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# CHANGES IN BIOACTIVE COMPOUNDS CONCENTRATION AND PHYSICOCHEMICAL PROPERTIES OF MANGO SMOOTHIES TREATED BY ULTRASOUND

# CAMBIOS EN LA CONCENTRACIÓN DE COMPUESTOS BIOACTIVOS Y PROPIEDADES FÍSICO-QUÍMICAS DE BATIDOS DE MANGO TRATADOS CON ULTRASONIDO

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**Abstract**
Mango smoothies prepared with whole milk (WM-MS) or soymilk (SM-MS) were processed by ultrasound (400 W, 24 kHz, 100 $\mu$m amplitude for 20 min at 35 and 55 °C) in order to evaluate the effects on physicochemical parameters (pH, total soluble solids, and total acidity) and bioactive compounds content (carotenoids and isoflavones). Regardless of the temperature applied and the milk used in the formulation, significant changes in physicochemical parameters were observed in sonicated smoothies. While the pH slightly decreased from 5.58 to 5.45 (WM-MS) and 5.64 to 5.59 (SM-MS), total soluble solids and acidity augmented approximately 5 - 6 % and 3 - 5 %, respectively. Carotenoid concentration significantly diminished in sonicated smoothies, observing higher degradation in the SM-MS (58 %) than in the WM-MS (40 %) when treated at 55 °C. Otherwise, a significant increase in total isoflavone content (6 %) was detected in SM-MS after processing. Additionally, higher aglycone than glucoside concentration was detected in sonicated SM-MS. The application of ultrasound combined with mild heat treatment in mango smoothies induces physical and chemical changes that can modify their bioactive compound profile.
*Keywords*: ultrasound, fruit-based smoothies, carotenoids, isoflavones.

**Resumen**
Se elaboraron dos batidos de mango con leche entera (WM-MS) o leche de soya (SM-MS), los cuales fueron procesados con ultrasonido (400 W, 24 kHz, 100 $\mu$m de amplitud durante 20 min a 35 y 55 °C) para evaluar el efecto sobre sus propiedades fisicoquímicas (pH, contenido de sólidos solubles, acidez total) y concentración de compuestos bioactivos (carotenoides e isoflavonas). Después del tratamiento e independientemente de la leche utilizada en la formulación, se observó que los valores de pH disminuyeron ligeramente de 5.58 a 5.45 (WM-MS) y de 5.64 a 5.59 (SM-MS); mientras que el contenido de solidos solubles y la acidez total incrementaron 5 - 6 % y 3 - 5 %, respectivamente. La concentración de carotenoides se redujo significativamente después del ultrasonido, con una mayor degradación en el SM-MS (58 %) que en el WM-MS (40 %). Por el contrario, el contenido total de isoflavonas aumentó significativamente (6 %) en el SM-MS sonicado. Además, el SM-MS tuvo mayor contenido de formas agliconas que de formas glucosídicas después del tratamiento. La aplicación de ultrasonido combinado con temperaturas medias en smoothies de mango produce cambios físicos y químicos que pueden modificar su perfil de compuestos bioactivos.
*Palabras clave*: ultrasonido, smoothies, carotenoides, isoflavonas.

## 1 Introduction

The term “smoothie” is given to a blended fruit beverage characterized by a pulpy consistency, containing one or more fruits, yogurt, cow’s milk or soymilk. Due to the nature of its composition, this kind of beverages is characterized by attractive flavors and high convenience. Furthermore, by the combination of different ingredients not only a high content of health-related compounds is achieved but also an enhanced mouthfeel of the final product. Therefore, fruit-smoothies, considered as functional beverages, are currently receiving great attention from consumers and their market has rapidly increased (Chatterjee *et*

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*al*., 2015).

Mango (*Mangifera indica*) is a tropical fruit highly appreciated for the elaboration of smoothies due to its excellent organoleptic properties such as exotic taste, attractive fragrance, and color (Siró *et al*., 2008). Also, it contains vitamins, minerals, and other health-related phytochemicals, such as carotenoids (Arauz, 2000). Carotenoid compounds are considered potent antioxidants with beneficial effects on human health (Gallicchio *et al*., 2008; Parisi *et al*., 2008; Perera and Yen, 2007). Likewise, milk and soymilk are the most common ingredients used for smoothies preparation, because they provide the smooth consistency to the final product. Moreover, they are sources of proteins, essential amino acids, and minerals. In addition, soymilk has a significant concentration of isoflavones, which are phytoestrogen substances associated with the low risk of the so-called Western diseases (Aldercreutz, 1990; Anderson *et al*., 1995; Alekel *et al*., 2000; Keinan-Boker *et al*., 2004; Bhathena and Velasquez, 2002). Hence, the development of mango-smoothies with milk or soymilk to obtain the best benefits of each ingredient is relevant. Up to date, different works have been focused on the development of mango-mixed beverages with cows' milk, yogurt, soymilk or whey (Arauz, 2000; Bhathena and Velasquez, 2002; Zafra-Rojas *et al*., 2013; Gogate, 2011; O'Donell *et al*., 2010), indicating that their sensorial and physicochemical attributes are of high acceptance.

Otherwise, preservation of fruit-smoothies requires the smart selection of pasteurization process to obtain safe products with fresh-like characteristics and clean-labels (Castorena-García *et al*., 2013). In this respect, ultrasound (US) technology has received special attention because it can be cheap, simple, environmental-friendly, and effective treatment in extending shelf-life of beverages, particularly when it is combined with mild heat (thermosonication) (Abdullah, 2014). Some authors have demonstrated that US processing caused minimal impact on quality parameters and great retention of health related compounds in orange, guava, and strawberry juices, purple cactus pear, and soymilk (Abdullah, 2014; Cheng *et al*., 2014; Fahmi *et al*., 2012; Sakhale *et al*., 2012; Ribeiro *et al*., 2014; Chauhan and Patil, 2013). However, information about the effects of US treatment together with the application of mild heat on physicochemical properties and health-related compounds of mango smoothies is not available.

The aim of this research was to evaluate the effects of US treatment combined with the application mild temperature on physicochemical parameters and bioactive compound concentration of mango smoothies.

## 2 Materials and methods

### 2.1 Soymilk preparation

Soybeans (*Glycine max*) were purchased in a local market (Monterrey, N.L., Mexico) and used to obtain the soymilk (SM) following the procedure suggested by Yeo and Liong (2013). Briefly, 200 g of soybeans were washed with running water and soaked in 600 mL of distilled water for 16 h at room temperature. Hydrated beans were drained, rinsed, and ground with 800 mL of distilled water using a Vita-mix blender (Vita-mix Corp., OH, USA) for 3 min. The slurry was filtered through cheesecloth with four layers to separate the okara from the SM. The resultant SM was heated at 60 °C for 30 min, to inactivate lipoxygenase enzyme. Finally, it was immediately cooled into an ice bath and chilled stored until smoothies elaboration.

### 2.2 Mango smoothies preparation

Mango (*Mangifera indica* L. cv. Ataulfo) fruits were purchased at edible ripeness state in a local supermarket (Monterrey, N.L. Mexico). They were washed with distilled water and the peel was removed. Immediately, mango pulp was extracted manually and then ground for 1 min in a Vita-mix blender (Vita-mix Corp., OH, USA) to obtain a homogeneous consistency. Mango pulp (400 g) was blended with 600 mL of commercial whole milk (Lala®, Monterrey, N.L., Mexico) or SM, obtaining the whole milk-mango smoothie (WM-MS) and the soymilk-mango smoothie (SM-MS), respectively. Smoothies formulation was selected based on a previous sensory evaluation (data not shown).

