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Impact of High-Pressure Processing on Vitamin E (α-, γ-, and δ-Tocopherol), Vitamin D (Cholecalciferol and Ergocalciferol), and Fatty Acid Profiles in Liquid Foods

Francisco J. Barba, Maria J. Esteve, and Ana Frigola*

Department of Nutrition and Food Chemistry, Universitat de València, Avenida Vicent Andrés Estellés s/n, 46100 Burjassot, Spain

ABSTRACT: In the present study, four high-pressure (HP) treatments (100, 200, 300, and 400 MPa) of 9 min duration were evaluated to assess their effect on the lipid fraction (fat-soluble vitamins and fatty acid profile) of an orange juice−milk and a vegetable beverage. After HP treatment, nonsignificant changes in vitamin D_2 and D_3 contents were observed for both beverages. An increase in vitamin E activity was observed in HP beverages when pressures >100 MPa were applied, mainly due to an increase in α-tocopherol content. Only a small reduction in fat content was found for the orange juice−milk beverage, but no changes were observed for the vegetable beverage. A significant decrease in SFA levels was observed in HP-treated (300−400 MPa) orange juice−milk. With regard to MUFA, a significant increase in oleic acid ($C_{18:1}$) was found in both liquid foods. Nonsignificant differences in the PUFA profiles were observed after HP processing.

KEYWORDS: *fatty acids, orange juice−milk beverage, vegetable beverage, high-pressure processing, fat-soluble vitamins*

INTRODUCTION

Fruit juice mixed with milk and vegetable-based beverages are ideal vehicles for bioactive food ingredients targeting lifestyle diseases.[1,2] Fat-soluble vitamins are provided by food intake, and the content of these vitamins will vary in relation to the foods of which it is made and the treatments to which they are subjected.[3] Tocopherols are naturally occurring lipid antioxidants that specifically inhibit the oxidation of polyunsaturated fatty acids (PUFA). γ-Tocopherol is the major tocopherol present in Western diets, but α-tocopherol is the most abundant form of vitamin E in milk. α-Tocopherol is a fat-soluble vitamin with higher antioxidant capacity, reacting with peroxy radicals and other free radicals.[4] High concentrations of antioxidants, including α-tocopherol, are associated with a reduction in the risk of disorders connected to free radicals, such as atherosclerosis, cancer, cataracts, and cell damage connected to ischemia and reperfusion. Reduction in the risk of coronary illness as a result of a high intake of vitamin E has also been indicated.[5] Numerous studies have observed that optimal vitamin D status has a positive effect on our health and may reduce cancers and cardiovascular diseases.[6] Obtaining sufficient vitamin D from natural food sources alone is difficult. For many people, consuming vitamin D-fortified foods and, arguably, being exposed to some sunlight are essential for maintaining a healthy vitamin D status.[7] Consumption of PUFA has been shown to reduce the risk of coronary heart disease and cancer to improve inflammatory conditions such as arthritis, to reduce plasma triacylglycerol levels, and to lower blood pressure.[8] Consequently, PUFA and their derivatives and analogues are important nutraceuticals and are becoming of increasing nutritional interest.[9] Among the recent trends, low-fat milk is commonly used for delivery of PUFA fatty acids. Drinks containing combinations of dairy and fruit juices with added bioactive components are becoming common in United States (U.S.) and European Union (EU) markets.[10] As some studies have shown previously,[11,12] the presence of PUFA-enriched foods on the market and the promotion of their consumption may significantly increase the level of PUFA intake. On the other hand, the consumption of monounsaturated fatty acids (MUFA), especially oleic acid, has been shown to decrease plasma triacylglycerol and cholesterol concentrations in healthy normolipidaemic subjects.[13]

High-pressure (HP) processing is one of the nonthermal, physical technologies that are being investigated and beginning to be used by the food industry as an alternative to thermal treatments because it inactivates and inhibits microorganisms and can activate or inactivate enzymes.[14] HP preserves nutritional value with only a minimal effect on the product quality and delicate sensory properties of fruits and vegetables owing to its limited effect on the covalent bonds of low molecular mass compounds such as color and flavor compounds.[15] Several studies have been conducted to apply HP to various food materials, including fruit juice, fruit juice mixed with milk, and vegetable beverages.[16−20] When any novel technological method is employed in the processing of food, it is important that key micronutrients such as vitamins are not adversely affected.[21] Moreover, some studies have demonstrated an increase in some antioxidant compounds' extractability after the application of HP in orange juice[22−24] and some modifications in fatty acid profile in HP-processed milk.[25,26] In addition, at this stage of development of HP technology, evaluating the influence of process variables on vitamins and fatty acid profile is a key factor in defining treatment conditions to avoid the loss of these important properties of foods and to obtain a food beverage with high benefits for the health of the consumer. Previously, Zulueta et al.[27] studied the effect of high-intensity pulsed electric fields on the fatty acid profile in an orange juice−milk beverage, and it is

