EFFECTS OF HIGH INTENSITY PULSED ELECTRIC FIELDS OR THERMAL PASTEURIZATION AND REFRIGERATED STORAGE ON ANTIOXIDANT COMPOUNDS OF FRUIT JUICE-MILK BEVERAGES. PART I: PHENOLIC ACIDS AND FLAVONOIDS

Authors:

Laura Salvia-Trujillo[1]

Mariana Morales-de la Peña[2]

Alejandra Rojas-Graü[1]

Olga Martín-Belloso[1,2]*

* Author to whom correspondence should be addressed: omartin@tecal.udl.es

1. Department of Food Technology

   University of Lleida – Agrotecnio Center

   Rovira Roure 191, 25198, Lleida, Spain

   Phone: +34 973 702593

   Fax: + 34 973 702596

2. Centro de Biotecnología, FEMSA

   Escuela de Ingeniería y Ciencias

   Tecnológico de Monterrey

   Av. Eugenio Garza Sada 2501, Col. Tecnológico, 64849

   Monterrey, NL, Mexico.

ABSTRACT

The effects of High Intensity Pulsed Electric Fields (HIPEF) or thermal pasteurization (TP) over phenolic compounds in mixed beverages were evaluated after processing and during chilled storage, having untreated beverage as reference. Total phenolic (TPC, 57.0 – 58.8 mg of galic acid/100mL) and flavonoid (TFC, 4.14 – 4.33 mg of quercetin/100mL) contents remained constant in fruit juice-skim milk (FJ-SM) and -whole milk (FJ-WM) beverages just after HIPEF or TP. Nonetheless, concentration of most individual phenolics augmented. TPC in HIPEF treated beverages remained constant through storage, while in thermally pasteurized beverages tended to decrease (5 – 15%). No significant changes were observed in TFC in untreated and treated beverages over time. The concentration of individual phenolics in fresh and treated beverages remained constant or decrease with time, except hesperidin, which significantly increased (19 – 61%) after 56 days. Hence, HIPEF is a feasible technology to obtain mixed beverages with antioxidant properties.

Key words: HIPEF, thermal pasteurization, phenolic acids, flavonoids, mixed beverages

PRACTICAL APPLICATIONS

Fruit juice-milk beverages can be considered as a great source of phenolic compounds with high antioxidant properties. Hence, this kind of beverages are attracting the attention of consumers to introduce healthy products in their diets. Currently, food scientist are focused on developing technologies able to preserve bioactive substances in foods. This study demonstrates that the concentration of phenolic acids and flavonoids in mixed beverages could be well preserved by the application of non-thermal technologies such as HIPEF. Although some fluctuation on phenolic compounds concentration in FJ-WM or FJ-SM beverages occur during the storage; at the end, HIPEF treated beverages had a higher content than those thermally treated. Hence, HIPEF processing might be considered as a potential alternative to thermal pasteurization for the preservation of mixed beverages with high content of antioxidant compounds.

INTRODUCTION

Phenolic compounds represent a group of secondary metabolites, being the most abundant antioxidants in human diets (Erlund, 2004). With over 8,000 structural variants, phenolic acids and flavonoids are the largest subclasses of polyphenols widely distributed in plant kingdom (Han et al., 2007). It is well known that fruits are a good source of these compounds, which possess antimicrobial, antiviral and anti-inflammatory properties (Ignat et al., 2011). Additionally they are considered as multifunctional antioxidants and can act as oxidative enzymes inhibitors, metal chelators or free radical scavengers (Boskou et al., 2006). Furthermore, it has been reported that phenolic acids and flavonoids are able to inhibit lipid peroxidation, protect low-density lipoproteins, reduce platelet aggregation and enhance vasodilatation (Vinson and Dabbagh, 1998; Fuhrman et al., 1995; Renaud and Delorgeril, 1992 and Fitzpatrick et al., 1993). In this way, it is believed that these compounds induce health benefits and have an important role in the protection against a number of pathological disturbances (Gordon, 1996).

