin E.

2.6. Phenolic Compounds

2.6.1. Proanthocyanidins. The cranberry food supplement was shaken for 2 min on a vortex shaker (Reax, Heidolph, Schwabach, Germany). Then, 10 mL of this sample was centrifuged at 5,000 rpm for 10 min. A total of 1 mL of this supernatant was diluted with 0.5 mL of methanol and then filtered ($0.2 \, \mu m$ nylon filter) and analyzed using HPLC (see vitamin B_{12} determination). The proanthocyanidins were analyzed using a C18 column (LiChroCART® 250-4 LiChrospher® 100 RP-18 ($5 \, \mu m$) (Merck Millipore, Billerica, MA, USA)). Chromatographic separation was carried out using a gradient with two solvents: ultrapure water and 0.1% phosphoric acid (solvent A) and methanol (solvent B). The following gradient was used: 95% A and 5% B for 70 s to reach 85% A and 15% B from 70 s to 140 s. The flow rate was $1 \, mL \, min^{-1}$ at 40°C, and the chromatograms were recorded at 525 nm. The proanthocyanidins were quantified by comparison with external standards of cyanidin-3-O-glucoside chloride.

2.6.2. Cynarin and Chlorogenic Acid. Cynarin and chlorogenic acid determination was performed as described below. Samples (5 mL) were added to 5 mL of pure methanol and homogenized for 1 min. The samples were then filtered through $0.22 \, \mu m$ filters into a vial for HPLC analysis (see vitamin B_{12} determination). A C18 stainless-steel column (Phenomenex Gemini-NX $5 \, \mu$ C18 110 A) with a C18 guard column (250×4.6 mm, Micron Analitica, S.A., Madrid, Spain) was used. The mobile phase was a solution of ultrapure water and 2.5% acetic acid and a solution of methanol and 2.5% acetic acid. The flow rate was 1.3 mL per min, and chromatograms were recorded at 316 nm with UV-Vis spectroscopy. The cynarin and chlorogenic acid were quantified using an external standard.

2.7. 10-Hydroxy-2-decenoic Acid (10-HDA). The royal jelly food supplement was shaken for 2 min on a vortex shaker (Reax, Heidolph, Schwabach, Germany). Then, 10 mL of the sample was acidified with 1 N HCl to pH 2.5–3.0. An aliquot of 1 mL was slowly filtered with the help of a vacuum pump through a column (Strata C18-E, Phenomenex, Madrid, Spain), which was previously activated with 3 mL of HPLC grade methanol. Then, 3 mL of ultrapure water was added. Finally, 1 mL of the sample was slowly filtered through the

column to dry it. Finally, the sample was dissolved in 5 mL of methanol and filtered through a $0.2 \, \mu m$ filter before analysis by HPLC (see vitamin B_{12} determination). Separation was conducted on a Zorbax SB-C18, $5 \, \mu m$, 150×4.6 mm column (Agilent 1100 System). Chromatographic separation was carried out by isocratic elution with 50% each of the following two solvents: solvent A made up of ultrapure water and trifluoroacetic acid (1 : 1) and solvent B made up of methanol. The injection volume was $20 \, \mu L$ with a flow rate of $1 \, mL \, min^{-1}$ at 20°C, and chromatograms were registered at 520 nm and quantified by comparison with an external standard of 10-HDA.

2.8. Iron (Fe). Samples from iron food supplement were injected into an inductively coupled plasma mass spectrometer (ICP-MS, Agilent Technologies 7500 ce, Japan), where samples were vaporized and ionized by argon plasma. The iron isotope 56 was determined and quantified by comparison with an external standard. For the extraction, three replicates of samples (0.1 mL) were placed into plastic tubes, 10 mL of water was added, and serial dilution was performed until a dilution of 1 : 1000 was reached.

2.9. Statistical Analysis. A randomized design with three replicates per treatment was used. To determine the effect of HPH on each sampling time of accelerated storage, a one-way analysis of variance (ANOVA, $p < 0.05$) was carried out (Statgraphics Plus, version 5.1, 2001, Manugistics Inc., Rockville, MD, USA). Mean values were compared by the Least Significant Difference (LSD) test to identify significant differences between pasteurization and HPH treatments.

3. Results and Discussion

Some parts of the discussion have been focused on HHP treatments, as there is currently a lack of information regarding the effect of HPH on functional supplements.