### 2.3 US processing of mango smoothies

A portion of 400 mL of WM-MS or SM-MS was placed in a 1000-mL double-walled vessel used as treatment chamber, having untreated smoothies as control samples. US treatment was carried out with a US processor (UP400S, Hielscher Inc., USA, Inc. Ringwood, N.J.) with a 22 mm diameter titanium sonotrode, which was immersed 3 cm into the smoothies. The treatment was performed at 24

kHz with 100 % of amplitude (100 $\mu$m) during 20 min and two temperatures (35 and 55 °C). The temperature in each treatment was controlled with thermostatic bath (Lauda Wobser Gmb & Co., Germany) and monitored through the whole processing with a thermocouple attached to the treatment chamber. Processing conditions were chosen by considering information available in the literature, where successful reduction of bacterial numbers applying US in different food matrices have been achieved (Cheng *et al*., 2007; Yeo and Liong, 2013; Bermudez-Aguirre *et al*., 2011; Bermudez-Aguirre and Barbosa-Cánovas, 2008).

### 2.4 *pH, titratable acidity and soluble solids content*

pH, total acidity (TA) and total soluble solids (TSS) content of the WM-MS and SM-MS were determined according to the AOAC procedures (Vercet *et al*., 2001).

### 2.5 *Carotenoid profile*

Carotenoids were extracted from the untreated and sonicated smoothies (WM-MS and SM-MS) according to the method described by Escobedo-Avellaneda *et al*. (2014). All the extracts were stored at -20 °C until chromatographic analysis. An HPLC system (1200 series, Agilent Technologies, Inc., Santa Clara, CA, USA) operated in reversed phase with a diode array detector, was used to identify the different carotenoids by comparing retention times, wavelength of maximum absorption ($\lambda_{max}$), and UV-vis spectral data of standards ($\beta$-carotene, zeaxanthin, and lutein) previously reported (Escobedo-Avellaneda *et al*., 2014; Giuffrida *et al*., 2010; Meléndez-Martínez *et al*., 2008). Quantification of carotenoids was done by the integration of the peak areas. Data were compared to a calibration curve (10 points) of $\beta$-carotene ($\beta$C) and results were expressed mg $\beta$C/100 mL of WM-MS or SM-MS.

### 2.6 *Isoflavone profile*

Isoflavones were extracted and quantified in the fresh and US-processed SM-MS according to Luthria *et al*. (2006), with some modifications. A portion of 0.5 g of freeze-dried SM-MS (0.024 mm Hg and -52 °C, Virtis FM 25 EL-85, SP Scientific freeze dryer group, Warminster, PA, USA) was mixed with 10 mL of methanol: water solution (80:20 v/v) and vigorously shaken for 1 min (VWR Digital Vortex Mixer, USA). Samples were immediately placed in a US bath (Branson 2510, Branson Ultrasonic Corporation, CT, USA) at room temperature during 15 min and centrifuged for 10 min at 8000 g and 4°C. Supernatant was decanted into a 50 mL flask and the residue was re-extracted with the extraction solution. Supernatants were combined, filtered through Whatman paper (No.1), and pooled in a 20 mL vial. Obtained extracts were concentrated using a Rocket evaporator (Genevac Ltd, Suffolk, UK). The residue was diluted with 1 mL methanol: water solution (80:20 v/v), passed through a 0.20 $\mu$m PVDF filter and placed in glass vials. All the extracts were stored at -20 °C until chromatographic analysis.

An HPLC system (1200 series, Agilent Technologies, Inc., Santa Clara, CA, USA) with a diode array detector was used to identify the isoflavones. Separation of isoflavones was achieved using a reversed phase XDB Eclipse C18 column (Agilent Technologies, CA, USA). Isoflavones were identified by comparison of their UV-vis spectra and retention time with those of the reference standards (Daidzein, Genistein, Daidzin, and Genistin) (Sigma Aldrich, Munich, Germany). Quantification was done by integration of the peak areas. Data were compared to calibration curves of each standard and results were expressed as mg of isoflavone/100 mL of SM-MS.

### 2.7 *Statistical analysis*

The treatments were conducted in duplicate for each condition and two replicate analysis were assayed on each parameter in order to obtain mean values. Analysis of the variance (ANOVA) was performed to compare means of treatments. Least significance difference (LSD) test was used to determine differences between means. The confidence level was set at 0.95 for analysis and procedures. Results were analyzed using a statistical software (Minitab Release 14.1).

## 3 Results and discussion

### 3.1 *Physicochemical analysis*

Physicochemical parameters of the ingredients used to prepare the mango smoothies (Table 1) are within the ranges previously reported in the literature for mango, whole milk, and soymilk (Sakhale *et al*., 2012; Pino, 2012). As expected, pH, TA and TSS values

Table 1. Physicochemical parameters of raw materials, untreated and US-treated mango smoothies: WM-MS (whole milk-mango smoothie) and SM-MS (soymilk-mango smoothie).

| Sample | pH | °Bx | TA (%) |
|---|---|---|---|
| Comercial whole milk | $6.60 \pm 0.01^{a}$ | $9.07 \pm 0.12^{a}$ | $0.150 \pm 0.030^{a}$ |
| Soymilk | $6.36 \pm 0.21^{b}$ | $9.60 \pm 0.17^{b}$ | $0.110 \pm 0.020^{a}$ |
| Mango | $4.01 \pm 0.01^{c}$ | $19.0 \pm 0.11^{c}$ | $0.370 \pm 0.010^{b}$ |
| Untreated WM-MS | $5.58 \pm 0.03^{d}$ | $13.8 \pm 0.3^{d}$ | $0.243 \pm 0.001^{c}$ |
| US-35°C WM-MS | $5.49 \pm 0.05^{e}$ | $14.4 \pm 0.2^{e}$ | $0.250 \pm 0.001^{d}$ |
| US-55°C WM-MS | $5.45 \pm 0.04^{e}$ | $14.5 \pm 0.2^{e}$ | $0.251 \pm 0.001^{d}$ |
| Untreated SM-MS | $5.64 \pm 0.01^{f}$ | $13.5 \pm 0.5^{d}$ | $0.284 \pm 0.001^{c}$ |
| US-35°C SM-MS | $5.60 \pm 0.01^{e}$ | $14.3 \pm 0.2^{e}$ | $0.291 \pm 0.002^{d}$ |
| US-55°C SM-MS | $5.59 \pm 0.02^{e}$ | $14.2 \pm 0.1^{e}$ | $0.299 \pm 0.002^{d}$ |

°Bx: Total soluble solids.

TA: Total acidity expressed in percentage of lactic acid for whole milk and soymilk, and in percentage of citric acid for mango and mango smoothies.

A different letter in the same column correspond to significant differences among the samples and the treatments ($p < 0.05$).

of both mango-smoothies were different from those of the raw materials. Obtained results are similar to the data presented by Sakhale *et al*. (2012) for a soymilk-mango mixed beverage with a pH of 5.22 - 6.05, TSS content around 13.0 - 14.1 °Bx, and total acidity of 0.137 - 0.241 %. In a recent study, Ribeiro *et al*. (2014) also developed a mixed beverage with soymilk and mango pulp and reported lower values of pH (5.18 - 5.48), TSS (9.17 - 10.83 °Bx), and total acidity (0.07 - 0.09 %) than those obtained in this work. These differences may be attributed to the origin and variety of the raw materials, to acid addition as preservation purpose, and to the different proportions of the ingredients used to formulate the smoothies.

