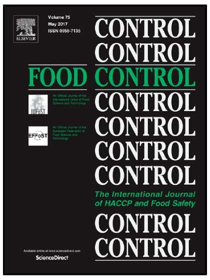

Mineral and fatty acid profile of high intensity pulsed electric fields or thermally treated fruit juice-milk beverages stored under refrigeration

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HIGHLIGHTS

- Ca, Zn, Fe, Mg, Cu, and Mn were identified in FJ-WM or FJ-SM bevearges
- Mineral content was not affected by HIPEF or TT, neither during storage
- 11 fatty acids were identified in the FJ-WM beverage
- Palmitic acid was the most abundant fatty acid in the FJ-WM bevearge
- HIPEF treated FJ-WM beverage kept more fatty acids during storage than that heated

MINERAL AND FATTY ACID PROFILE OF HIGH INTENSITY PULSED ELECTRIC FIELDS OR THERMALLY TREATED FRUIT JUICE-MILK BEVERAGES STORED UNDER REFRIGERATION

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ABSTRACT

The influence of High Intensity Pulsed Electric Fields (HIPEF) or Thermal Treatment (TT) on minerals and fatty acids of fruit juice-whole (FJ-WM) or skimmed milk (FJ-SM) beverages was assessed after processing and during chilled storage. Mineral profile of both beverages was characterized by Ca, Zn, Fe, Mg, Cu, and Mn; being Ca the macroelement detected at the highest concentration (3.06-3.17mg/100mL). Neither HIPEF nor TT significantly affected mineral concentration of the beverages, except Fe, which augmented after HIPEF (300%) or TT (43%). During storage (56 days), mineral content in both bevearges remained highly stable, regardless of the treatment applied. 11 fatty acids were identified in untreated and processed FJ-WM beverages. Palmitic acid was detected at highest concentration (21.83-24.37mg/100g of fat). Immediately after HIPEF or TT, most fatty acids remained with no significant changes, only linoleic acid increased (20%) in HIPEF treated beverage. Fatty acid content of HIPEF treated FJ-WM beverages was kept constant along storage; only palmitic, linoleic and linolenic acids showed lower concentrations (12-20%) at day 56. Conversely, the concentration of most fatty acids in the heated beverage underwent a significant reduction (7-19%) with time, except palmitic acid, which remained constant. HIPEF can be considered as a potential alternative to conventional pasteurization to obtain stable mixed beverages with significant concentrations of health-related compounds.

Key words: HIPEF; thermal treatment; mixed beverages; fatty acids; minerals.

1. INTRODUCTION

Through the last decades, different studies have demonstrated that fruits and vegetables consumption has important beneficial effects on health due to their high content of antioxidants compounds and phytochemical substances (Barba, Esteve, & Frigola, 2012; Andrés, Villanueva, & Tenorio, 2016; Morales-de la Peña, Salvia-Trujillo, Rojas-Graü, & Martín-Belloso, 2011). According to Barroso et al. (2009) and Navarro and Rohan (2007), the intake of macro (Ca, K, Na, Mg) and micro (Fe, Cu, Zn) elements is considered necessary for the maintenance of human health. These minerals are involved in physiological and biological processes affecting water and electrolyte balance, metabolic catalysis, oxygen binding and hormone functions (Gutzeit et al., 2008). Furthermore, they play an important role in the maintenance of pH, osmotic pressure, nerve conductance, and energy production (Barroso et al., 2009; Biziuk and Kuczynska, 2007) and are important factors for bone and membrane structure formation (Gutzeit et al., 2008). On the other hand, the ingestion of monounsaturated fatty acids (MUFA), especially oleic acid, helps to decrease plasma triacylglycerol and cholesterol concentrations in healthy normolipidaemic subjects (Feldman, 1999; Kris-etherton et al., 1999); while polyunsaturated fatty acids (PUFA) consumption reduces the risk of coronary heart disease (Harris, 2003), cancer (Larsson, Kumlin, Ingelman-sundberg, & Wolk, 2004) and plasma triacylglycerol levels (Funk, 2001). In this sense, it can be suggested that an adequate intake of minerals and fatty acids is of high importance to decrease or avoid the risk of chronic disorders.

Food industry is continuously looking for market opportunities through the development of new products with improved sensorial, nutritional and functional attributes. In this regard, and considering that fruit juices and milk are excellent sources of minerals and fatty acids, respectively (Claeys et al., 2014; Winiarska-mieczan & Nowak, 2008), they can be used as potential ingredients for the development of mixed beverages with functional properties and attractive flavor. In previous papers, it was demonstrated that fruit juice-milk beverages are rich in water-soluble vitamins, phenolic compounds and carotenoids, possessing at the same time, high antioxidant capacity (Morales-de la Peña, Salvia-Trujillo, Rojas-Graü, & Martín-Belloso, 2016a, 2016b; Salvia-trujillo, Rojas-Graü, & Martín-belloso, 2011; Barba, Cortés, Esteve, & Frígola, 2012; Barba, Esteve, & Frígola, 2011; Barba, Esteve, Tedeschi, Brandolini, & Frígola, 2013; Zulueta, Barba, Esteve, & Frígola, 2013). Hence, this kind of beverages are currently receiving considerable attention by the consumers and are achieving high popularity around the world, especially in the US and EU markets (Cook, 2003; Sharma, 2005).