3.1. Water Soluble Vitamins

3.1.1. Ascorbic Acid. Before processing, the initial ascorbic acid content was $7.5 \, mg \, mL^{-1}$ in the cranberry food supplement, $9 \, mg \, mL^{-1}$ in the royal jelly, and $7.5 \, mg \, mL^{-1}$ in the iron food supplement (Figure 1, Table 1). The HPH-treated samples had the best ascorbic acid retention levels, particularly in the sample treated with 80 MPa. On the contrary, the levels of this bioactive compound decreased significantly (between 12 and 23%) in the thermally treated samples, showing significant differences ($p < 0.05$) with respect to the HPH-treated ones. This is due to the effect of the intensity and time of the thermal treatment applied on the heat-sensitive ascorbic acid [18]. Similar results were published by Barba et al. [12], who observed that the use of 100 MPa (120–540 s) on a vegetable beverage resulted in better retention of ascorbic acid as compared to thermal treatment (90–98°C, 15–21 s). The decrease in vitamin C content in the thermal treatment can be explained by the oxidation of ascorbic acid into dehydroascorbic acid, which is irreversibly converted into 2,3-diketogulonic acid [19]. Heat accelerates these processes
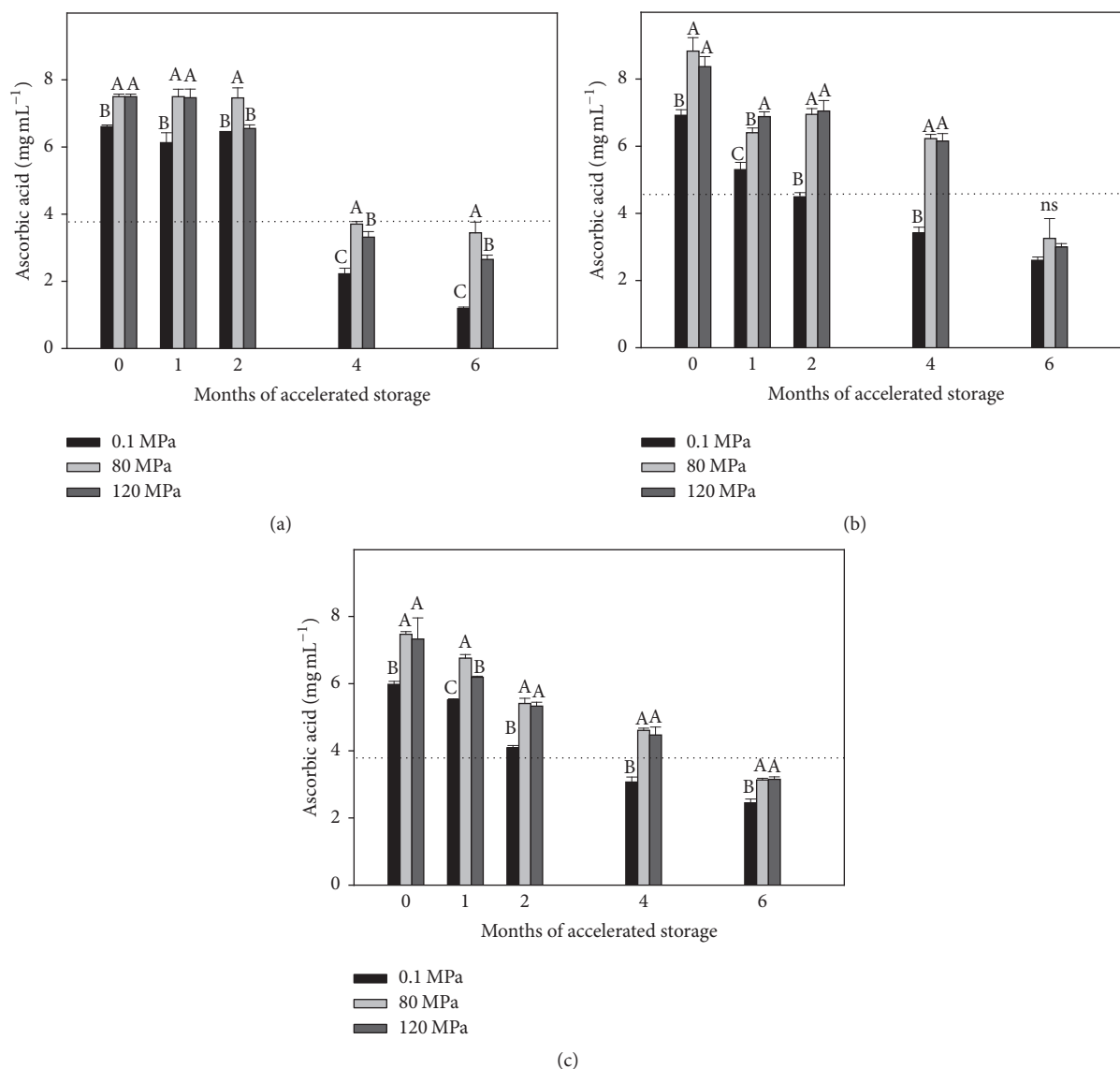
(a)

(b)

(c)

FIGURE 1: The effect of HPH treatments on the ascorbic acid content of cranberry (a), royal jelly (b), and iron food supplements (c) stored at $40°C \pm 2°C$ and $75\% \pm 5\%$ RH. Mean ($n = 3 \pm$ SD). Different letters within the same day indicate significant differences between pasteurization and HPH treatments at $p \leq 0.05$. ns: not significant. The dotted line indicates 50% vitamin losses with respect to the initial contents shown in Table 1.