Immediately after US treatments, a slight but significant change ($p < 0.05$) in pH, TA and TSS values of both mango-smoothies was produced regardless of the temperature applied and the milk used in the formulation (Table 1). Sonicated smoothies had lower pH and higher TA and TSS content than those untreated. Interestingly, there is controversial information related to the effects of US in physicochemical parameters of fruit-based beverages. On one hand, it has been reported that pH, TA and TSS values of different fruit juices were not affected after sonication process (Tiwari *et al*., 2008; Cheng *et al*., 2014; Abid *et al*., 2013; Adekunte *et al*., 2010). However, Bermudez-Aguirre and Barbosa-Cánovas (2012) observed significant changes in pH and TA after US processing of whole milk and pineapple, grape, and cranberry juices. These authors elucidate that the cavitation phenomenon occurring during US produces chemical reactions that might be related to the decrease of pH and the related change of TA in US-processed products. According to Walstra *et al*. (2006), hydrolysis and lipolysis reactions could take place during sonication due to specific enzyme activity. As a result, some substances such as nitrite, hydrogen peroxide, nitrate, esters, and free fatty acids from triglycerides are released and/or formed within the medium; producing changes in pH and acidity values (Walstra *et al*., 2006; Supeno and Kruus, 2000). On the other hand, the increment of TSS observed in sonicated smoothies could be associated with the physical-mechanical effects of acoustic cavitation, which causes the disruption of large organic particles, in to smaller size particles (Pilli *et al*., 2011), leading to a rise of soluble solid content.

## *3.2 Carotenoid profile*

Fig. 1 shows the carotenoid profile of the mango smoothies elaborated with whole milk (Fig. 1a) and soymilk (Fig. 1b). Regardless of the treatment applied, carotenoid profile of both smoothies was characterized by three epoxycarotenoids: violaxanthin, luteoxanthin, and 9-*cis*-violaxanthin; one dihydroxy-carotenoid (all-*trans*-zeaxanthin); and three carotenes: 13-*cis*-$\beta$-carotene, all-*trans*-$\beta$-carotene, and 9-*cis*-$\beta$-carotene. The most abundant carotenoids in the untreated and US-treated smoothies were all-*trans*-$\beta$-carotene (15.8 - 82.9 $\mu$g/100 mL), violaxanthin (6.34 - 23.5 $\mu$g/100 mL), and 9-*cis*-violaxanthin (4.54 - 10.22 $\mu$g/100 mL).

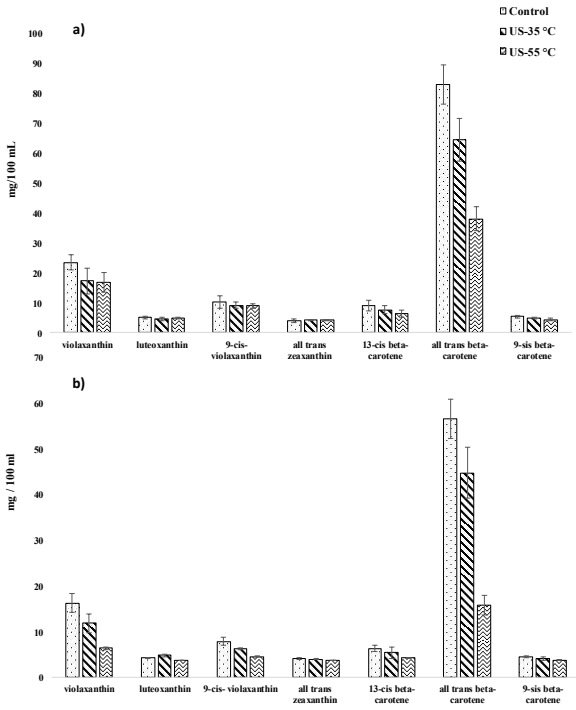


Fig. 1 Changes on the carotenoid profile of whole milk-mango smoothie (a) and soymilk-mango smoothie (b) after US processing (400W, 24 kHz, and 100 $\mu$m amplitude for 20 min) at 35 and 55 °C.

It has been reported that fruit-based beverages have a widely different carotenoid profile, which mainly depends on the ingredients used in the formulation under study (Morales-de la Peña *et al*., 2016; Morales-de la Peña *et al*., 2011). In this regard, Ornelas-Paz *et al*. (2007) indicated that the most abundant carotenoids in mango *Ataulfo* are all-*trans*-$\beta$-carotene, all-*trans*-violaxanthin, and 9-*cis*-violaxanthin, which were the main carotenoids identified in the mango-smoothies. Furthermore, a significant concentration of $\beta$-carotene and lutein has been reported in dairy and soy-based products, respectively (Morales-de la Peña *et al*., 2011; Correa *et al*., 2010; Toda *et al*., 2011). Morales-de la Peña *et al*. (2011) stated that $\beta$-carotene was the predominant carotenoid followed by lutein and zeaxanthin in different fruit juice-milk beverages containing mango, orange, and pineapple juices. The differences in the carotenoid profile of the fruit juice-milk beverages and the mango-smoothies are clearly associated with the diverse ingredients used in their formulations.

Total carotenoid concentration, calculated by the sum of the individual carotenoids identified in the mango smoothies, was significantly ($p < 0.05$) higher in the WM-MS (140.2 ± 2.1 mg/100 mL) than in the SM-MS (99.8 ± 1.20 mg/100 mL). There is no information comparing the carotenoid profile of mango-smoothies prepared with milk or soymilk. Nonetheless, some studies have evaluated the carotenoid profile of different beverages formulated with whole or skimmed milk. Cilla *et al*. (2012) showed that total carotenoid content of a fruit juice-based beverage prepared with orange, kiwi, pineapple, and mango and mixed with whole milk, was 11 % higher than the same beverage blended with skimmed milk. Likewise, Morales-de la Peña *et al*. (2011) observed that the concentration of most individual carotenoids was higher in a fruit juice-whole milk beverage than in that prepared with skimmed milk. Cilla *et al*. (2012) suggested that the higher fat content of the mixed beverage containing whole milk (3 %) compared to the fat content of skimmed milk (0.3 %) might enhance carotenoid extraction due to the lipophilic nature of these compounds. The results of the present study corroborate this statement since the fat content of the SM-MS (0.49 %) was lower than that in the WM-MS (1.1 %), resulting in a smaller carotenoid concentration in the smoothie elaborated with soymilk.

Immediately after US processing at 35 and 55 °C, the concentration of all-*trans*-$\beta$-carotene and violaxanthin of the WM-MS (Fig. 1a) was significantly reduced ($p < 0.05$) in 22.2 - 54.1 % and 26.4 - 28.5 %, respectively. In the same way, the content of these compounds present in the SM-MS (Fig. 1b) was diminished ($p < 0.05$) in 20.9 - 72.1 % (all-*trans*-$\beta$-carotene) and 27.6 - 60.9 % (violaxanthin). In addition, after sonication at 35 and 55 °C the amount of 9-*cis*-violaxanthin and 13-*cis*-$\beta$-carotene reduced ($p < 0.05$) approximately 21 - 42.3 % and 14.2 - 33.1 %, respectively, in the SM-MS. The rest of the identified carotenoids in both smoothies remained with no significant changes. As a result of the changes in the individual carotenoid concentration of US processed mango smoothies, their total carotenoid content also decreased around 19 - 20 % and 40 - 58% at 35 and 55 °C, respectively. As can be noticed, the highest the temperature applied the biggest the carotenoid degradation. Likewise, higher losses of carotenoids were detected in the SM-MS (19 - 58%) than in the WM-MS (20 - 40%).