Though heating is the simplest and most reliable method to obtain safe and shelf-stable mixed beverages, the high temperatures achieved during processing cause significant losses of most of their desirable health-related compounds and affects physicochemical and sensorial properties of the final product. Thus, the study of novel technologies for preservation purposes at low temperatures is a marked trend in food research. Among these novel technologies, high intensity pulsed electric fields (HIPEF) has been investigated by different research groups as non-thermal treatment for mixed beverage preservation (Carbonell-Capella et al., 2013; Morales-De La Peña et al., 2012; Morales-de la Peña et al., 2011; Morales-De La Peña et al., 2011; Salvia-trujillo et al., 2011a, 2011b; Barba, Parniakov, et al., 2015; Zulueta, Barba, Esteve, & Frígola, 2010). Interestingly, results from these studies have demonstrated that most of the bioactive compounds identified in mixed beverages such as water-soluble vitamins, phenolic acids, flavonoids, and carotenoids are better retained after HIPEF processing than using conventional pasteurization methods. However, to the best of the authors' knowledge, there is no information related to the effects of HIPEF on the mineral and fatty acid profile of beverages containing fruit juices and whole or skim milk. Hence, this work attempts to evaluate the effects of a HIPEF treatment over the mineral composition and the fatty acid profile of fruit juice-milk beverages, immediately after processing and throughout the storage (56 days) at 4°C, in comparison to the untreated and thermally treated (TT) beverages.

2. MATERIAL AND METHODS

2.1 Beverage preparation

Orange, mango, kiwi and pineapple fruits were purchased in a local supermarket (Lleida, Spain) at commercial ripeness stage. Fruits were sanitized in a 200 ppm sodium hypochlorite solution for 2 min and rinsed with tap water. Then the juice from each fruit was extracted, mixed and added with commercial pasteurized whole (3.5% fat) or skim (0.3% fat) milk and sugar in the following proportions: orange (30%), kiwi (25%), mango (10%), pineapple (10%), milk (17.5%) and sugar (7.5%). These proportions were selected in basis of previous studies in order to maximize the content of vitamin C in the beverages (Salvia-trujillo et al., 2011a). Fruit juice-whole milk (FJ-WM) or –skim milk (FJ-SM) beverages were filtered through a cheese cloth, and their pH was adjusted to 3.35 with citric acid.

2.2 HIPEF processing

A continuous flow bench-scale system OSU-4F (The Ohio State University, Colombus, OH), delivering square-wave pulses, was used for HIPEF processing. On the basis of previous work (Salvia-Trujillo et al., 2011b), the HIPEF process was conducted at 35 kV/cm electric field strength for 1800 μs, a pulse frequency of 200 Hz, and 4 μs bipolar pulses with an electric energy density input of 1276.7 JL^{-1}. Electric field strength, pulse duration and frequency were controlled through a pulse generator (model 9410, Quantum Composers, Inc., Bozeman, MT) and measured with an oscilloscope (TEKScope, Tektronix Inc., Beaverton, OR). The samples were pumped through the system at a flow rate of 60 mL/min with a variable gear pump (model 752210-26, 106 Cole Palmer Instrument Co., Vermon Hills, IL). The system was composed of eight collinear treatment chambers serial connected, each with two stainless steel electrodes separated by 0.292 cm. Each treatment chamber has a diameter of 0.23 cm and a volume of 0.0121 cm^3. Between each treatment chamber the product was refrigerated in an ice-water bath. The temperature of the product during the process and at the end of the treatment was always below 40°C, which was measured with thermocouples at the inlet and outlet of each treatment chamber.

2.3 Thermal treatment

Beverages were thermally treated (TT) at 90 °C for 60 seconds to ensure the inactivation of spoilage microorganisms and to simulate a conventional preservation process based on literature (Nagy et al., 1993). The samples were pumped with a peristaltic pump (model D-21 V, Dinko, Barcelona, Spain) at a flow rate of 40 mL/min and passed through a tubular stainless steel heat exchanger coil system (0.037 cm^2 transversal section and 1100 cm long) submerged in a hot water bath settled at 90°C (Universitat de Lleida, Spain). Then the heated beverages were immediately cooled in a heat exchange coil immersed in an ice water-bath.

2.4 Packaging and storage

HIPEF and thermal systems were disinfected first with 4% of NaOH and then with 10% chlorine and 20% ethanol, aqueous solutions prior to processing. Polypropylene sterile bottles of 25 mL were used to store the untreated and treated FJ-WM and FJ-SM beverages. Once filled, the receptacles were tightly close and stored at 4 ± 1 °C in darkness and with minimal headspace volume. Because of microbial growth, treated and untreated samples were stored for 56 and 14 days, respectively (Salvia-Trujillo et al., 2011b).

2.5 Identification and quantification of minerals

Mineral profile was determined following a procedure established by Alwakeel and Al-Humaidi (2008). FJ-WM or FJ-SM beverages (20 mL) were mixed with 10 mL of HCl and made up to 100 mL with water in a volumetric flask. The mixture was vigorously shaken, transferred to centrifuge tubes and then centrifuged to removed solid particles to obtain solution (A) for minor minerals identification (Zn, Fe, Cu and Mn). In order to detect Ca and Mg, further dilutions were made. In the case of Ca, 12 mL of solution A were mixed with 10 mL of Lanthanum solution (5 %), 5 mL of HCl and made up to 100 mL with water in a volumetric flask to obtain solution B. Regarding Mg identification, 12.5 mL of solution A were mixed with 5 mL of HCl and made up to 100 mL with water in a volumetric flask to obtain solution C.

A Spectrophotometer (PerkinElmer AAnalyst 200, Conneticut, USA) with air-acetylene oxidizing flame and hollow cathode lamps (Varian) was used to analyse solutions A, B and C. Ca, Mg, Zn, Fe, Cu and Mn were detected at wavelengths of 422.7, 285.2, 213.9, 248.3, 324.8, and 279.5 nm, respectively. Quantification of the different elements was done using the calibration curves of the respective standard solution for each mineral.

2.6 Identification and quantification of fatty acids

The extraction and quantification of fatty acids was carried out in the FJ-WM beverage following the procedure established by Folch et al. (1957). The method consisted in fat extraction from the sample using a mixture of chloroform and methanol (2:1, v/v). After fat was extracted, FAMEs (fatty acid methyl esters) were prepared according to Golay et al. (2006).