of oxidation. For instance, in orange juice, the content of L-ascorbic acid decreased by 2% (100 MPa/45°C), 5% (200 MPa/70°C), and 11% (300 MPa/94°C) [20]. It has been reported that the high pressure used in HHP technology has null effects on low-weight molecules with high energy bonds, such as vitamins [8]. This is because HPH and HHP have different mechanisms of action. In HPH, the fluid is forced to pass through a narrow gap in the homogenizer valve [10], while in HHP, the food, which has been previously sealed in its flexible final package, is subjected to high levels (up to 600 MPa) of hydrostatic pressure (transmitted by water) to alter the structure of molecules. The fact that ascorbic acid levels decreased to a lesser extent with the HPH treatment as compared to heat-treated samples could be also explained by

the short retention time of food supplements at the treatment temperature after the homogenization process (<0.5 s) [21].

The level of ascorbic acid degradation was slightly different for each functional compound, depending on the functional compound's matrix. This could be due to the fact that the ingredients in the food matrix can have influence on the bioactive compounds' activity, degradation, or release. For instance, the application of HPH treatment (20–100 MPa) to mandarin juice with trehalose addition (10–30%) degraded the vitamin C content between 2 and 4% during storage, but this trehalose addition resulted in less flavonoid degradation during storage [22].

Storage under accelerated conditions strongly reduced the ascorbic acid content in all food supplements, although
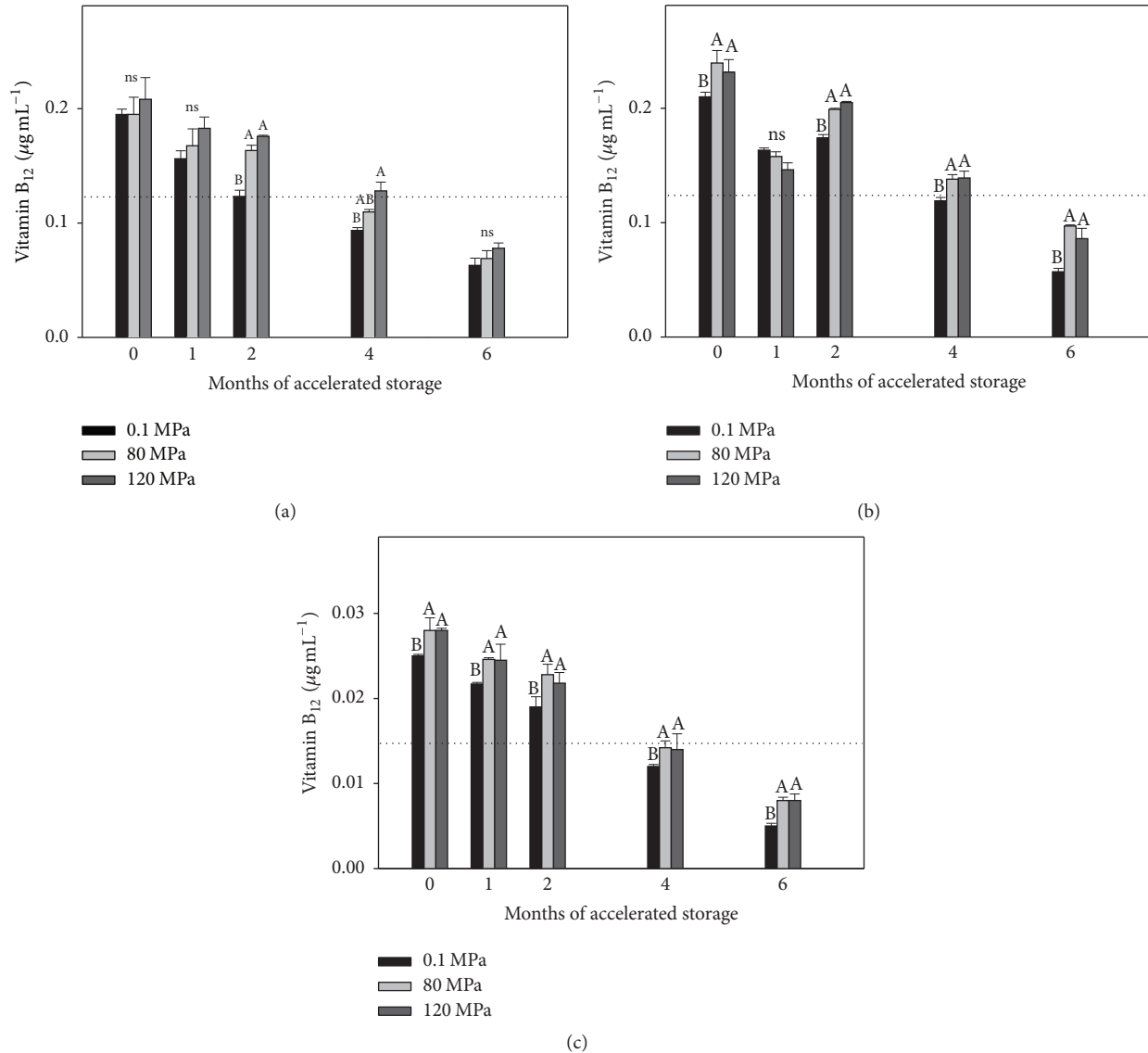
(a)