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necessary to investigate other nonthermal technologies in these kinds of matrices.

The objective of this study was to evaluate the impact of high-pressure processing on vitamin E (α-, γ-, and δ-tocopherol), vitamin D (cholecalciferol and ergocalciferol), and fatty acid profiles in different liquid foods such as orange juice−milk and vegetable beverages.

MATERIALS AND METHODS

Samples. Oranges (*Citrus sinensis* L.), Navel variety, and ultrahigh-temperature (UHT) skimmed milk (0.1% fat) (Grupo Leche Pascual, SA, Burgos, Spain) with EPA, DHA (60 mg/100 mL), oleic acid (1.30 g/100 mL), and vitamins A (120 mg/100 mL), D (0.75 mg/100 mL), and E (1.50 mg/100 mL) were purchased from a local supermarket. Orange juice was extracted after appropriate washing and hygienization of the fruits. The orange juice−milk beverage was prepared according to the method of Zulueta et al.[27] by mixing 50% (v/v) of orange juice with the pulp removed, 20% (v/v) of UHT skimmed milk, and 30% (v/v) of distilled water. Sugar (7.5% w/v), citric acid (0.1% w/v), and high-methoxyl citrus pectin (0.3% w/v) were added as sweetener, preservative, and homogenizer of the samples, respectively. Solid ingredients were placed in water in the weight proportions indicated. The beverage was prepared just before use.

The vegetable beverage was prepared according to the method of Barba et al.[18] by mixing the following ingredients purchased from a local supermarket in Valencia (Spain): tomato (*Lycopersicon esculentum* Mill., 33%), green pepper (*Capsicum annuum* L., Italian pepper, 17%), green celery (*Apium graveolens* L., 8.5%), cucumber (*Cucumis sativus* L., 4%), onion (*Allium cepa* L., 4%), carrot (*Daucus carota* L., 4%), lemon (*Citrus limon* L, 1.7%), salt (1.7%), virgin olive oil (SOS Cuétara, SA, Madrid, Spain, 0.8%), and water to 100%.

Chemicals. Ethanol, methanol, acetonitrile, hexane, diethyl ether (HPLC grade), and phenolphthalein 1% (w/v) were obtained from J. T. Baker (Deventer, The Netherlands). Potassium hydroxide and sodium sulfate were obtained from Scharlab (Barcelona, Spain), chloroform and ascorbic acid from Merck (Darmstadt, Germany), and BHT, cholecalciferol, α-tocopherol, δ-tocopherol, and γ-tocopherol from Sigma-Aldrich (Steinheim, Germany). Ergocalciferol was from Fluka (Buchs, Switzerland). Nylon filters with a pore size of 0.22 μm and a diameter of 47 mm were used for mobile phases, and filters with a pore size of 0.20 μm and a diameter of 13 mm were used for filtering samples (Millipore Co., Bedford, MA).

Thermal Treatment System. An Armfield FT74P unit with a plate exchanger was used to treat the vegetable beverage. The beverage, placed in a feed tank, was pumped through the heat exchanger to achieve the treatment conditions (90 °C for 15 s). All of the treatments were applied in duplicate, with three bottles per treatment. Immediately after thermal treatment, the samples were transferred to an ice/water bath (Armfield FT61, U.K.), packed, and then stored under refrigeration (4 ± 1 °C) until needed for analysis. Thermal treatment was selected among different thermal treatments according to the results of previous studies.[18,19]