A gas chromatograph (Carlo, Erba, Milan, Italy) was used to perform the analysis of FAMEs. The chromatograph was equipped with split/splitless injector, a flame ionization detector (300 °C) and a CP-Sil 88 capillary column of 100 m length and 0.25 μm of film thickness (Varian, Palo Alto, CA, USA). The oven temperature was raised from 65 °C in the first 5 min up to 165 °C with an increase rate of 15 °C/min, immediately the temperature was increased to 225 °C (rate of 2 °C/min) and it was maintained during 17 min. Gas pressure was 200 kPa. The different FAMEs were identified by comparing retention times with those obtained for standards using a Chrom-Card Data System (Thermo Fisher Scientific).

2.7 Statistical analysis

Treatments were conducted in duplicate and two replicate analyses were performed for each sample. Analysis of the variance (ANOVA) was done to compare treatments. Least significance difference (LSD) test was employed to determine differences between means immediately after processing and throughout the storage. The confidence interval was set at 0.95 for analysis and procedures. Results were analysed using Statgraphics Plus v.5.1 Windows package (Statistical Graphics Co., Rockville, Md).

3. RESULTS AND DISCUSSION

3.1 Mineral profile

Six minerals were detected in the FJ-WM and FJ-SM beverages, being Ca the macroelement identified at highest concentration (3.06 to 3.17 mg/100 mL), followed by Zn, Fe, Mg, Cu, and Mn. As can be seen in tables 1 and 2, the concentration of these minerals was similar in both beverages, irrespectively of the milk, whole or skimmed, used in their formulation; except Cu content, which was a little higher in the FJ-WM beverage (0.35 ± 0.04 mg/100mL) than in the FJ-SM (0.27 ± 0.03 mg/100mL). Our results are different to those reported in a recent study conducted by Andrés et al. (2015). These authors observed that the mineral profile of a fruit juice smoothie mixed with milk or soymilk was constituted by K, Zn, Fe, Cu, Mn, Na, Ca and Mg; being K the main macroelement in both milk- and soymilk-smoothies. The Ca content of the milk smoothie was significantly lower (1.08 mg/100mL) than the level quantified in the FJ-WM or FJ-SM beverages. These differences could be mainly attributed to the ingredients and proportions used the formulations of the mixed bevearges evaluated in both studies. While the milk smoothie evaluated by Andres et al. (2015) had only 10% of skimmed milk, the FJ-WM or FJ-SM beverages prepared in this study contained 17.5% of milk. Chassaing et al. (2016) demonstrated that Ca was the mineral present at highest concentration in different milk samples. Furthermore, the USDA (2009) has reported that the main elements in orange, kiwi, pineapple and mango juices are K, P, Ca, Mg, Na, Fe, Zn, Cu and Mn at different concentrations depending on their variety and ripeness stage. Therefore, the mineral profile of a mixed beverage will be highly influenced by the raw materials used on its formulation as well as the percentage of each one.

According to Bonjour et al. (2009), the intake of adequate amount of Ca has a potential relevance in health, since this macroelement plays an important role in bone strength. The WHO and FAO (2004) have established that the minimum daily amount of Ca required for human metabolism is 1000 mg. On the other hand, it has been reported by the IDF (2013) that the sole consumption of milk in the European Union (170 mL/day) provides approximately 20 – 25% of Ca. Then, the ingestion of this kind of bevearges as a supplementation of traditional consumers' diets will increase the percentage of Ca intake at day (8 mg/250mL/day), contributing to the health of the population.

Neither HIPEF nor TT significantly affected the concentration of the minerals identified in FJ-WM (Table 1, day 0) and FJ-SM beverages (Table 2, day 0), with the exception of Fe. Immediately after HIPEF or TT, the Fe content was significantly increased in both beverages, reaching values of 1.52 – 1.64 mg/100mL and 0.59 mg/100mL, respectively. Although Fe is an essential element for various metabolisms reactions, it can become toxic if consumed at elevated concentrations (Barroso et al., 2009; Lukaski, 2004; Navarro and Rohan, 2007). The WHO and FAO (2003) established that the tolerable intake of this mineral is 0.8 $mg/kg_{bodyWeigth}/day$. Considering that the average weight of an adult person in Europe is 70 kg (Quilty-Harper, 2012), then the tolerable intake of Fe would be approximately 56 mg/day. In this sense, even though the Fe concentration in treated FJ-WM and FJ-SM beverages significantly increased after HIPEF or thermal processing, a standard serve (250 mL) will maintain Fe content (1.47 – 4.1 mg) at adequate levels for being consumed with no health risks for humans.

To the best of the authors' knowledge, there are few studies reporting the effects of HIPEF or thermal processes on the mineral profile of fruit based beverages. On one hand, it has been stated that HIPEF treatments at different processing conditions did not induce significant changes on the mineral concentration of sour cherry juice (Akin & Evrendilek, 2009), carrot juice (Altuntas, Evrendilek, Sangun, & Zhang, 2010) or a mixed beverage prepared with orange and whey powder (Monico et al., 2006). Otherwise, Evrendilek et al. (2004) and Morales-de la Peña et al. (2011) indicated that a HIPEF processed orange juice or fruit juice-soymilk beverage, respectively, have higher concentrations of Fe and Zn than the untreated ones. Moreover, it has been found that conventional thermal processes could also modify the mineral content in foods to an extent depending on the severity of the treatment. Bamishaiye et al. (2011) observed that, Ca, Na, K and Fe content of a non-alcoholic beverage significantly increased after thermal treatments applied at different conditions. These authors stated that, minerals might have been released from mineral-macromolecules interactions during thermal processing, without being affected by heat.