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FIGURE 2: The effect of HPH treatments on the total vitamin B_{12} content of artichoke (a), royal jelly (b), and iron food supplements (c) stored at $40°C \pm 2°C$ and $75\% \pm 5\%$ RH. Mean ($n = 3 \pm$ SD). Different letters within the same day indicate significant differences between pasteurization and HPH treatments at $p \leq 0.05$. ns: not significant. The dotted line indicates 50% vitamin losses with respect to the initial contents shown in Table 1.

the remaining vitamin C in both HPH treatments was significantly higher ($p < 0.05$) than in the thermally treated samples (Figure 1). At the end of the accelerated storage, the greatest differences between the control and HPH treatments were found in the cranberry food supplement (Figure 1(a)). A greater decrease was observed in control samples (0.1 MPa) at 4 months of accelerated storage (more than 50%), and in general, this same degree of change was observed in all HPH samples at 6 months of accelerated storage (Figure 1). Regarding the time of storage, Barba et al. [12] also found that ascorbic acid content was preserved in cranberry juice that was HHP-treated with 200 MPa for 5–15 min. Nevertheless, the ascorbic acid levels in juice treated with 400 and 600 MPa for the same amount of time were significantly lower than in fresh samples (maximum temperature under HHP was 42°C).

3.1.2. Vitamin B_{12}. Before processing, the initial vitamin B_{12} content in both artichoke and royal jelly supplements was $0.25 \, \mu g \, mL^{-1}$, and it was $0.03 \, \mu g \, mL^{-1}$ in the iron supplement (Figure 2, Table 1). After processing, the concentration of vitamin B_{12} decreased in all treatments, although the effect of the treatments depended on the functional food's matrix. In the artichoke supplement, all the processing treatments resulted in similar decreases, ranging from 17 to 24% (Figure 2(a)). However, in the royal jelly and the iron supplements, the use of HPH reduced vitamin B_{12} losses, which were around 4–7% in HPH at 80 and 120 MPa, respectively, as compared to 16% in the thermally treated samples (Figures 2(b) and 2(c)). Accordingly, Ottaway [23] had previously reported on the potential for substantial vitamin B_{12} losses after prolonged heating during milk
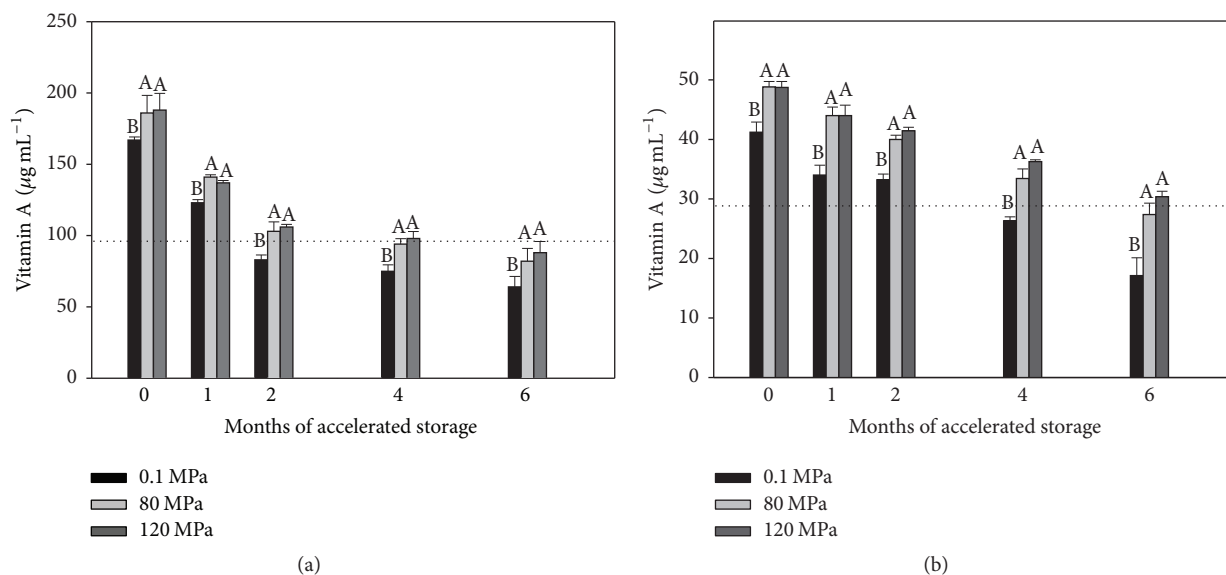
FIGURE 3: Effect of HPH treatments on the total vitamin A content of royal jelly (a) and iron food supplements (b) stored at $40°C \pm 2°C$ and $75\% \pm 5\%$ RH. Mean ($n = 3 \pm$ SD). Different letters within the same day indicate significant differences between pasteurization and HPH treatments at $p \leq 0.05$. ns: not significant. The dotted line indicates 50% vitamin losses with respect to the initial contents shown in Table 1.