HP Treatment System. The samples, inserted in PE-LD bottles, were placed in polyethylene bags filled with water and heat-sealed (MULTIVAC Thermosealer, Hünenberg, Switzerland) before being placed in the HP unit (High-Pressure Food Processor; EPSI NV, Belgium). The equipment consists of a vessel with an internal diameter of 100 mm and 300 mm high, with an operation pressure vessel of 689 MPa, an operation temperature vessel of −20 to 100 °C, and a volume of 2.35 L. The pressure medium was a water−ethyleneglycol mixture (80:20). The samples were pressurized at 100, 200, 300, and 400 MPa for a time of 9 min. Pressure level, pressurization time, and temperature were controlled automatically. Pressure increase rate was 300 MPa/min, and the depressurization time was <1 min. The initial temperature was 15 °C, the final temperature after pressurization at highest pressure was 32 °C, the final temperature after holding time at highest pressure was 26.6 °C, and the final temperature after decompression at highest pressure was 12.5 °C. Come-up time was 90 s, and decompression time was 15 s. All of the treatments were applied in duplicate, with three bottles per treatment. Immediately after pressurization, the samples were transferred to an ice/water bath (Armfield FT61, U.K.), packed, and then stored under refrigeration (4 ± 1 °C) until needed for analysis. According to preliminary results of the group, these processing conditions were selected due to their effect on food bioactives extraction and microbial reduction.[19,28]

Extraction and Identification of Vitamins E (α-, γ-, and δ-Tocopherol) and D (Cholecalciferol and Ergocalciferol). The method of Barba et al.,[29] consisting of saponification with KOH in MeOH and subsequent liquid−liquid extraction of fat-soluble vitamins with organic dissolvent, was used. The LC system consisted of two isocratic pumps (Prostar 210, Varian Inc., Palo Alto, CA, USA) with a degasser (Degassit, MetaChem, USA), column thermostat (Prostar 510, Varian), and UV−vis detector (Varian Inc.). The whole LC system was operated by a Varian STAR Chromatography Workstation ver. 6.0. A Kromasil 100 C18 precolumn (guard column) (5 μm, 150 × 4.6 mm) and a Kromasil 100 C18 column (5 μm, 150 × 4.6 mm) (Scharlab) were used.

The vitamin E activity was calculated using the factors for conversion of tocopherols to RRR-α-tocopherol equivalents:[30]

$$\text{vitamin E activity (α-TE/100mL)} = \text{α-tocopherol (mg)} \times 1.0 + \text{γ-tocopherol (mg)} \times 0.1 + \text{δ-tocopherol (mg)} \times 0.03$$

Extraction and Identification of Fatty Acids. The method of Folch et al.,[31] consisting of the extraction of fat from the sample with a mixture of chloroform and methanol (2:1, v/v), with various modifications,[27] was used. The fatty acid methyl esters were separated in a 30 m, 0.25 mm (0.25 μm film thickness), column of CP-WAX 52CB (Varian, Spain) using a Focus CG Thermo Finnigan system (Thermo Finnigan, Italy). The initial temperature was 70 °C and increased to 180 °C at 15 °C/min over a period of 8 min, after which the temperature was increased to 210 °C at 5 °C/min during 5 min and held at 250 °C for 12 min. The total analysis time was 25 min. The fatty acid methyl esters were identified by comparing their retention times with those obtained for standards using a Chrom-Card Data System.

Physicochemical Determinations. Some characteristic parameters of beverages, such as pH and °Brix, were determined before and after processing. Quantification of the pH was determined using a Crison GLP 21 pH-meter (Barcelona, Spain) equipped with a temperature compensation sensor at 20 °C, and °Brix was determined with an Atago RX-1000 digital refractometer (Atago Co. Ltd., Tokyo, Japan).

Statistical Analysis. A two-way analysis of variance (ANOVA) was applied to the results obtained to verify whether there were significant differences in the parameters studied in relation to treatment pressure and food matrix and to ascertain possible interactions between the factors (differences at $p < 0.05$ were considered to be significant). When there were differences, an LSD test was applied to indicate the samples between which there were differences. All statistical analyses were performed using SPSS (Statistical Package for the Social Sciences) v. 13.0 for Windows (SPSS Inc., Chicago, IL, USA).