In accordance to Reddy and Love (1999), minerals can either be removed from foods during processing by physical separation or added as ingredients or from the instruments used during processing. Their stability depends on the nature of the food in which are found, the particle size, and the exposure to heat and air (Akhtar, Anjum, Salim-Ur-Rehman, & Sheikh, 2010). Roodenburg et al. (2005) indicated that elements of the HIPEF treatment chamber electrodes are able to be dissolved in treated products by electrochemical reactions that occur during the processing. According to them, the extend of the element transfer depends on the current magnitude, pulse duration and shape, as well as the product constitution. The obtained results in this work, evidence that during HIPEF treatment, metal elements from the chamber electrodes were transferred to the mixed beverages and as a result, the concentration of Fe significantly rose. On the other hand, the increase of Fe content observed in the heated mixed bevearges may be due to the hydrolysis reactions caused by temperature, releasing Fe from protein-iron complexes or to the metal element transfer from the pipe in which the beverage was processed.

During the storage at 4°C, the concentration of all the minerals identified in FJ-WM and FJ-SM bevearges did not suffer noticeable changes (Tables 1 and 2). Similar results have been reported by de Vasconcelos et al. (2010), Morales-de la Peña et al. (2011), Zhang et al. (2007) and Andrés et al. (2015) who agreed that minerals are generally stable in food throughout storage time. In this sense, our data support that minerals contained in mixed bevearges with fruit juices and whole milk or skimmed milk are stable compounds along chilled storage, regardless of the treatment used for their preservation. Furthermore, when consumers drink a HIPEF or TT mixed beverage they will receive similar amount of minerals contained in the untreated beverage.

3.2 Fatty acid profile of FJ-WM beverage

As can be seen in tables 3 and 4 (day 0), five saturated (SFA): capric (C10:0), lauric (C12:0), myristic (C14:0), palmitic (C16:0) and stearic (C18:0); four monounsaturated (MUFA): myristoleic (C14:1), palmitoleic (C16:1), elaidic (C18:1*trans*), oleic (C18:1*cis*), and two polyunsaturated (PUFA): linoleic (C18:2*cis*) and α-linolenic (C18:3n3), fatty acids were identified in untreated, HIPEF and TT FJ-WM beverages. Regardless of the treatment applied, palmitic acid was detected at highest concentration (21.83 – 24.37 mg/100 g of fat), followed by oleic acid (14.50 – 16.94 mg/100 g of fat), myristic acid (7.28 – 8.07 mg/100 g of fat) and stearic acid (6.59 – 7.58 mg/100g of fat). At present, there is not available information of the fatty acid profile of blended beverages containing different fruit juices and whole milk. Nevertheless, other research works have been carried out separately in dairy products and some fruits, such as mango pulp (Cappozzo et al., 2015; Nunes and Torres, 2010; Pestana et al., 2015; Bandyopadhyay and Gholap 1973; Morales-de la Peña et al., 2011; Zulueta et al., 2007; Barba, Esteve, & Frigola, 2012). On the one hand, similar to our results, Cappozzo et al. (2015), Nunes and Torres (2010) and Pestana et al. (2015) agreed that the principal fatty acids in dairy products are palmitic, oleic, stearic, and myristic acids. Pestana et al. (2015) observed that oleic acid was the main MUFA in bovine milk, contributing to 25.3 - 26.4% of total fatty acid concentration. On the other hand, Bandyopadhyay and Gholap (1973) characterized the fatty acid profile of mango pulp during ripening and identified the following fatty acids C12:0, C14:0, C16:0, C16:1, C18:0, C18:1, C18:2 and C18:3 at different concentrations. These authors observed that the content of the different fatty acids is highly related with the ripeness stage of the fruit. For example, palmitic acid (C16:0) was the major fatty acid (28.9%) present in raw mango pulp; nonetheless, when the mango was full ripe palmitic acid concentration decrease in 2%, while the content of palmitoleic acid (C16:1) augmented in 13.6%. Another similar study conducted by Morales-de la Peña et al. (2011) reported the fatty acid profile of a mixed beverage containing orange, pineapple and kiwi juices, and soymilk. Different to our results, palmitic, stearic, oleic, linoleic, and linolenic acids were the fatty acids detected at highest concentration in that beverage. The use of soymilk, which is rich in linoleic and linolenic acids, in the mixed beverage elaborated by Morales-de la Peña et al. (2011) instead of whole milk, may be the main factor related to the differences observed in the fatty acid profile of both mixed beverages. Otherwise, Zulueta et al. (2007) and Barba et al. (2012) elaborated a blended beverage containing orange juice and milk fortified with linolenic and oleic acids. Both authors reported similar fatty acid profiles in the evaluated drinks with a percentage of SFA, MUFA and PUFA was 16 - 17.4%, 64.2 - 66.4% and 13.5 – 16.3%, respectively. Due to the addition of linolenic and oleic acids in the beverage, their results were significantly different from those obtained in this research. Hence, it could be stated that the combination of fruit juices with milk might modify the characteristic fatty acid profile of whole milk, depending on the fruits used for the beverage formulation and their maturity stage. In this case, the high concentration of palmitic acid (34.4 - 35.3%), could be attributed to the use of whole milk and mango pulp.

Initial concentration of SFA (63.2-64.2%), MUFA (29.4-30.1%) and PUFA (6.4-6.7%) of the FJ-WM beverage remained with no significant changes after HIPEF or TT. These results imply that concentration of individual fatty acids was not altered during processing, regardless of the treatment applied; except for linoleic acid content, which was significantly higher in HIPEF treated beverages (Table 4, day 0). Garde-Cerdán et al. (2007) and Zulueta et al. (2007) compared the effects of HIPEF and thermal treatments on the fatty acid concentration of grape juice and an orange juice-milk beverage, respectively. Similar to the results obtained in the present study, these authors observed that neither thermal nor HIPEF processing have a significant influence in the fatty acid content of the evaluated samples. Likewise, other authors have reported that fatty acid profile of milk is little affected after pasteurization, commercial sterilization or ultraviolet light processing (Cappozzo et al., 2015; Cilliers et al., 2014; Costa et al., 2011; De Souza et al., 2003; Pestana et al., 2015).