sterilization ($120°C$, 13 min). As with vitamin C, vitamin B_{12} was mainly degraded during the storage period. The following significant decreases in vitamin B_{12} were observed in the different functional products during storage: around 66–69% in the artichoke supplement, without significant differences between samples; between 63% and 73% in the royal jelly supplement; and around 72–80% in the iron food supplement. In all supplements, HPH treatments helped maintain vitamin B_{12} levels. Similarly, Yamada et al. [24] reported that the vitamin B_{12} content in dietary supplements was also affected by storage time, temperature, and light exposure. The thermolability of vitamin B_{12} would explain the benefits of using HPH in royal jelly and iron food supplements. However, the nonsignificant effect found in artichoke supplements could be explained by the lemon juice content in this supplement (Table 1). The presence of acidic components can accentuate the degradation of this vitamin [25].

3.2. Fat Soluble Vitamins

3.2.1. Vitamin A. Before processing, the initial vitamin A content was $190 \, \mu g \, mL^{-1}$ in royal jelly and $50 \, \mu g \, mL^{-1}$ in the iron food supplement (Figure 3, Table 1). Just after processing, the level of vitamin A decreased significantly ($p < 0.05$) when using the thermal treatment (12% losses in royal jelly and 29% in the iron food supplements). Our result is in agreement with the results found by Amador-Espejo et al. [21] who observed that ultra HPH-treated milks (300 MPa, 45 and $55°C$) had a similar retinol content to raw milk. In the present study, samples treated under both HPH treatments showed the highest vitamin A content, with a decrease after treatment of about 2% in royal jelly and 16% in iron supplements.

In all treatments, the concentration of vitamin A sharply decreased with time of storage, yet the drop occurred faster in the thermally treated samples than in the HPH-treated samples. The royal jelly supplement, for example, suffered a 50% reduction in vitamin A at around 2 months of accelerated storage (Figure 3(a)), and the iron food supplement showed the same losses at around 6 months of accelerated storage (Figure 3(b)). Other researchers have also found better retention of vitamin A using HPH (100–200 MPa at $4°C$) as compared to heat treatments [18]. According to Rodríguez-Amaya [26], vitamin A degradation depends on the availability of oxygen, water activity, light exposure, temperature, and the presence of antioxidants, metals, and enzymes. HPH could also preserve vitamin A content since the short time of processing helps reduce the oxygen content in the sample.

3.2.2. Vitamin E. Before processing, the initial vitamin E content was $2 \, mg \, mL^{-1}$ in the artichoke supplement, $1.6 \, mg \, mL^{-1}$ in royal jelly, and $0.54 \, mg \, mL^{-1}$ in the iron food supplement (Figure 4, Table 1). The use of HPH resulted in significant vitamin E retention in artichoke and iron supplements (Figures 4(a) and 4(c)). In these supplements, vitamin E losses in the thermally treated samples were around 13% and 15%, respectively, while the HPH samples only lost 2 to 7%, respectively. In the royal jelly supplements, however, a similar decrease in vitamin E (5%) was found in both the control and the HPH treatments (Figure 4(b)). Likewise, Moltó-Puigmartí et al. [27] also observed that levels of tocopherols did not vary in mature human milk with high-pressure processing or higher-temperature pasteurization.