RESULTS AND DISCUSSION

To establish the effect of the HP treatment, different pressures (100, 200, 300, and 400 MPa) were applied during 9 min. To compare the effects of HP and thermal treatments, the optimum conditions for reaching a 5-log reduction of *Lactobacillus plantarum* CECT 220 and *Escherichia coli* CECT 433 in orange juice−milk and vegetable beverage, respectively, were selected. A 5-log reduction of *L. plantarum* CECT 220 and *E. coli* CECT 433 was demonstrated in the orange juice−milk and vegetable beverage after HP at 200 MPa/9 min,[19,28] obtaining the minimum reduction level demanded by the U.S. FDA.[15] This result was compared with thermal treatment at 90 °C for 15 s,

Table 1. Vitamin E Content (μg/100 mL) in Untreated, High-Pressure-Treated (100, 200, 300, and 400 MPa during 9 min), and Thermally Treated (90 °C/15 s) Orange Juice−Milk and Vegetable Beverages[a]

	δ-T	γ-T	α-T	vitamin E
orange juice−milk				
untreated	63.42 ± 0.34 a	26.01 ± 0.72 a	362.58 ± 0.87 a	367.08 ± 0.93 a
90 °C/15 s	63.06 ± 0.24 a	25.95 ± 0.11 a	361.81 ± 0.38 a	366.30 ± 0.38 a
100 MPa	68.45 ± 1.77 ab	30.71 ± 3.45 a	399.15 ± 0.39 b	404.27 ± 0.79 b
200 MPa	83.08 ± 4.64 c	40.27 ± 3.80 b	463.64 ± 2.01 c	470.16 ± 1.49 c
300 MPa	73.18 ± 0.09 b	34.08 ± 0.30 ab	466.94 ± 3.78 c	472.54 ± 3.75 c
400 MPa	75.56 ± 1.59 bc	30.24 ± 0.25 a	387.46 ± 2.06 d	392.75 ± 2.04 d
vegetable beverage				
untreated	18.44 ± 0.18 A	9.81 ± 0.04 A	307.14 ± 4.43 A	308.67 ± 4.44 A
90 °C/15 s	16.81 ± 0.35 B	9.74 ± 0.09 A	239.33 ± 6.46 B	240.80 ± 6.45 B
100 MPa	23.65 ± 0.02 C	10.57 ± 0.01 B	246.92 ± 0.74 B	248.69 ± 0.73 B
200 MPa	26.37 ± 0.20 D	14.41 ± 0.10 C	351.31 ± 3.75 C	353.54 ± 3.77 C
300 MPa	17.33 ± 0.13 B	15.98 ± 0.07 D	368.35 ± 4.42 C	370.46 ± 4.43 C
400 MPa	17.28 ± 0.36 B	15.89 ± 0.21 D	368.46 ± 9.96 C	370.56 ± 9.99 C

[a]Different lower case letters in the same column indicate significant differences as a function of the applied treatment in orange juice−milk beverage; different upper case letters in the same column indicate significant differences as a function of the applied treatment in vegetable beverage; δ-T, δ-tocopherol; γ-T, γ-tocopherol; α-T, α-tocopherol.

which also reached 5-log reduction and proved to be the most effective heat treatment for preserving ascorbic acid.

The values of pH and °Brix were 3.90 ± 0.03 and 4.21 ± 0.02 for unprocessed orange juice−milk and 14.40 ± 0.40 and 4.20 ± 0.01 for untreated vegetable beverage, respectively. No statistically significant changes were obtained for pH and °Brix values when the various HP and thermal treatments were applied in both beverages (data not shown).

Vitamin D_2 and D_3 contents in unprocessed orange juice−milk were 0.63 ± 0.11 and 0.66 ± 0.27 μg/100 mL, respectively. These values were in the range of those previously reported by Barba et al.[29] in other fruit juice−milk beverages enriched with vitamin D having characteristics similar to those of the beverage studied in the present work. After application of the different HP treatments (100−400 MPa) and thermal processing (90 °C, 15 s), nonsignificant changes in vitamin D content of the orange juice−milk were observed (data not shown). On the other hand, the vitamin D_2 value in untreated vegetable beverage was 0.18 ± 0.01 μg/100 mL, with no statistically significant modifications when the various HP and thermal treatments were applied (data not shown).

Tocopherols concentrations and resultant vitamin E activity of untreated, HP-treated, and thermally treated orange juice−milk and vegetable beverage are shown in Table 1. Letters indicate the existence of statistically significant differences ($p < 0.05$) between the results obtained by applying an ANOVA test.