According to davis and Kris-Etherton (2003), the ratio ω-6 /ω-3 is often used to assess the balance between essential fatty acids. Lower values of ω-6 /ω-3 are more desirable in reducing risk of chronic and degenerative diseases and, according to Simopoulos (2002) it cannot be over than 4. The ω-6 /ω-3 ratios of untreated, HIPEF and TT FJ-WM beverages were 2.31, 2.37 and 2.19, respectively. These values agree well with ω-6 /ω-3 ratios reported for whole raw (2.10), pasteurized (1.97) and commercially sterilized milks (2.07) (Costa et al., 2011). Hence, despite of the high levels of SFA quantified in the FJ-WM beverage, it can be considered still a great source of fatty acids and the preservation processes applied will not negatively affect the quality of the final product maintaining the proportion of essential fatty acids.

Table 3 shows the concentration of the individual SFA observed in untreated, HIPEF and TT FJ-WM beverages during refrigerated storage (4 °C, 56 days). As can be seen, the concentration of most SFA in untreated and treated beverages remained with no significant changes through the time, compared to their initial values, except capric, myristic and palmitic acid content which was significantly lower (8 – 11%) in the TT beverage after 56 days. Regarding MUFA and PUFA (Table 4), their concentrations were kept constant after 14 and 56 days in untreated and HIPEF treated beverages, respectively. Only palmitoleic, linoleic and linolenic acids content of FJ-WM processed with HIPEF, decreased around 12 - 20% regarding their initial concentration. Likewise, the content of most of the MUFA and PUFA identified in the TT FJ-WM beverage, but palmitic acid, significantly diminished (7 – 19%) over time. According to Claeys et al. (2014), oxidation reactions may occur even at low temperatures. Lipid oxidation can be initiated by enzymes present in the milk or by traces of metal ions, such as Fe, from processing equipment (Giroux, St-Amant, Fustier, Chapuzet, & Britten, 2008; Kristensen, Hedegaard, Nielsen, & Skibsted, 2004). Hence, it could be possible that the presence of Fe, which was detected at high concentration (0.59 – 1.64 mg/100mL) in HIPEF and TT beverages, catalyses the oxidation process of MUFA and PUFA along the time, thus reducing their concentration at the end of the storage.

Some research works have been conducted to evaluate the fatty acid profile of different treated products such as cow’s milk, mixed beverages containing fruit juices and soymilk, and fermented beverages during storage, reporting different results depending on the food matrix and treatment applied. Similar to our observations, Capozzo et al. (2015), Timmons et al. (2001) and Yue et al. (2016) mentioned that fatty acid composition in whole milk remained unchanged during 7, 8 or 14 days of storage. Otherwise, Regula (2007) stated that the amount of most fatty acids detected in fermented beverages (yogurt, sour milk and kefir) was lower on the last day of storage period (14 days at 4 °C) than on the first day of analysis. Conversely, total fatty acid content of HIPEF or TT whole milk (Odriozola-Serrano, Bendicho-Porta, & Martín-Belloso, 2006) or a fruit juice-soymilk beverage (Morales-de la Peña et al., 2011) enhanced as storage time increased, irrespective of the treatment applied. Odriozola-Serrano, et al. (2006) and Morales-de la Peña, et al. (2011) attributed this effect to the spoilage of the product by microorganisms during the storage, that would contribute to an increase in fat degradation and release of fatty acids. The processing conditions applied to the whole milk or the fruit juice-soymilk beverage were different to those used in this study; therefore, the growth of microorganisms during the storage was better controlled avoiding fat degradation.

4. CONCLUSIONS

Mixed beverages containing fruit juices and milk could be considered good sources of minerals and fatty acids. Calcium was the main mineral identified in the FJ-WM and FJ-SM beverages, while palmitic acid was the main fatty acid detected in FJ-WM beverage, irrespective of the treatment applied. The most relevant change observed in the mineral profile of the FJ-W and FJ-SM beverages, was the significant increase of Fe concentration after HIPEF and TT. Likewise, most fatty acids in the FJ-WM beverage remained with no significant changes immediately after HIPEF or thermal processing, only linoleic acid content increased in the HIPEF treated beverages. Along storage, mineral concentration in both bevearges remained highly stable, irrespective of the processing applied. Conversely, linoleic and linolenic acids content of FJ-WM beverage treated by HIPEF diminished with time; while TT beverage showed lower concentration of most fatty acids compared to their initial values. Even though both treatments caused specific changes in the concentration of minerals and fatty acids in mixed beverages, those HIPEF treated had always higher concentration than the heat treated ones. Hence, HIPEF process is a potential treatment to develop functional beverages with high content of health related substances, such as fatty acids.

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Table 1. Mineral profile (mg/100 mL) of the untreated, high intensity pulsed electric field (HIPEF: 35 kV/cm with 4 µs bipolar pulses at 200 Hz for 1800 µs) and thermally (TT: 90 °C, 60 s) treated fruit juice-whole milk (FJ-WM) beverages along refrigerated storage