Despite the effects of US processing on vitamin C, anthocyanins, phenolic acids, and carotenoids of fruit and vegetable juices have been established; no information is currently available on the changes of the carotenoid profile of more complex food

matrixes, such as mango-smoothies. Santhirasegaram *et al*. (2013) and Martínez-Flores *et al*. (2015) reported that the application of US produces a slight but significant increase of 4 - 9 % and 2.71 - 3.44 %, respectively, in carotenoid concentration of mango (40 kHz/130 W/15 - 30 min/25 °C) and carrot (24 kHz/120 $\mu$m amplitude/10 min/50 - 58 °C) juices. However, other studies report a significant degradation of carotenoid content of fruit and vegetable-based products after exposure to conventional thermal treatments or US (Pingret *et al*., 2013). Both processes induce carotenoid degradation; nonetheless, the magnitude of the losses is related to the intensity of the treatments. Morales-de la Peña *et al*. (2011) observed that, after thermal treatment at 90 °C during 60 s, the individual carotenoid concentration of mixed beverages, containing fruit juices and milk or soymilk, significantly diminished around 13 - 40 % compared to fresh beverages. Similarly, the total carotenoid concentration of US treated mango juice (40 kHz frequency, 130 W at 25 °C, 60 min) was significantly diminished in 5 % after processing (Santhirasegaram *et al*., 2013). Likewise, degradation of (*all-E*)-astaxanthin and lycopene from tomato and watermelon juices treated by ultrasound has been reported (Zhao *et al*., 2006; Eh and Teoh, 2012; Rawson *et al*., 2011). It might be possible that the higher carotenoid degradation levels achieved in the sonicated mango smoothies, compared to those reported in the above-mentioned studies are due to the high intensity US treatment conditions combined with mild temperature.

It is well known that cavitation is the main phenomenon occurring during sonication of a liquid medium and involves the formation, growth, and collapse of microbubbles. It leads to various physical, chemical, and biochemical reactions increasing diffusion rates, dispersing aggregates or generating the breakdown of susceptible particles (Keenan *et al*., 2012a). Different authors agree that during cavitation, the formation of highly reactive radicals, such as OH· and H·, occurs, accelerating oxidation pathways (Abdullah, 2014). As a result, some alterations in the sonicated products such as off-flavor production, molecule structure modifications, degradation of bioactive compounds and metallic taste are caused (Keenan *et al*., 2012). Nonetheless, the degree of this phenomenon depends on the frequency, amplitude and treatment time, as well as the temperature and the properties of food matrix under study (Keenan *et al*., 2012; Sivansankar *et al*., 2007). In addition, carotenoids are highly unsaturated compounds with polyisoprenoid structure, consisting of a long conjugated chain of C-C double bonds (Shi and Le Maguer, 2000). Due to their nature, they are very sensitive to degradation and susceptible to geometric isomerization and oxidation reactions during processing (Meléndez-Martínez *et al*., 2007). In this sense, the highly reactive radicals (OH· and H·) generated at the surface of the bubbles during sonication can react with carotenoids producing their oxidation and, therefore, a significant reduction of their concentration. Furthermore, the high shearing effect occurred during sonication might induce carotenoid isomerization (Santhirasegaram, 2013). Other physical phenomena such as propagation, attenuation, and reverberation produced during US treatment could also be related to the carotenoid degradation (Sun *et al*., 2010). Then, it could be stated that the changes in the carotenoid concentration of sonicated products are directly related to chemical and physical effects occurring during the processing. Therefore, more carotenoid content could be retained in sonicated fruit-based products by regulating the US treatment intensity, processing temperature and controlling cavitation phenomenon.

Moreover, the higher fat content of the whole milk in WM-MS might protect the carotenoid compounds from the cavitation phenomenon; fat globules may act as a shield or protective barrier on carotenoids and decrease the penetration and energy distribution during US processing. Hence, the combination of mango pulp with different ingredients such as whole milk could lead to distinct molecule arrangement between carotenoids and macromolecules, improving their retention or their extraction performance. However, further research is required in order to elucidate the possible mechanisms of interaction between bioactive compounds, such as carotenoids, and other macromolecules in complex food matrices treated by US.

### *3.3 Isoflavone profile*

Isoflavone profile of the untreated SM-MS was characterized by two isoflavones in their glucoside form, daidzin (Din) and genistin (Gin), and their corresponding aglycones, daidzein (Da) and genistein (Ge); being Gin (12.53 ± 0.7 mg/100 mL) the most abundant (Fig. 2). Similar to the results obtained in this study, different authors have stated that the main isoflavones present in soymilk are Din, Gin, Da and Ge (Ishihara *et al*., 2007; Xu and Chang, 2009).

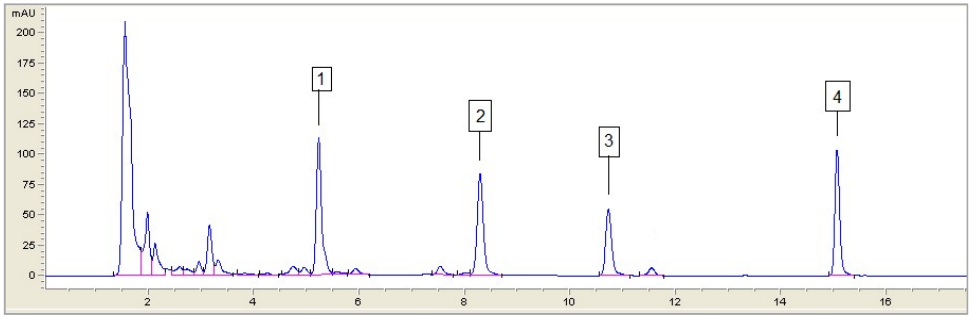


Fig. 2 HPLC chromatogram of isoflavones detected in the untreated soymilk-mango smoothie (SM-MS). *1 Daidzin, 2 Genistin, 3 Daidzein and 4 Genistein.

Consistently, Morales-de la Peña *et al*. (2010) reported that a mixed beverage containing soymilk and orange, pineapple, and kiwi juices had a significant concentration of Din, Gin, Da, and Ge, being Gin the isoflavone present at the highest concentration. Total isoflavone content of the SM-MS, calculated by the sum of the individual isoflavones, was 43.4 mg/100 mL, which is within the range of isoflavone concentration reported for different soy-based products (50 - 20000 $\mu$g/g) (Song *et al*., 1998). However, De Almedia *et al*. (2010), Rostagno *et al*. (2007), and Morales-de la Peña *et al*. (2010) calculated lower levels of total isoflavone concentration in different soy-beverages blended with fruits, varying from 0.5 - 2.5 mg/100 mL, 0.7 - 5.82 mg/100 mL and 16.21 mg/100mL, respectively. It is clearly noticed that isoflavone content of soy-based products, such as soy-beverages, is highly dependent on the soybean cultivar, processing parameters (soaking time, soybean:water ratio, grinding, temperature, and heat treatment time), and storage conditions (De Almedia *et al*., 2010; Rostango *et al*., 2007; Chun *et al*., 2008; Jung *et al*., 2008). In this sense, the differences observed between the total isoflavone content of the SM-MS and the data reported by other authors could be mainly attributed to the variety of the raw materials, proportions, and treatment conditions used for the elaboration of each soy-based beverage.