Vitamin E activity values were 367.1 and 308.7 μg/100 mL for the untreated orange juice−milk and vegetable beverage, respectively. These values were similar to those previously reported by Barba et al.[29] in this kind of food matrix. After application of HP treatment, a significant increase in vitamin E activity (7−28%) of orange juice−milk was observed for all pressures (100−400 MPa), mainly due to an increase in α-tocopherol content, reaching a maximum at 200−300 MPa (28−29%). However, in the vegetable beverage, a significant decrease (19%) in vitamin E activity was observed when HP at 100 MPa was applied, whereas a significant increase (15−20%) after the application of HP (200−400 MPa) was obtained. It could be inferred that the increase in the α-tocopherol content found in these samples could be due to the disruption of the chloroplasts where α-tocopherol is confined. Along this line, some authors have suggested that micronutrients and bioactive compounds in certain fruits and vegetables may be more extractable by HP treatments.[16,32] However, these results were in contrast to those found by Molto-Puigmartí et al.,[33] who did not find significant changes in δ-, γ-, and α-tocopherols when they applied HP (400−600 MPa/22−27 °C/5 min) in mature human milk. On the other hand, nonsignificant modifications in vitamin E activity of orange juice−milk were obtained after the application of thermal treatment (90 °C, 15 s). These results were in accordance with those reported by Van Zoeren-Grobben et al.;[34] they did not find modifications in vitamin E of human milk after Holder pasteurization. However, Romeu-Nadal et al.[35] observed a decrease of 17% in vitamin E content after thermal treatment in mature human milk. Furthermore, in the vegetable beverage analyzed in the present study, a decrease (22%) was observed in vitamin E activity after thermal treatment in comparison with untreated beverage.

Fat content and fatty acid profile of untreated, HP-treated, and thermally processed orange juice−milk and vegetable beverage are shown in Table 2. Fat content did not decrease significantly in orange juice mixed with milk after HP for all of the pressures applied. Along this line, some authors have observed that the size of the fat globule was not significantly affected by HP treatment.[36,37] This contrasts with other research, which suggested that pressures above 500 MPa produced some alterations in the size and distribution of the fat globule in whole milk.[38,39] In addition, in the vegetable beverage, no changes were obtained in fat content after HP and thermal treatments. These results were in accord with those found by Garde-Cerdán et al.[40] in grape juice processed at 90 °C during 60 s.

The percentage in the untreated orange juice−milk beverage of saturated fatty acids (SFA) was 16.1%, that of MUFA was 64.2%, and that of PUFA was 16.3%. These values were in the range of those previously reported by Zulueta et al.[27] Analysis of the variation in the SFA, MUFA, and PUFA profiles in each pressure applied shows a significant increase ($p < 0.05$) in SFA at 100 and 200 MPa, mainly due to increases in $C_{16:0}$ and $C_{18:0}$ levels. SFA percentage changes decreased when pressures (300 and 400 MPa) were higher. With regard to MUFA, a small reduction ($p < 0.05$) in the percentage of oleic acid ($C_{18:1}$) was observed after HP treatment. However, nonsignificant changes in PUFA profile were obtained after the application of HP treatment. Gervilla et al.[38] observed free fatty acid levels lower

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Table 2. Fat Content (Grams per 100 g; Mean ± Standard Deviation) and Free Fatty Acids (Percent) Found in Untreated, High-Pressure-Treated (100, 200, 300, and 400 MPa during 9 min), and Thermally Treated (90 °C/15 s) Orange Juice Mixed with Milk and Vegetable Beverage[a]