Storage time (days)	Sample	Ca	Cu	Mg	Fe	Mn	Zn
0	**FJ-WM untreated**	3.06 ± 0.21a	0.35 ± 0.04a	0.35 ± 0.02a	0.44 ± 0.01a	0.17 ± 0.01a	0.50 ± 0.06a
	FJ-WM HIPEF	3.09 ± 0.06a	0.39 ± 0.01a	0.34 ± 0.01a	1.52 ± 0.04b	0.18 ± 0.01a	0.49 ± 0.04a
	FJ-WM TT	3.06 ± 0.03a	0.38 ± 0.02a	0.31 ± 0.02a	0.59 ± 0.04c	0.17 ± 0.01a	0.60 ± 0.13a
7	**FJ-WM untreated**	3.18 ± 0.16a	0.37 ± 0.01a	0.34 ± 0.01a	0.07 ± 0.02a	0.16 ± 0.01a	0.49 ± 0.01a
	FJ-WM HIPEF	2.84 ± 0.01b	0.36 ± 0.01a	0.35 ± 0.01a	1.50 ± 0.11b	0.18 ± 0.04a	0.56 ± 0.07a
	FJ-WM TT	2.93 ± 0.14ab	0.36 ± 0.01a	0.33 ± 0.01a	0.18 ± 0.02c	0.17 ± 0.03a	0.53 ± 0.03a
14	**FJ-WM untreated**	3.17 ± 0.10ab	0.31 ± 0.01a	0.34 ± 0.02a	0.23 ± 0.01a	0.18 ± 0.02a	0.47 ± 0.02a
	FJ-WM HIPEF	3.22 ± 0.10a	0.38 ± 0.01b	0.30 ± 0.01b	1.48 ± 0.01b	0.16 ± 0.03a	0.40 ± 0.01b
	FJ-WM TT	3.10 ± 0.01b	0.33 ± 0.01a	0.33 ± 0.01a	0.35 ± 0.01c	0.16 ± 0.03a	0.51 ± 0.02a
21	**FJ-WM HIPEF**	3.09 ± 0.03a	0.32 ± 0.01a	0.33 ± 0.01a	1.60 ± 0.12a	0.18 ± 0.02a	0.50 ± 0.05a
	FJ-WM TT	3.07 ± 0.04a	0.35 ± 0.04a	0.33 ± 0.01a	0.31 ± 0.01b	0.19 ± 0.03a	0.50 ± 0.01a
28	**FJ-WM HIPEF**	3.18 ± 0.01a	0.34 ± 0.05a	0.32 ± 0.01a	1.65 ± 0.07a	0.19 ± 0.01a	0.53 ± 0.05a
	FJ-WM TT	3.24 ± 0.12a	0.35 ± 0.03a	0.32 ± 0.01a	0.33 ± 0.01b	0.16 ± 0.01b	0.49 ± 0.04a
35	**FJ-WM HIPEF**	3.10 ± 0.13a	0.39 ± 0.03a	0.31 ± 0.01a	1.48 ± 0.01a	0.16 ± 0.04a	0.48 ± 0.04a
	FJ-WM TT	3.23 ± 0.04a	0.35 ± 0.01a	0.30 ± 0.01a	0.33 ± 0.02b	0.18 ± 0.04a	0.51 ± 0.04a
42	**FJ-WM HIPEF**	3.15 ± 0.13a	0.35 ± 0.05a	0.30 ± 0.03a	1.45 ± 0.02a	0.18 ± 0.02a	0.41 ± 0.01a
	FJ-WM TT	3.15 ± 0.04a	0.33 ± 0.03a	0.32 ± 0.03a	0.34 ± 0.01b	0.16 ± 0.03a	0.57 ± 0.13b
49	**FJ-WM HIPEF**	3.01 ± 0.01a	0.36 ± 0.01a	0.31 ± 0.03a	1.39 ± 0.01a	0.16 ± 0.04a	0.39 ± 0.06a
	FJ-WM TT	3.02 ± 0.11a	0.33 ± 0.03a	0.32 ± 0.03a	0.33 ± 0.01b	0.16 ± 0.02a	0.49 ± 0.08a
56	**FJ-WM HIPEF**	3.37 ± 0.43a	0.37 ± 0.01a	0.34 ± 0.01a	1.47 ± 0.01a	0.17 ± 0.01a	0.48 ± 0.01a
	FJ-WM TT	3.22 ± 0.16a	0.36 ± 0.04a	0.35 ± 0.03a	0.31 ± 0.01b	0.16 ± 0.04a	0.56 ± 0.11a

Values in the same column with different letters were significantly different ($p < 0.5$)

Table 2. Mineral profile (mg/100 mL) of the untreated, high intensity pulsed electric field (HIPEF: 35 kV/cm with 4 µs bipolar pulses at 200 Hz for 1800 µs) and thermally (TT: 90 °C, 60 s) treated fruit juice-skimmed milk (FJ-SM) beverages along refrigerated storage