Immediately after US processing, Din and Gin concentration of SM-MS diminished, while the content of Da and Ge tended to increase, regardless of the temperature applied (35 and 55°C) (Fig. 3). As a result, total glucoside content underwent a significant reduction ($p < 0.05$) around 9 - 18 %, while the aglycone concentration enhanced by 22 - 27 %. Unfortunately, similar studies evaluating the isoflavone profile of fruit-smoothies with soymilk as affected by US processing have not been reported up today. Nonetheless, other works conducted in soymilk and a fruit juice-soymilk beverage treated with thermal pasteurization, high hydrostatic pressures or pulsed electric fields describe interesting information (Fahmi *et al*., 2012; Morales-de la Peña *et al*., 2010; Jung *et al*., 2008; Giri and Mangaraj, 2012; Murphy *et al*. 1999; Yen and Kao 2002). For instance, Yen and Kao (2002) evaluated the changes on the isoflavone profile of soymilk obtained from black soybean soaked at different temperatures (30 and 50 °C) and times (6, 8 and 12 h). Similar to the obtained results, these authors observed that when black soybeans were soaked in water at 30 and 50 °C for various periods of time, the content of Da and Ge increased, while Din and Gin concentration was diminished. Conversely, Morales-de la Peña *et al*. (2010) and Jung *et al*. (2008) did not detect any significant change in the isoflavone profile of a HIPEF-treated fruit juice-soymilk beverage (35 kV/cm, 1400 - 1800 $\mu$s) and HHP-processed soymilk (400 - 750 MPa, 25 and 75°), respectively. Isoflavone content and profile of soy-based products could be affected in different ways by processing conditions, chemical configuration, medium pH, and endogenous enzyme activity (Jung *et al*., 2008; Kao *et al*., 2004; Grün *et al*., 2001; Coward *et al*., 1998). Moreover, according to and Niamnuy *et al*. (2012) isoflavones are generally more susceptible to inter-conversion reactions such as i) decarboxylation of malonate to acetate, ii) de-esterification of malonate to underivatized glycosides, and iii) generation of aglycones through the breakdown of glycoside isoflavones; than to degradation during processing. Hence, it might be possible that US treatment induced some physical or biochemical reactions within the SM-MS, leading to the changes observed in its isoflavone profile.

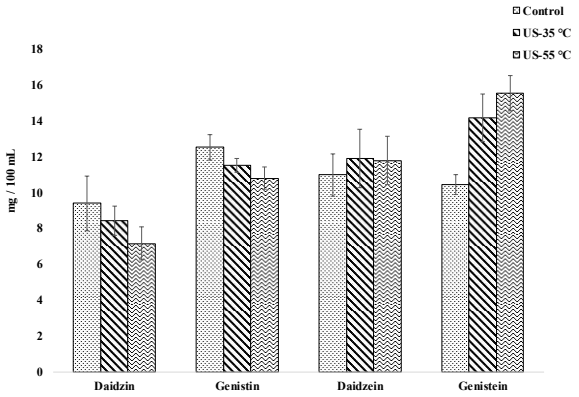


Fig. 3 Changes on the isoflavone profile of soymilk-mango smoothie after US processing (400W, 24 kHz, and 100 $\mu$m amplitude) at 35 and 55 °C.

Just like the achieved results, a significant interconversion of isoflavone glucosides to aglycones during processing was reported by Fahmi *et al*. (2012). These authors elucidated that the ratio of aglycones to glucosides increased by 19 - 23 % in soymilk obtained from a sonicated slurry. Yeo and Liong (2013) established that $\beta$-glucosidase is an important enzyme related to the bioconversion of glucosides to bioactive aglycones in soy-based products. According to them, the application of US increased the extracellular $\beta$-glucosidase activity of L. casei, leading to the bioconversion of isoflavones in mannitol-soymilk. Likewise, Sánchez de Lima and Ida (2014) observed lower glucoside and higher aglycone content in soybeans soaked at 55 °C during 6 h, attributing this effect to the soybean $\beta$-glucosidase activity. Thus, the observed changes in the isoflavone profile of the US-processed SM-MS at 35 and 55 °C could be correlated to the action of $\beta$-glucosidase enzyme, which possesses its maximum activity at 45 - 55 °C (Matsuura *et al*., 1995; Góes-Favoni *et al*., 2010; Wardhani *et al*., 2008), leading to hydrolysis of glucosides and enhancing the aglycone concentration.

As can be observed in Fig. 3, Ge was the isoflavone mostly affected after US processing irrespective of the temperature applied; its content increased around 36 to 49 % compared to the untreated SM-MS. Otieno *et al*. (2006) observed that in some cases, the endogenous $\beta$-glucosidase has shown a preferential hydrolysis for Gin. They assumed that high concentration of Gin in unfermented soymilk led to a high concentration of Ge after fermentation. Similar remarks have been made by Ismail and Hayes (2005) who noticed that $\beta$-glucosidase apparently had a higher affinity for Gin and Din as compared to Glyn, leading to a significant increase in their hydrolysis. In this sense, the high concentration of Ge in the sonicated SM-MS is probably due to the action of the $\beta$-glucosidase over Gin, which was the isoflavone present at highest concentration in the untreated SM-MS.

According to Ismail and Hayes (2005) and Xu *et al*. (2000), aglycones are absorbed faster and in greater amounts than their glucoside forms because their low molecular weight improves diffusion. Consistently, Cederroth and Nef (2009) elucidated that only the aglycone forms are absorbed by the intestinal tract and are, therefore, biological active. Consequently, aglycone rich products may be more effective than glucoside rich products in preventing chronic diseases (Izumi *et al*., 2000; Kano *et al*., 2006). Additionally, among aglycones present in soy-based foods, Ge has been reported as the most biological active (Mazur *et al*., 1998). Thus, it can be established that US processing applied in combination with mild heat is able to improve soy-based smoothies functionality and biological activity due to the higher concentration of aglycones compared to the untreated smoothies.

Finally, as a consequence of the changes in the individual isoflavone concentration caused by US processing, total isoflavones content significantly increased 6 % ($p < 0.05$). This phenomenon was also observed by Fahmi *et al*. (2012) in a US treated-soymilk slurry at different frequencies (35 and 130 kHz), treatment temperatures (20 - 40 °C) and times (20, 40 and 60 min). These authors indicated that, by increasing treatment time (from 20 to 60 min) and temperature (from 20 to 40 °C), isoflavone content of soymilk obtained from the sonicated slurry significantly increased; however, the changes were more noticeable at 35 kHz. Cavitation, specifically the localized high temperatures generated by the collapse bubbles, may increase the cell disruption (Quiroz-Reyes *et al*., 2013), leading to a better mass transfer of intracellular isoflavones into the treated medium. Therefore, total isoflavone concentration enhancement might be attributed to the mechanical and chemical effects of cavitation phenomena produced during sonication. Moreover, since soy isoflavones are associated with globular proteins (Achouri *et al*., 2005) it could be possible that mechanical forces originated during cavitation induce the rupture of the isoflavone-protein interactions, resulting in the release of isoflavones into the SM-MS. A similar effect was reported by Nufer *et al*. (2009), they suggested that at high temperature, the dissociation of the protein-isoflavone interaction occur with the release of isoflavones in the extracting solvent

and, consequently, there was a significant increase of the isoflavone content.