	untreated	90 °C/15 s	100 MPa	200 MPa	300 MPa	400 MPa
			Orange Juice−Milk			
fat	0.40 ± 0.04 a	0.35 ± 0.03 a	0.38 ± 0.02 a	0.36 ± 0.02 a	0.35 ± 0.02 a	0.34 ± 0.03 a
$C_{12:0}$	0.14 ± 0.01 a	0.14 ± 0.01 a	0.13 ± 0.01 ab	0.13 ± 0.01 ab	0.12 ± 0.01 ab	0.11 ± 0.01 b
$C_{14:0}$	0.98 ± 0.01 a	1.14 ± 0.03 b	1.09 ± 0.01 ab	1.06 ± 0.01 ab	0.60 ± 0.06 c	0.58 ± 0.04 c
$C_{16:0}$	10.70 ± 0.03 a	11.45 ± 0.21 b	11.48 ± 0.35 b	11.15 ± 0.11 ab	10.63 ± 0.04 a	10.59 ± 0.06 a
$C_{18:0}$	3.92 ± 0.04 a	4.46 ± 0.06 b	4.36 ± 0.18 ab	4.26 ± 0.20 ab	3.22 ± 0.08 c	3.25 ± 0.02 c
$C_{16:1}$	0.69 ± 0.03 a	0.63 ± 0.04 a	0.58 ± 0.02 a	0.67 ± 0.08 a	0.61 ± 0.04 a	0.74 ± 0.01 a
$C_{18:1}$	63.46 ± 0.27 a	63.97 ± 0.32 ab	64.77 ± 0.11 c	65.37 ± 0.22 c	64.39 ± 0.13 b	64.56 ± 0.12 bc
$C_{18:2}$	13.64 ± 0.13 ab	13.47 ± 0.18 a	14.13 ± 0.13 b	13.90 ± 0.13 ab	13.65 ± 0.15 ab	13.69 ± 0.18 ab
$C_{18:3}$	0.48 ± 0.01 ab	0.44 ± 0.02 a	0.52 ± 0.02 b	0.49 ± 0.01 ab	0.47 ± 0.01 ab	0.47 ± 0.01 ab
$C_{20:4}$	0.35 ± 0.01 ab	0.34 ± 0.01 abc	0.37 ± 0.01 a	0.33 ± 0.01 bc	0.31 ± 0.01 c	0.30 ± 0.01 d
$C_{20:5}$	1.48 ± 0.03 a	1.47 ± 0.01 a	1.50 ± 0.01 a	1.51 ± 0.01 a	1.49 ± 0.02 a	1.48 ± 0.01 a
$C_{20:6}$	0.69 ± 0.01 a	0.68 ± 0.01 a	0.69 ± 0.01 a	0.69 ± 0.00 a	0.68 ± 0.01 a	0.68 ± 0.01 a
SFA	16.08 ± 0.01 a	17.53 ± 0.33 b	17.42 ± 0.19 b	16.93 ± 0.08 b	14.87 ± 0.06 c	14.82 ± 0.01 c
MUFA	64.15 ± 0.30 a	64.59 ± 0.35 ab	65.35 ± 0.09 bc	66.04 ± 0.30 c	65.04 ± 0.16 ab	65.30 ± 0.11 bc
PUFA	16.28 ± 0.15 ab	16.05 ± 0.18 a	16.83 ± 0.18 b	16.59 ± 0.13 ab	16.27 ± 0.18 ab	16.32 ± 0.17 ab
			Vegetable Beverage			
fat	0.41 ± 0.01 a	0.40 ± 0.01 a	0.40 ± 0.01 a	0.40 ± 0.01 a	0.40 ± 0.01 a	0.40 ± 0.01 a
$C_{12:0}$	ND	ND	ND	ND	ND	ND
$C_{14:0}$	ND	ND	ND	ND	ND	ND
$C_{16:0}$	14.79 ± 0.17 a	14.75 ± 0.17 a	14.70 ± 0.33 a	14.63 ± 0.10 a	14.49 ± 0.02 a	14.25 ± 0.28 a
$C_{18:0}$	2.93 ± 0.11 ab	2.99 ± 0.04 a	2.90 ± 0.08 ab	2.89 ± 0.06 ab	2.66 ± 0.07 b	2.94 ± 0.04 ab
$C_{16:1}$	1.44 ± 0.05 a	1.27 ± 0.05 b	1.26 ± 0.01 b	1.31 ± 0.01 b	1.29 ± 0.02 b	1.30 ± 0.06 b
$C_{18:1}$	63.11 ± 0.70 ab	62.85 ± 0.14 a	64.19 ± 0.13 c	64.29 ± 0.13 bc	64.22 ± 0.09 c	64.38 ± 0.35 c
$C_{18:2}$	14.13 ± 0.31 ab	13.98 ± 0.11 ab	14.25 ± 0.14 b	14.44 ± 0.21 b	14.05 ± 0.21 ab	13.88 ± 0.10 a
$C_{18:3}$	1.71 ± 0.03 a	1.68 ± 0.04 a	1.71 ± 0.01 a	1.73 ± 0.01 a	1.70 ± 0.04 a	1.71 ± 0.04 a
SFA	17.72 ± 0.06 a	17.74 ± 0.21 a	17.60 ± 0.41 a	17.52 ± 0.04 a	17.15 ± 0.05 a	17.18 ± 0.31 a
MUFA	64.54 ± 0.65 a	64.12 ± 0.09 a	65.45 ± 0.13 a	65.59 ± 0.13 a	65.50 ± 0.11 a	65.68 ± 0.29 a
PUFA	15.84 ± 0.28 ab	15.65 ± 0.07 abc	15.96 ± 0.13 a	16.17 ± 0.23 bc	15.75 ± 0.16 bc	15.59 ± 0.06 bc

[a]Different letters in the same file indicate significant differences as a function of the applied treatment. ND, non-detectable.