Storage time (days)	Sample	Ca	Cu	Mg	Fe	Mn	Zn
0	**FJ-SM untreated**	3.17 ± 0.10ab	0.27 ± 0.03a	0.33 ± 0.01a	0.41 ± 0.03a	0.16 ± 0.01a	0.42 ± 0.02a
	FJ-SM HIPEF	3.16 ± 0.03a	0.28 ± 0.04a	0.32 ± 0.02a	1.64 ± 0.29b	0.17 ± 0.03a	0.47 ± 0.06a
	FJ-SM TT	3.03 ± 0.09b	0.25 ± 0.01a	0.34 ± 0.01a	0.59 ± 0.01c	0.17 ± 0.03a	0.45 ± 0.02a
7	**FJ-SM untreated**	2.97 ± 0.01a	0.28 ± 0.03a	0.32 ± 0.01a	0.19 ± 0.05a	0.16 ± 0.01a	0.47 ± 0.03a
	FJ-SM HIPEF	3.17 ± 0.01b	0.29 ± 0.05a	0.32 ± 0.01a	1.64 ± 0.05b	0.17 ± 0.03a	0.45 ± 0.01a
	FJ-SM TT	2.96 ± 0.06a	0.24 ± 0.03a	0.32 ± 0.01a	0.19 ± 0.01a	0.14 ± 0.04a	0.44 ± 0.01a
14	**FJ-SM untreated**	3.15 ± 0.01a	0.26 ± 0.02a	0.31 ± 0.02a	0.25 ± 0.01a	0.17 ± 0.03a	0.41 ± 0.01a
	FJ-SM HIPEF	2.99 ± 0.06a	0.29 ± 0.01a	0.32 ± 0.01a	1.51 ± 0.01b	0.15 ± 0.01a	0.43 ± 0.01a
	FJ-SM TT	3.04 ± 0.09a	0.27 ± 0.06a	0.31 ± 0.02a	0.26 ± 0.03a	0.17 ± 0.01a	0.44 ± 0.01a
21	**FJ-SM HIPEF**	3.01 ± 0.03a	0.29 ± 0.01a	0.34 ± 0.01a	1.55 ± 0.05a	0.17 ± 0.03a	0.48 ± 0.05a
	FJ-SM TT	3.11 ± 0.03b	0.23 ± 0.01b	0.33 ± 0.01a	0.16 ± 0.01b	0.16 ± 0.04a	0.47 ± 0.01a
28	**FJ-SM HIPEF**	3.03 ± 0.11a	0.28 ± 0.01a	0.32 ± 0.01a	1.60 ± 0.01a	0.19 ± 0.01a	0.46 ± 0.02a
	FJ-SM TT	3.18 ± 0.09a	0.27 ± 0.04a	0.34 ± 0.02a	0.20 ± 0.01b	0.15 ± 0.04a	0.48 ± 0.01a
35	**FJ-SM HIPEF**	3.15 ± 0.05a	0.29 ± 0.01a	0.30 ± 0.01a	1.68 ± 0.23a	0.18 ± 0.05a	0.45 ± 0.04a
	FJ-SM TT	3.17 ± 0.16a	0.30 ± 0.01a	0.29 ± 0.01a	0.31 ± 0.01b	0.16 ± 0.01a	0.42 ± 0.01a
42	**FJ-SM HIPEF**	3.00 ± 0.05a	0.29 ± 0.04a	0.30 ± 0.01a	1.49 ± 0.01a	0.16 ± 0.03a	0.41 ± 0.02a
	FJ-SM TT	3.02 ± 0.04a	0.25 ± 0.02a	0.32 ± 0.01a	0.24 ± 0.01b	0.17 ± 0.04a	0.44 ± 0.01a
49	**FJ-SM HIPEF**	3.13 ± 0.01a	0.30 ± 0.05a	0.34 ± 0.01a	1.59 ± 0.01a	0.18 ± 0.05a	0.42 ± 0.01a
	FJ-SM TT	3.13 ± 0.01a	0.27 ± 0.01a	0.34 ± 0.01a	0.30 ± 0.01b	0.16 ± 0.02a	0.45 ± 0.01a
56	**FJ-SM HIPEF**	3.15 ± 0.12a	0.30 ± 0.04a	0.35 ± 0.01a	1.55 ± 0.01a	0.15 ± 0.02a	0.51 ± 0.01a
	FJ-SM TT	3.17 ± 0.05b	0.25 ± 0.01a	0.34 ± 0.01a	0.26 ± 0.01b	0.13 ± 0.03a	0.46 ± 0.01b

Values in the same column with different letters were significantly different ($p < 0.5$)

Table 3. Saturated fatty acids (SFA) (mg/100 g of fat) profile of the untreated, high intensity pulsed electric field (HIPEF: 35 kV/cm with 4 µs bipolar pulses at 200 Hz for 1800 µs) and thermally (TT: 90 °C, 60 s) treated fruit juice-whole milk (FJ-WM) beverages along refrigerated storage

Storage time (days)	Sample	C10:0	C12:0	C14:0	C16:0	C18:0
0	**FJ-WM untreated**	1.91 ± 0.01a	2.32 ± 0.07a	7.28 ± 0.17a	21.8 ± 0.45a	6.86 ± 0.16a
	FJ-WM HIPEF	2.14 ± 0.24a	2.58 ± 0.34a	8.07 ± 1.12a	24.4 ± 3.38a	7.58 ± 1.07a
	FJ-WM TT	1.87 ± 0.05a	2.22 ± 0.15a	7.29 ± 0.05a	21.6 ± 0.25a	6.59 ± 0.34a
7	**FJ-WM untreated**	2.00 ± 0.14a	2.41 ± 0.18ab	7.57 ± 0.57a	22.8 ± 1.83ab	7.19 ± 0.63ab
	FJ-WM HIPEF	2.12 ± 0.04a	2.55 ± 0.01a	8.06 ± 0.44a	24.2 ± 0.14a	7.72 ± 0.06a
	FJ-WM TT	1.99 ± 0.13a	2.41 ± 0.11b	7.57 ± 0.43a	22.7 ± 1.34b	7.10 ± 0.38b
14	**FJ-WM untreated**	2.15 ± 0.50a	2.57 ± 0.61a	8.07 ± 1.89a	23.9 ± 5.58a	7.46 ± 1.73a
	FJ-WM HIPEF	2.16 ± 0.04a	2.59 ± 0.03a	8.07 ± 0.10a	24.1 ± 0.24a	7.63 ± 0.12a
	FJ-WM TT	2.02 ± 0.25a	2.42 ± 0.28a	7.65 ± 0.84a	23.0 ± 2.49a	7.19 ± 0.87a
21	**FJ-WM HIPEF**	2.10 ± 0.05a	2.52 ± 0.04a	7.89 ± 0.16a	23.5 ± 0.64a	7.29 ± 0.18a
	FJ-WM TT	2.03 ± 0.17a	2.46 ± 0.19a	7.86 ± 0.62a	23.7 ± 2.00a	7.78 ± 0.64a
28	**FJ-WM HIPEF**	1.79 ± 0.04a	2.13 ± 0.04a	6.70 ± 0.12a	20.1 ± 0.40a	6.31 ± 0.13a
	FJ-WM TT	1.85 ± 0.10a	2.21 ± 0.12a	6.95 ± 0.40a	20.6 ± 1.30a	6.51 ± 0.45a
35	**FJ-WM HIPEF**	1.88 ± 0.17a	2.30 ± 0.01a	7.06 ± 0.03a	20.7 ± 0.09a	6.11 ± 0.02a
	FJ-WM TT	1.99 ± 0.02a	2.43 ± 0.02b	7.49 ± 0.02b	22.1± 0.09b	6.65 ± 0.04a
42	**FJ-WM HIPEF**	1.78 ± 0.03a	2.11 ± 0.02a	6.47 ± 0.11a	18.8 ± 0.36a	5.62 ± 0.16a
	FJ-WM TT	1.95 ± 0.01b	2.31 ± 0.01b	7.21 ± 0.06b	20.7 ± 0.12b	6.19 ± 0.11b
49	**FJ-WM HIPEF**	1.99 ± 0.04a	2.39 ± 0.01a	7.42 ± 0.08a	21.6 ± 0.24a	6.68 ± 0.10a
	FJ-WM TT	2.12 ± 0.14a	2.53 ± 0.15a	7.84 ± 0.46a	23.1 ± 1.51a	7.04 ± 0.42a
56	**FJ-WM HIPEF**	1.89 ± 0.02a	2.39 ± 0.02a	7.61 ± 0.03a	22.6 ± 0.12a	7.08 ± 0.02a
	FJ-WM TT	1.68 ± 0.07b	2.13 ± 0.02b	6.78 ± 0.13b	20.0 ± 0.36b	6.29 ± 0.11b