## Conclusions

US processing in combination with mild temperature (35 and 55 °C) caused significant changes in pH, TA and TSS of mango smoothies, irrespectively of the milks used in the formulation. However, the observed changes on physicochemical parameters in sonicated smoothies were not higher than 6 %, retaining their fresh-like characteristics. On the other hand, a significant decrease in carotenoids concentration in the WM-MS and SM-MS was observed just after processing, which was related to cavitation phenomena. Interestingly, US induced the modification of the isoflavone profile of the SM-MS. There was an interconversion between glucoside isoflavones to aglycones. As a result, SM-MS functionality was enhanced, having higher Ge concentration in sonicated smoothie than in that without treatment. According to the results, a US treatment of 24 kHz with 100 % of amplitude during 20 min at 35 °C will be recommended to obtain WM-MS or SM-MS with the optimal retention of carotenoids and isoflavones. However, further research is needed to better elucidate the mechanisms related to the changes of these bioactive compounds observed during sonication processing and to corroborate that the US processing at defined conditions does not affect sensory properties of the mango smoothies.

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## References

Abdullah, N. (2014). Application of thermosonication treatment in processing and production of high quality and safe-to-drink fruit juices. *Agriculture and Agricultural Science Procedia 2*, 320-327.

Abid, M., Jabbar, S., Wu, T., Hashim, M.M., Hu, B., Lei, S., Zhang, X., and Zeng, X. (2013). Effect of ultrasound on different quality parameters of apple juice. *Ultrasonic Sonochemistry 20*, 1182-1187.

Achouri, A., Boye, J.I., and Belanger, D. (2005). Soybean isoflavones: Efficacy of extraction conditions and effect of food type on extractability. *Food Research International 38*, 1199-1204.

Adekunte, A.O., Tiwari, B.K., Cullen, P.J., Scannell, A.G.M., and O'Donnell, C.P. (2010). Effect of sonication on colour, ascorbic acid and yeast inactivation in tomato juice. *Food Chemistry 122*, 500-507.

Aldercreutz, H. (1990). Western diet and western diseases: Some hormonal and biochemical mechanisms and associations. *Scandinavian Journal of Clinial and Laboratory Investgation 50*, 3-23.

Alekel, D.L., Germain, A.S., Peterson, C.T., Hanson, K.B., Stewart, J.W., and Toda, T. (2000). Isoflavone-rich soy protein isolate attenuates bone loss in the lumbar spine of perimenopausal women. *American Journa of Clinical Nutrition 3*, 844-852.

Anderson, J.W., Johnstone, B.M., and Cook-Newel, M.E. (1995). Meta-analysis of the effects of soy protein intake on serum lipids. *The New England Journal of Medicine 5*, 276 - 282.

Arauz, L.F. (2000). Mango Anthracnose: Economic Impact and Current Options for Integrated Management. *Plant Disigne 84*, 600-611.

Bermúdez-Aguirre, D. and Barbosa-Cánovas, G. V. (2008). Study of butter fat content in milk on the inactivation of *Listeria innocua* ATCC 51742 by thermo-sonication. *Innovative Food Science and Emerging Technology 9*, 176 - 185.

Bermúdez-Aguirre, D., Mawson R., and Barbosa-Cánovas, G. V. (2011). Study of possible mechanisms of inactivation of Listeria innocua in thermo-sonicated milk using scanning electron microscopy and transmission electron microscopy. *Journal of Food Processing and Preservation 35*, 767-777.

Bermúdez-Aguirre, D., and Barbosa-Cánovas, G. V. (2012). Inactivation of Saccharomyces cerevisiae in pineapple, grape and cranberry juices under pulsed and continuous thermo-sonication treatments. *Journal Food Engineering 108*, 383-392.

Bhathena, S.J., and Velasquez, M.T. (2002). Beneficial role of dietary phytoestrogens in obesity and diabetes. *American Journa of Clinical Nutrition 76*, 1191-1201.

Castorena-García, J.H., Martínez-Montes, F.J., Robles-López, M.R., Welti-Chanes, J., Hernández-Sánchez, H., and Robles-de la Torre, R.R. (2013). Effect of electric fields on the activity of polyphenol oxidases. *Revista Mexicana de Ingeniería Química 12*, 1665 - 2738

Cederroth, C.R., and Nef, S. (2009). Soy, phytostrogens and metabolism: A review. *Molecular and Cellular Endocrinology 304*, 30 - 42.

Chatterjee, G., De Neve, J., Dutta, A., and Das, S. (2015). Formulation and statistical evaluation of ready-to-drink whey based orange beverage and its storage stability. *Revista Mexicana de Ingeniería Química 14*, 253 - 264.

Chauhan, A.K., and Patil, V. (2013). Effect of packaging material on storage ability of mango milk powder and the quality of reconstituted mango milk drink. *Powder Technology 239*, 86-93.

Cheng, L.H., Soh, C.Y., Liew, S.C., and The, F.F. (2007). Effects of sonication and carbonation on guava juice quality. *Food Chemistry 104*, 1396 - 1401.

Cheng, X.-F., Zhang, M., and Adhikari, B. (2014). Changes in quality attributes of strawberry purees processed by power ultrasound or thermal treatments. *Food Science and Technology Research 20*, 1033-1041.

Chun, J., Kim, J.S., and Kim, J.H. (2008). Enrichment of isoflavone aglycones in soymilk by fermentation with single and mixed cultures of *Streptococcus infantarius* 12 and *Weissella* sp. 4. *Food Chemistry 109*, 278-284.x

Cilla, R.A., Alegría, A., de Ancos, B., Sánchez-Moreno, C., Cano, M.P., Plaza, L., Clemente, G., Lagarda, M.J., Barberá, T. (2012). Bioaccessibility of tocopherols, carotenoids, and ascorbic acid from milk- and soy-based fruit beverages: Influence of food matrix and processing. *Journal of Agriculture and Food Chemistry*, 7282-7290.

Correa, C.R., Li, L., Aldini, G., Carini, M., Oliver Chen, C.Y., Chun, H.K., Cho, S.M., Park, K.M., Russell, R.M., Blumberg, J.B., and Yeum, K.J. (2010). Composition and stability of phytochemicals in five varieties of black soybeans (*Glycine max*). *Food Chemistry 123*, 1176-1184.

Coward, L., Smith, M., Kirk, M., and Barnes, S. (1998). Chemical modification of isoflavones in soyfoods during cooking and processing. *American Journal of Clinical Nutrition 68*, 1486-1491.

De Almeida Callou K. R., Sadigov, S., Lajolo, F.M., and Genovese, M.I. (2010). Isoflavones and antioxidant capacity of commercial soy-based beverages: Effect of storage. *Journal of Agricultural and Food Chemistry 58*, 4284-4291.

Eh, A.L.S., and Teoh, S.G. (2012). Novel modified ultrasonication technique for the extraction of lycopene from tomatoes. *Ultrasonic Sonochemistry 19*, 151-159.