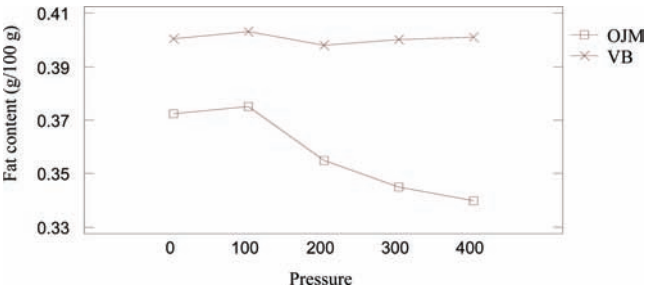

Figure 1. Fat content in high-pressure-treated (100, 200, 300, and 400 MPa during 9 min) orange juice−milk (OJM) and vegetable beverage (VB): interactions.

in HP-treated whole sheep's milk (100−500 MPa/4−50 °C/15−30 min) than in untreated milk. On the other hand, Molto-Puigmartí et al.[33] did not find significant alterations in fatty acids in the HP-treated human milk (400−600 MPa/22−27 °C/5 min) in comparison with fresh milk. Moreover, Kim et al.,[26] after applying HP treatments (200 MPa/-4 °C/10−30 min) in milk, also did not observe changes in short-chain free fatty acid contents, when the treatment time was <20 min, but the values did increase at 20 and 30 min, which they attributed to the possible activation of lipolysis. Lipolysis in milk can be produced by natural lipases such as lipoprotein lipase (LPL). The LPL hydrolyzes preferentially positions 1 and 3 of long-chain triglycerides,[25] which is related to the higher levels of $C_{16:0}$, $C_{18:0}$, and $C_{18:1}$ free fatty acids.

On the other hand, the percentage in the untreated vegetable beverage of SFA was 17.7%, that of MUFA was 64.5%, and that of PUFA was 15.8%. Analysis of variance shows nonsignificant differences with HP treatment when fatty acids were grouped in SFA, MUFA, and PUFA. However, an increase in the MUFA percentage (5%) after HP treatment at 400 MPa was observed. It is important to enhance the increase in oleic acid percentage after application of the different pressures, whereas a decrease in $C_{16:1}$ was obtained for all HP treatments. Similar results were reported by Kowalsky et al.;[41] they did not observe statistically significant changes in linolenic acid after 600 MPa. On the other hand, these results were not confirmed by the observation of Porreta et al.,[42] who reported losses of linoleic and linolenic acid in tomato juice of 40 and 26%, respectively, after HP treatment.

With regard to thermal treatment, fat content decreased in thermally processed orange juice−milk. Furthermore, the two-way ANOVA shows a significant increase in SFA content, whereas nonsignificant differences were obtained for MUFA and PUFA in comparison to untreated beverage. Thermal treatment is known to inactivate milk lipases, which means that triglycerides in pasteurized samples will not be hydrolyzed by the action of these enzymes and therefore will be less susceptible to further oxidation.[43] Similar results were reported by Moltó-Puigmartí et al.[33] in mature human milk, and also other studies reported no alteration in human milk

fatty acid proportions after Holder pasteurization.[35,43] Moreover, in the vegetable beverage, nonsignificant modifications were obtained in fatty acid profile after HP and thermal treatment. These results were in accord with those found by Garde-Cerdán et al.[40] in grape juice processed at 90 °C during 60 s.