Values in the same column with different letters were significantly different ($p < 0.5$)

Table 4. Monounsaturated and polyunsaturated fatty acids (mg/100 g of fat) profile of the untreated, high intensity pulsed electric field (HIPEF: 35 kV/cm with 4 μs bipolar pulses at 200 Hz for 1800 μs) and thermally (TT: 90 °C, 60 s) treated fruit juice-whole milk (FJ-WM) beverages along refrigerated storage

Storage time (days)	**Sample**	**C14:1**	**C16:1**	**C18:1trans**	**C18:1cis**	**C18:2cis**	**C18:3n3**
0	**FJ-WM untreated**	0.98 ± 0.02a	1.30 ± 0.04a	1.57 ± 0.05a	14.6 ± 0.42ab	2.79 ± 0.06a	1.21 ± 0.03a
	FJ-WM HIPEF	1.09 ± 0.14a	1.53 ± 0.18b	1.72 ± 0.17a	16.9 ± 2.06a	3.35 ± 0.41b	1.41 ± 0.18a
	FJ-WM TT	0.98 ± 0.01a	1.27 ± 0.09ab	1.57 ± 0.02a	14.5 ± 0.12b	2.75 ± 0.14a	1.25 ± 0.04a
7	**FJ-WM untreated**	1.03 ± 0.09ab	1.34 ± 0.10a	1.65 ± 0.17a	15.3 ± 1.36ab	2.77 ± 0.03a	1.13 ± 0.08a
	FJ-WM HIPEF	1.11 ± 0.01a	1.42 ± 0.01a	1.76 ± 0.03a	16.6 ± 0.15a	3.03 ± 0.02b	1.22 ± 0.01a
	FJ-WM TT	1.03 ± 0.06b	1.37 ± 0.05a	1.65 ± 0.09a	15.2 ± 0.87b	2.89 ± 0.07a	1.23 ± 0.08a
14	**FJ-WM untreated**	1.10 ± 0.27a	1.40 ± 0.35a	1.71 ± 0.38a	15.9 ± 3.76a	2.78 ± 0.64a	1.12 ± 0.24a
	FJ-WM HIPEF	1.10 ± 0.01a	1.42 ± 0.01a	1.76 ± 0.01a	16.2 ± 0.18a	2.85 ± 0.03a	1.15 ± 0.01a
	FJ-WM TT	1.06 ± 0.12a	1.37 ± 0.15a	1.64 ± 0.21a	15.2 ± 1.82a	2.85 ± 0.41a	1.23 ± 0.14a
21	**FJ-WM HIPEF**	1.07 ± 0.03a	1.39 ± 0.04a	1.68 ± 0.02a	15.4 ± 0.28a	2.73 ± 0.06a	1.10 ± 0.03a
	FJ-WM TT	1.09 ± 0.10a	1.34 ± 0.12a	1.79 ± 0.13a	16.1 ± 1.20a	2.56 ± 0.21a	0.93 ± 0.08b
28	**FJ-WM HIPEF**	0.90 ± 0.01a	1.22 ± 0.04a	1.45 ± 0.01a	13.6 ± 0.26a	2.66 ± 0.04a	1.14 ± 0.01a
	FJ-WM TT	0.93 ± 0.05a	1.23 ± 0.07a	1.45 ± 0.11a	14.1 ± 0.81a	2.76 ± 0.17a	1.23 ± 0.07b
35	**FJ-WM HIPEF**	0.94 ± 0.01a	1.29 ± 0.01a	1.43 ± 0.01a	13.4 ± 0.10a	2.66 ± 0.01a	1.23 ± 0.01a
	FJ-WM TT	1.00 ± 0.01b	1.37 ± 0.01b	1.54 ± 0.01b	14.6± 0.13a	2.85 ± 0.01b	1.32 ± 0.04b
42	**FJ-WM HIPEF**	0.87 ± 0.02a	1.14 ± 0.02a	1.30 ± 0.05a	12.1 ± 0.36a	2.22 ± 0.05a	0.97 ± 0.02a
	FJ-WM TT	1.02 ± 0.12a	1.24 ± 0.01b	1.48 ± 0.02b	13.5 ± 0.09b	2.50 ± 0.04b	1.11 ± 0.01b
49	**FJ-WM HIPEF**	1.00 ± 0.01a	1.26 ± 0.01a	1.55 ± 0.01a	14.2 ± 0.17a	2.4 ± 0.03a	0.96 ± 0.01a
	FJ-WM TT	1.05 ± 0.07a	1.40 ± 0.10b	1.64 ± 0.07b	15.1 ± 1.12a	2.83 ± 0.13b	1.26 ± 0.05b
56	**FJ-WM HIPEF**	1.02 ± 0.01a	1.35 ± 0.01a	1.65 ± 0.01a	15.1 ± 0.18a	2.69 ± 0.02a	1.13 ± 0.01a
	FJ-WM TT	0.92 ± 0.02b	1.21 ± 0.03b	1.48 ± 0.02b	13.5 ± 0.27b	2.38 ± 0.05b	1.02 ± 0.02b

Values in the same column with different letters were significantly different ($p < 0.5$)