Escobedo-Avellaneda, Z., Gutiérrez-Uribe, J., Valdez-Fragoso, A., Torres, J.A., and Welti-Chanes, J. (2014). Phytochemicals and antioxidant activity of juice, flavedo, albedo and comminuted orange. *Journal of Functional Foods 6*, 470-481.

Fahmi, R., Khodaiyan, F., Pourahmad, R., and Emam-Djomeh, Z. (2012). Effect of ultrasound assisted extraction upon the Genistin and Daidzin contents of resultant soymilk. *Journal of Food Science and Technology 51*, 2857-2861.

Gallicchio, L., Boyd, K., Matanoski, G., Tao, X., Chen, L., Lam, T.K., Shiels, M., Hammond, E., Robinson, K.A., Caulfield, L.E., Herman, J.G., Guallar, E., and Alberg, A.J. (2008). Carotenoids and the risk of developing lung cancer: a systematic review. *American Journal of Clinical Nutrition 88*, 372-383.

Giuffrida, D., Dugo, P., Salvo, A., Saitta, M., and Dugo, G. (2010). Free carotenoid and carotenoid ester composition in native orange juices of different varieties. *Fruits 65*, 277-284.

Giri, S.K., and Mangaraj, S. (2012). Processing influences on composition and quality attributes of soymilk and its powder. *Food Engineering Reviews 4*, 149-164.

Góes-Favoni, S.P., Carrão-Panizzi, M.C., and Beleia, A. (2010). Changes of isoflavone in soybean cotyledons soaked in different volumes of water. *Food Chemistry 119*, 1605-1612.

Gogate, P.R. (2011). Hydrodynamic cavitation for food and water processing. *Food Bioprocess and Technology 4*, 996-1011.

Grün, I.U., Adhikari, K., Li, C., Li, Y., Lin, B., Zhang, J., and Fernando, L.N. (2001). Changes in the profile of genistein, daidzein, and their conjugates during thermal processing of tofu. *Journal of Agricuture and Food Chemistry 49*, 2839-2843.

Ishihara, C., Singh, M., Ching, H., and Tam, G. (2007). Content composition and antioxidant activity of isoflavones in commercial and homemade soymilk and tofu. *Journal of Food Science and Agricutlure 87*, 2844-2852.

Ismail, B., and Hayes, K. (2005). $\beta$-glycosidase activity toward different glycosidic forms of isoflavones. *Journal of Agricultural and Food Chemistry 53*, 4918 - 4924.

Izumi, T., Piskula, M.K., Osawa, S., Obata, A., Tobe, K., Saito, M., Kataoka, S., Kubota, Y., and Kikuchi, M. (2000). Human nutrition and metabolism soy isoflavone aglycones are absorbed faster and in higher amounts than their glucosides in humans. *Journal of Nutrition 130*, 1695-1699.

Jung, S., Murphy, P.A., and Sala, I. (2008). Isoflavone profiles of soymilk as affected by high-pressure treatments of soymilk and soybeans. *Food Chemistry 111*, 592-598.

Kano, M., Takayanagi, T., Harada, K., Sawada, S., and Ishikawa, F. (2006). Bioavailability of isoflavones after ingestion of soy beverages in healthy adults. *Journal of Nutrition 136*, 2291-2296.

Kao, T.H., Lu, Y.F., Hsieh, H.C., and Chen, B.H. (2004). Stability of isoflavone glucosides during processing of soymilk and tofu. *Food Research International 37*, 891 - 900

Keenan, D.F., Tiwari, B.K., Patras, A., Gormley, R., Butler, F., and Brunton, N.P. (2012). Effect of sonication on the bioactive, quality and rheological characteristics of fruit smoothies. *International Journal of Food Science and Technology 47*, 827-836.

Keinan-Boker, L., van Der Schouw, Y.T., Grobbee, D.E., and Peeters, P.H.M. (2004). Dietary phytoestrogens and breast cancer risk. *The American Journal of Clinical Nutrition 79*, 282 - 288.

Luthria, D.L., Biswas, R., and Natarajan, S. (2007). Comparison of extraction solvents and techniques used for the assay of isoflavones from soybean. *Food Chemistry 105*, 325-333.

Matsuura, M., and Sasaki, J. (1995). Studies on $\beta$-glucosidase from soybenas that hydrolyze daidzin and genistin: isolation and characterization of an isozyme. *Bioscience, Biotechnology and Biochemistry 59*, 1623-1627.

Mazur, W.M., Duke, J.A., Wahala, K., Rasku, S., and Adlercreutz, H. (1998). Isoflavonoids and lignans in legumes: Nutritional and health aspects in humans. *Journal of Nutrition and Biochemistry 9*, 193 - 200.

Meléndez-Martínez, A.J., Britton, G., Vicario, I.M., and Heredia, F.J. (2008). The complex carotenoid pattern of orange juices from concentrate. *Food Chemistry 109*, 546-553.

Martínez-Flores, H.E., Garnica-Romo, M.G., Bermúdez-Aguirre, D., Pokhrel, P.R., and Barbosa-Cánovas, G. V. (2015). Physico-chemical parameters, bioactive compounds and microbial quality of thermo-sonicated carrot juice during storage. *Food Chemistry 172*, 650-656.

Meléndez-Martínez, A.J., Vicario, I.M., and Heredia, F.J. (2007). Review: Analysis of carotenoids in orange juice. *Journal of Food Composition and Analysis 20*, 638-649.

Morales-De La Peña, M., Salvia-Trujillo, L., Rojas-Graü, M.A., and Martín-Belloso, O. (2010). Isoflavone profile of a high intensity pulsed electric field or thermally treated fruit juice-soymilk beverage stored under refrigeration. *Innovative Food Science and Emerging Technology 11*, 604-610.

Morales-De La Peña, M., Salvia-Trujillo, L., Rojas-Graü, M.A., and Martín-Belloso, O. (2011). Changes on phenolic and carotenoid composition of high intensity pulsed electric field and thermally treated fruit juice-soymilk beverages during refrigerated storage. *Food Chemistry 129*, 982-990.

Morales-de la Peña, M., Salvia-Trujillo, L., Rojas-Graü, M.A., Martín-Belloso, O. (2016). Effects of high intensity pulsed electric fields or thermal Pasteurization and refrigerated storage on antioxidant compounds of fruit juice-milk beverages. Part II: Carotenoids. *Journal of Food Processecing and Preservation*.

Murphy, P.A., Song, T., Buseman, G., Barua, K., Beecher, G.R., Trainer, D., and Holden, J. (1999). Isoflavones in retail and institutional soy foods. *Journal of Agricultural and Food Chemistry 47*, 2697-2704.

Niamnuy, C., Nachaisin, M., Poomsa-Ad, N., and Devahastin, S. (2012). Kinetic modelling of drying and conversion/degradation of isoflavones during infrared drying of soybean. *Food Chemistry 133*, 946-952.

Nufer, K.R., Ismail, B., and Hayes, K.D. (2009). The effects of processing and extraction conditions on content, profile, and stability of isoflavones in a soymilk system. *Journal of Agricultural and Food Chemistry 57*, 1213-1218.

O'Donnell, C.P., Tiwari, B.K., Bourke, P., and Cullen, P.J. (2010). Effect of ultrasonic processing on food enzymes of industrial importance. *Trends Food Science and Technology 21*, 358-367.