The time of storage led to a decrease in vitamin E, but this liposoluble vitamin was much more stable than the rest
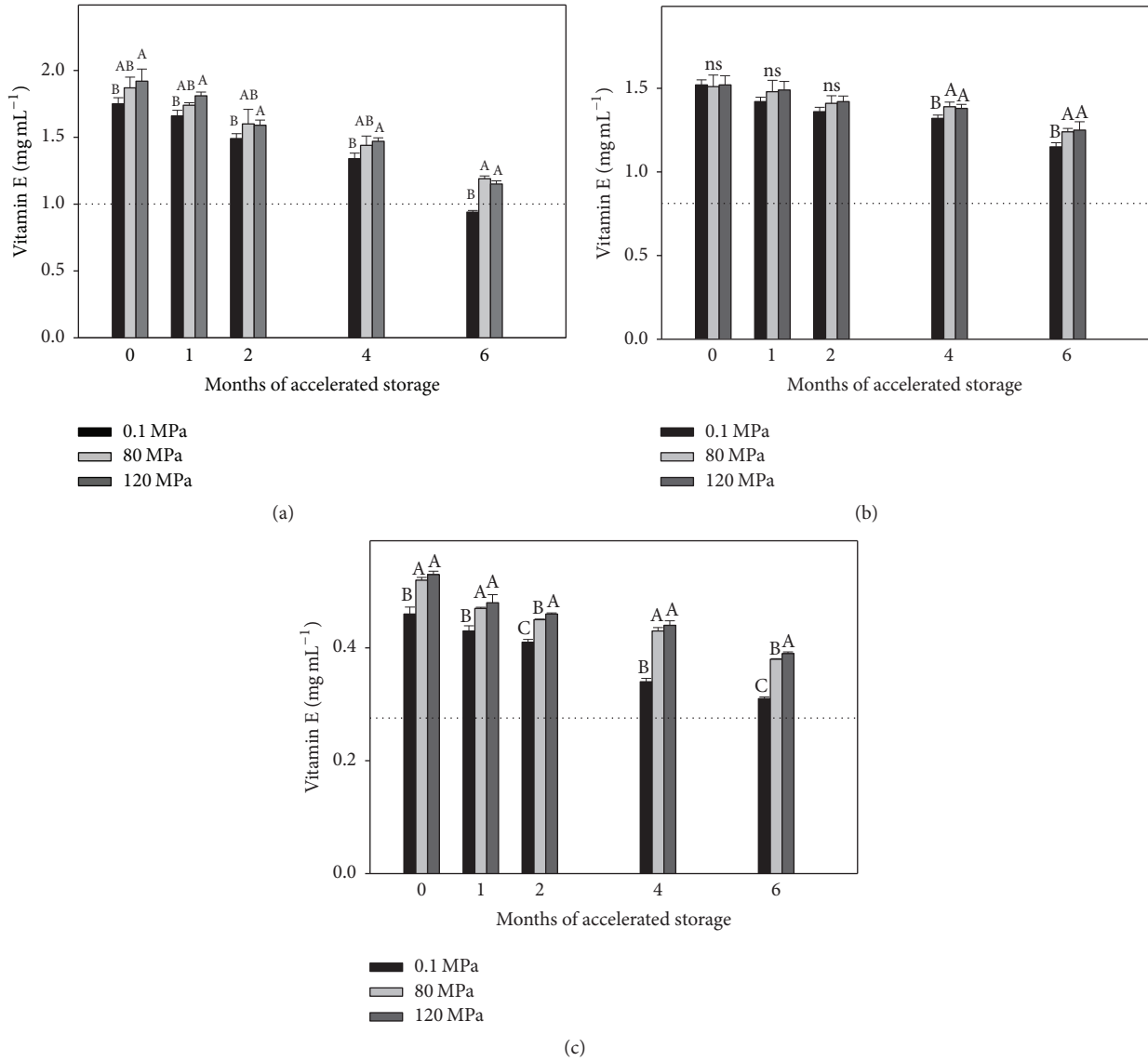
(a)

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FIGURE 4: Effect of HPH treatments on the total vitamin E content of artichoke (a), royal jelly (b), and iron food supplements (c) stored at $40°C \pm 2°C$ and $75\% \pm 5\%$ RH. Mean ($n = 3 \pm$ SD). Different letters within the same day indicate significant differences between pasteurization and HPH treatments at $p \leq 0.05$. ns: not significant. The dotted line indicates 50% vitamin losses with respect to the initial contents shown in Table 1.

of the analyzed vitamins particularly in the royal jelly food supplement, where final losses ranged from 18 to 24% with significant differences observed between treatments. In all food supplements, samples treated under HPH had the best vitamin E retention with storage time. It is interesting to note that, in royal jelly, vitamin E showed greater stability with storage time than vitamin A (Figure 3(a) versus Figure 4(b)).

3.2.3. Phenolic Compounds. The initial proanthocyanidin content present in the cranberry dry extract used was 5.50 mg mL^{-1} (Figure 5(a), Table 1). Just after processing, this amount dropped by around 3% in both thermally treated and HPH samples. During the storage time, the proanthocyanidin content decreased significantly in all samples but was much