To compare the effect of pressure depending on food matrix studied, an ANOVA of two factors, treatment pressure and studied matrix (orange juice mixed with milk and vegetable beverage), was applied. For fat content, the two-way ANOVA did not show interactions ($p > 0.05$) between the two factors studied, obtaining a decrease in fat content when the pressure of treatment augments in both matrices (Figure 1). With regard to vitamin E, nonsignificant interactions ($p > 0.05$) were observed for α-tocopherol content, obtaining an increase when

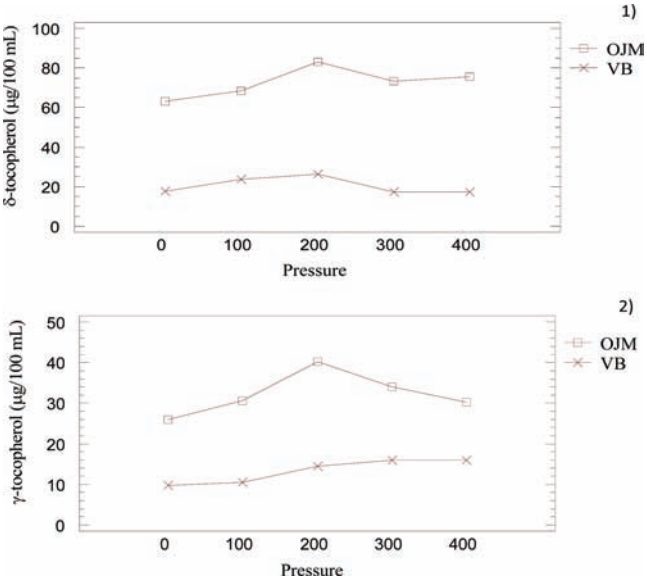

Figure 2. (1) δ- and (2) γ-tocopherol in high-pressure-treated (100, 200, 300, and 400 MPa during 9 min) orange juice−milk (OJM) and vegetable beverage (VB): interactions.

treatment intensity was increased. When the pressure was >200 MPa, nonsignificant changes were obtained in the α-tocopherol content of the HP-treated vegetable beverage. However, a slight decrease in α-tocopherol content was obtained in HP-treated orange juice−milk when the pressure was >200 MPa. On the other hand, significant interactions between the two factors studied were obtained for γ- and δ-tocopherol contents in both matrices after HP treatment (Figure 2). A significant increase ($p < 0.05$) in γ-tocopherol content was found for the vegetable beverage and orange juice−milk after application of HP treatments. However, the behavior of δ-tocopherol followed different trends after HP depending on food matrix, obtaining a significant increase in HP orange juice−milk for all treatments (100−400 MPa), whereas for the vegetable beverage a significant decrease was found when the pressure was >200 MPa. With regard to fatty acids, SFA behavior was similar in both matrices, obtaining significant interactions ($p < 0.05$). However, a different trend was observed for MUFA and PUFA depending on the food matrix studied. Nonsignificant interactions ($p > 0.05$) were observed in these cases (Figure 3). In conclusion, the results obtained after the application of HP treatments in orange juice mixed with milk and vegetable beverages show that high-pressure processing, in general, does not affect the total concentration of

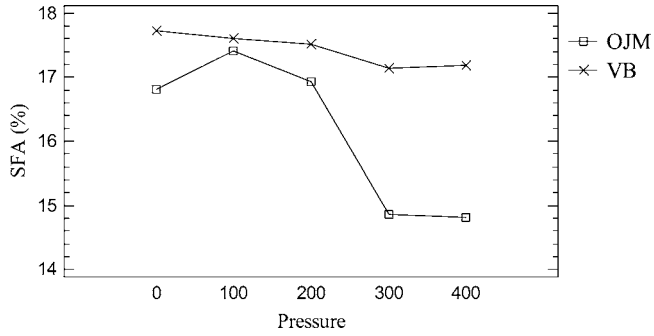

Figure 3. Saturated fatty acids (SFA) in high-pressure-treated (100, 200, 300, and 400 MPa during 9 min) orange juice−milk (OJM) and vegetable beverage (VB): interactions.

fatty acids or even increase levels of oleic acid, and from the potential nutritional point of view, the changes observed are negligible. On the other hand, in the vegetable beverage HP treatment increase MUFA, and PUFA remained unaffected or even increased. Thus, orange juice mixed with milk and vegetable beverage processed by high pressure could be beneficial for health. Such changes might also result in altered bioavailability with possible nutritional consequences.

AUTHOR INFORMATION

Corresponding Author

*Phone: +34 963 544955. Fax: +34 963 544954. E-mail: ana.frigola@uv.es.

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Notes

The authors declare no competing financial interest.

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