Ornelas-Paz, J.D.J., Yahia, E.M., and Gardea-Bejar, A. (2007). Identification and quantification of xanthophyll esters, carotenes, and tocopherols in the fruit of seven Mexican mango cultivars by liquid chromatography-atmospheric pressure chemical ionization-time-of-flight mass spectrometry [LC-(APcI+)-MS]. *Journal of Agricultural and Food Chemistry 55*, 6628-6635.

Otieno, D.O., Ashton, J.F., and Shah, N.E. (2006). Stability of $\beta$-glucosidase activity produced by *Bifidobacterium* and *Lactobacillus* spp. in fermented soymilk during processing and storage. *Journal of Food Science 70*, M236 - M241.

Parisi, V., Tedeschi, M., Gallinaro, G., Varano, M., Saviano, S. and Piermarochi, S. (2008). Carotenoids and antioxidants in age-related maculopathy italian study: Multifocal electroretinogram modifications after 1 year. *Ophthalmology 115*, 324 - 333.

Perera, C.O. and Yen, G.M. (2007). Functional properties of carotenoids in human health. *International Journal of Food Properties 10*, 201 - 230.

Pilli, S., Bhunia, P., Yan, S., LeBlanc, R.J., Tyagi, R., and Surampalli, R.Y. (2011). Ultrasonic pretreatment of sludge: A review. *Ultrasonic Sonochemistry 18*, 1 - 18.

Pingret, D., Fabiano-Tixier, A.S., and Chemat, F. (2013). Degradation during application of ultrasound in food processing: A review. *Food Control 31*, 593-606.

Pino, J.A. (2012). Odour-active compounds in mango (*Mangifera indica* L. cv. Corazón). *International Journal of Food Science and Technology 47*, 1944-1950.

Quiroz-Reyes, C.N., Aguilar-Méndez, M.A., Rámirez-Ortíz, M.E., and Ronquillo-De Jesus, E. (2013). Comparative study of ultrasound and maceration techniques for the extraction of polyphenols from cocoa beans (*Theobroma cacao* L.). *Revista Mexicana de Ingeniería Química 12*, 11 - 18

Rawson, A., Tiwari, B.K., Patras, A., Brunton, N., Brennan, C., Cullen, P.J. and O'Donnell, C. (2011). Effect of thermosonication on bioactive compounds in watermelon juice. *Food Research International 44*, 1168-1173.

Ribeiro, G.P., de Andrade, A.P.C., Daniels, J., and Seibel, N.F. (2014). Desenvolvimento de bebidas à base de soja com polpas de mamão e

manga. *Acta Scientiarum - Technology 36*, 341-347.

Riesz, T., and Kondo, P. (1992). Free radical formation induced by ultrasound and its biological implications. *Free Radical Biology and Medicine 13*, 247-270.

Rostagno, M.A., Palma, M., and Barroso, C.G. (2007). Ultrasound-assisted extraction of isoflavones from soy beverages blended with fruit juices. *Analitical Chemical Acta 597*, 265-272.

Santhirasegaram, V., Razali, Z., and Somasundram, C. (2013). Effects of thermal treatment and sonication on quality attributes of Chokanan mango (*Mangifera indica* L.) juice. *Ultrasonic Sonochemistry 20*, 1276-1282.

Sakhale, B.K., Pawar, V.N., and Ranveer, R.C. (2012). Studies on development of soymilk based mango RTS beverage. *Electronic Journal of Environmental, Agriculture and Food Chemistry 11*, 523-528.

Sánchez de Lima, F., and Ida, E.I. (2014). Optimisation of soybean hydrothermal treatment for the conversion of $\beta$-glucoside isoflavones to aglycones. *LWT - Food Science and Technology 56*, 232-239.

Shi, J., and Le Maguer, M. (2000). Lycopene in tomatoes: Chemical and physical properties affected by food processing. *Critical Reviews in Food Science and Nutrition 40*, 1 - 42.

Siró, I., Kápolna, E., Kápolna, B., and Lugasi, A. (2008). Functional food. Product development, marketing and consumer acceptance - A review. *Appetite 51*, 456-467.

Sivasankar, T., Paunikar, A.W., Moholkar, V.S. (2007). Mechanistic aproach to enhancement of the yield of a sonochemical reaction. *AIChE Journal 53*, 1132 - 1143.

Song, T., Baura, G., Buseman, G., Murphy, P.A. (1998). Soy isoflavone analysis: Quality control and new internal standard. *American Journal of Clinical Nutrition 68*, 1474 - 1479.

Sun, Y., Ma, G., Ye, X., Kakuda, Y., and Meng, R. (2010). Stability of all-*trans*-$\beta$-carotene under ultrasound treatment in a model system: Effects of different factors, kinetics and newly formed compounds. *Ultrasonic Sonochemistry 17*, 654-661.

Supeno, P. Kruus. (2000). Sonochemical formation of nitrate and nitrite in water. *Ultrasonic Sonochemistry 7*, 109-113.

Tiwari, B.K., O'Donell, C.P., Muthukumarappan, K., and Cullen, P.J. (2008). Effect of ultrasound processing on the quality and nutritional properties of fruit juices. *Steward Postharvest Review 5*, 1 - 6.

Toda, K., Takahashi, R., Iwashina, T., and Hajika, M. (2011). Difference in chilling-induced flavonoid profiles, antioxidant activity and chilling tolerance between soybean near-isogenic lines for the pubescence color gene. *Journal of Plant Research 124*, 173-182.

Vercet, A., Burgos, J., and López-Buesa, P.(2001). Manothermosonication of foods and food-resembling systems: Effect on nutrient content and nonenzymatic browning. *Journal of Agricultural and Food Chemistry 49*, 483-9.

Walstra, P., Wouters, J.T.M., and Geurts, T.J. (2006). *Dayri Science and Technolgy*, 2nd Ed. CRC Taylor and Frances, Boca Ratón, F.L.

Wardhani, D.H., Vázquez, J.A., and Pandiella, S.S. (2008). Kinetics of daidzin and genistin transformations and water absorption during soybean soaking at different temperatures. *Food Chemistry 111*, 13-19.

Xu, X., Wang, H., Murphy, P.A., and Hendrich, S. (2000). Neither background diet nor type of soy food affects short-term isoflavone bioavailability in women. *The Journal of Nutrition 2*, 798-801.

Xu, B., and Chang, S.K.C. (2009). Isoflavones, flavan-3-ols, phenolic acids, total phenolic profiles, and antioxidant capacities of soy milk as affected by ultrahigh-temperature and traditional processing methods. *Journal of Agricultural and Food Chemistry 57*, 4706-4717.

Yen, G., and Kao, C. (2002). Influence of processing conditions on isoflavones content of soybean products. *American Chemical Society*, 73-84.

Yeo, S.K., and Liong, M.T. (2013). Effect of ultrasound on bioconversion of isoflavones and probiotic properties of parent organisms and subsequent passages of Lactobacillus. *LWT - Food Science and Technology 51*, 289-295.

Zafra-Rojas, Q.Y., Cruz-Cansino, N., Ramírez-Moreno, E., Delgado-Olivares L., Villanueva-Sánchez, J., and Alanís-García, E. (2013). Effects of ultrasound treatment in purple cactus pear (*Opuntia ficus-indica*) juice. *Ultrasonic Sonochemistry 20*, 1283-1288.

Zhao, L., Zhao, G., Chen, F., Wang, Z., Wu, J., and Hu, X. (2006). Different effects of microwave and ultrasound on the stability of (all-E)-astaxathin. *Journals of Agriculture and Food Chemistry 54*, 8346 - 8351.