better preserved in the HPH treatments (Figure 5(a)). At the end of the storage period, proanthocyanidin degradation was around 58% in control samples versus 28% in HPH samples. Previous studies have similarly reported negative effects on proanthocyanidin content after thermal food processing [28]. Proanthocyanidins are heat-sensitive nutrients that are also vulnerable to enzyme-catalyzed oxidation. Patras et al. [29], for example, did not find a significant change in the anthocyanin content between pressure-treated (HHP) and unprocessed strawberry and blackberry purées, whereas conventional thermal treatments significantly reduced the anthocyanin levels. In our case, a difference between thermal and HPH treatments was not found after processing, but the protective effect of HPH on the proanthocyanidin content throughout the storage period could be explained by the
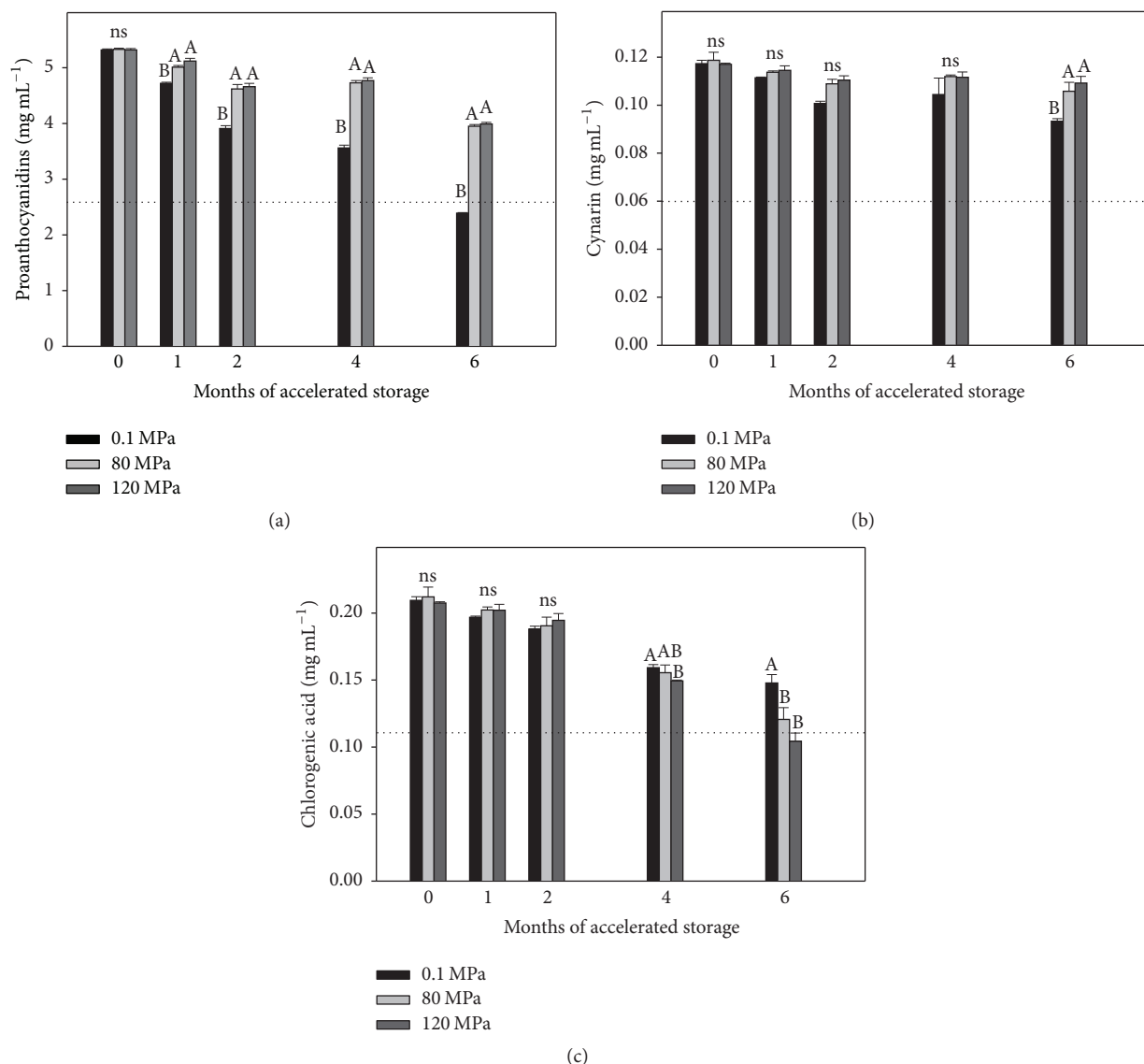
(a)

(b)

(c)

FIGURE 5: Effect of HPH treatments on the phenol content of cranberry (a) and artichoke food supplements (b, c) stored at $40°C \pm 2°C$ and $75\% \pm 5\%$ RH. Mean ($n = 3 \pm SD$). Different letters within the same day indicate significant differences between pasteurization and HPH treatments at $p \leq 0.05$. ns: not significant. The dotted line indicates 50% phenolic losses with respect to the initial contents shown in Table 1.

higher vitamin content present in the HPH samples that could protect the HPH-treated supplements from proanthocyanidin degradation.

The polyphenols studied in the artichoke food supplement were cynarin and chlorogenic acid. Both polyphenols were naturally present in the artichoke dry extract. The initial cynarin content was $0.12 \, mg \, mL^{-1}$ with small reductions (1 to 2.5%) after thermal and HPH treatment. This compound was well preserved until the end of storage, although there were significantly higher losses under thermal (18%) than under HPH treatments (9%) (Figure 5(b)). The effect of HPH on chlorogenic acid, however, was the opposite. Final losses were higher under HPH (43% at 80 MPa and 50% at 120 MPa) than in the control samples (30%) (Figure 5(c)). Barba et al. [12] observed similar results in a HHP-treated (100 MPa,

120–540 s) vegetable beverage, where no marked changes in total phenols were observed with respect to the thermal treatment (90–98°C, 15–21 s). On the contrary, Velázquez-Estrada et al. [20] reported no significant differences on the content of total polyphenols between the fresh and HPH-treated (100, 200, and 300 MPa) orange juice. However, significant differences were detected between them and the pasteurized orange juice samples. On the other hand, a study comparing mulberry juice processed with HPH at 200 MPa with juice processed via heat treatment (95°C, 1 min) concluded that there was a greater decrease in the phenolic acid content during HPH processing [30]. These results suggest that HPH treatment may either increase, decrease, or have no effect on phenolic compounds, depending on the commodity, treatment pressure, and time.
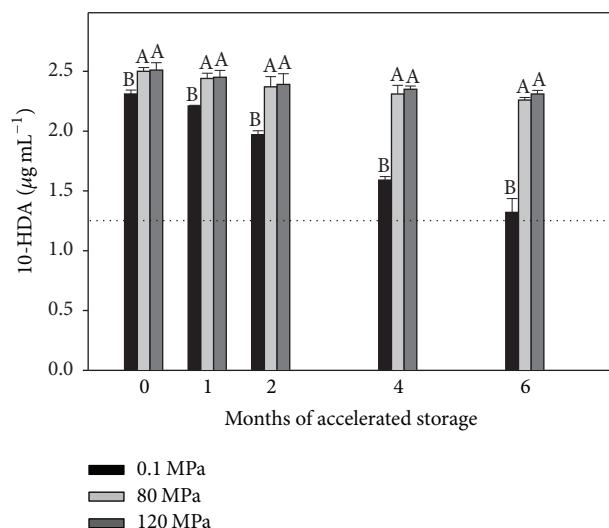
FIGURE 6: Effect of HPH treatments on the 10-HDA content of the royal jelly food supplement stored at $40°C \pm 2°C$ and $75\% \pm 5\%$ RH. Mean ($n = 3 \pm$ SD). Different letters within the same day indicate significant differences between pasteurization and HPH treatments at $p \leq 0.05$. ns: not significant. The dotted line indicates 50% 10-HDA losses with respect to the initial contents shown in Table 1.

3.2.4. 10-HDA. Before processing, the initial 10-HDA content in the royal jelly supplement was 2.50 μg/mL (Figure 6, Table 1). The heat process negatively affected the 10-HDA content, resulting in a decrease of 8% (Figure 6), while HPH treatments maintained the initial 10-HDA level. The degradation of the 10-HDA content with storage time was markedly high in the pasteurized samples, with losses of about 43% versus 8–10% in HPH samples. Similarly, Vervoort et al. [31] reported that unsaturated fatty acid oxidation occurred to a much greater extent under thermal processing. Butz et al. [32] studied the influence of pressures of up to 600 MPa on a model system closest to milk fatty acid decomposition. These researchers concluded that oleic acid was not affected by such pressures but that the autoxidation of linoleic acid increased with pressures starting from 350 MP, although the effects were small compared with the thermal treatment.

3.2.5. Iron. The iron levels in the supplements studied ranged from 1.3 to 1.5 mg mL^{-1}, and no significant differences were found between thermal and HPH treatments at any length of storage time (data not shown). The storage losses were around 16% in all samples, which indicated that this mineral was quite stable as compared to the vitamins studied. Recently, Hemery et al. [33] reported that, in fortified wheat flour, vitamin A degradation was high and occurred rapidly, whereas iron and zinc contents did not change over the 6-month storage at $40°C$. Similarly, in a previous apple study, the application of HHP (500 MPa) preserved and even improved the availability of minerals and antioxidants [34]. In our experiment, the pressures applied were not high enough to cause changes in the content of irons, as they were much lower than the 500 MPa used in the study mentioned.

4. Conclusions

HPH is a recommended alternative to thermal treatments for functional supplements containing heat-sensitive compounds. HPH also showed a positive effect on vitamins B_{12} and E, but this effect depended on the functional matrix. The optimization of HPH conditions depended on the bioactive compounds and functional food supplements.

Conflicts of Interest

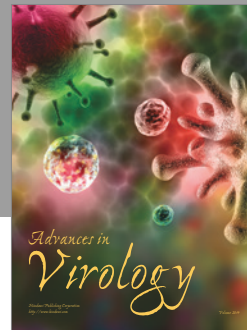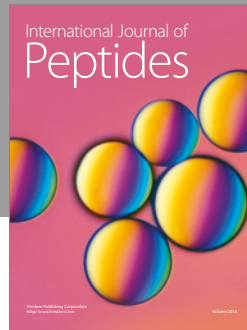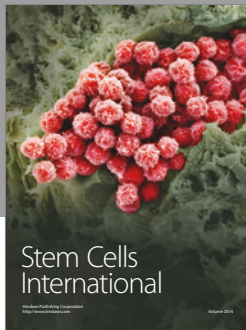The authors declare that they have no conflicts of interest.

Acknowledgments

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