Nowadays, dietary habits of modern population are changing and consumers are showing a growing interest on health promoting foods. As a result, the development of new fruit-based products such as functional beverages is increasing worldwide, not only to boost their sensory acceptability but also their nourishing properties through the combination of different ingredients such as fruit juices and milk. Mixed beverages represent a potential way to contribute to consumers health due to their elevate concentration of phytochemicals from fruits and other bioactive compounds of milk. Different studies demonstrate that fruit juice-milk beverages are a good source of vitamins, having at the same time, high antioxidant capacity (Salvia-Trujillo et al., 2011; Zulueta et al., 2010).

Though thermal pasteurization (TP) is the most used method to make this kind of beverages safe and shelf-stable, the high temperatures achieved during processing cause detrimental effects on some desirable food constituents, physicochemical characteristic, flavor attributes and antioxidant properties (Plaza et al., 2006). Thus, overcoming these changes is one of the major challenges in the food industry to fulfil consumers demand. At present, emerging technologies are being explored to process foods at low temperatures avoiding the negative effects induced by heat. Among them, high intensity pulsed electric fields (HIPEF) has demonstrated to be a gentle food preservation technique capable of providing fresh-like characteristics and shelf-stable products with minimum changes on their nutritional, physicochemical, and sensorial properties (Elez-Martinez and Martín-Belloso, 2007; Soliva-Fortuny et al., 2009).

To the best of the authors' knowledge, limited information is available concerning the effects of HIPEF on health-related compounds in mixed beverages containing fruit juices and milk (Zulueta, et al., 2010; Rivas et al., 2007) and data on phenolic acids and flavonoids content is scarce. Hence, the aim of this research was to evaluate the phenolic acid and flavonoid profile of fruit juice-milk beverages prepared with whole or skim milk and compare the effects of HIPEF and TP over these compounds immediately after processing and during the storage (56 days) at 4 °C.

MATERIAL AND METHODS

Beverage preparation

Orange, mango, kiwi and pineapple fruits were purchased in a local supermarket (Lleida, Spain) at commercial maturity stage. Fruit was sanitized in a 200 ppm sodium hypochlorite solution for 2 min and rinsed with a tap water. Then the juice was extracted from each fruit separately and mixed with commercial pasteurized whole (3.5% fat) or skim (0.3% fat) bovine milk with the following proportions: orange

(30%), kiwi (25%), mango (10%), pineapple (10%), milk (17.5%), and sugar (7.5%) These proportions, of each ingredient, 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 (FJ-SM) beverages were filtered through a cheese cloth, and their pH was adjusted to 3.35 with citric acid in order to have an acidified product and avoid microbial growth. Physicochemical characterization of the beverages was previously evaluated in terms of electrical conductivity, pH, total acidity and soluble solids content (Salvia-Trujillo et al., 2011a).

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. According to a previous study, 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 in order to inactivate *Listeria innocua*, guaranteeing product safety (Salvia-Trujillo et al., 2011a). 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 760 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 so that the temperature of the product was always below 40 °C, which was measured with thermocouples at the inlet and outlet of each treatment chamber.

Thermal treatment

Beverages were thermally pasteurized at 90 °C for 1 min to ensure the inactivation of spoilage microorganisms and to simulate a conventional preservation treatment 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 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 water bath with ice passing through a stainless steel coil.

Packaging and storage

HIPEF and TP fluid systems were disinfected first with 4% of NaOH and then with 10% chlorine and 20% ethanol solutions prior to processing. Polypropylene sterile bottles of 100 mL were used to store the FJ-WM and FJ-SM beverages. Once filled, the receptacle was tightly close and stored at 4 ± 1 °C in the absence of light and with minimal headspace volume. According to a previous study (Salvia-Trujillo et al., 2011a). HIPEF and TP treatments are able to inactivate mesophilic, mould and yeast populations, leading to a shelf-life of 56 days. Non-treated beverages were stored for just 14 days owing to the rapid growth of spoilage microorganisms.

Total flavonoid content

Total flavonoids compounds (TFC) were extracted with 5% $NaNO_2$, 10% $AlCl_3$ and NaOH 1M and measured spectrophotometrically at 415 nm using quercetin as standard (Dae-Ok et al., 2003; Meda et al., 2005). The results were expressed as mg of quercetin equivalents (QE) per 100 mL of beverage.

Total phenolic content

Total phenolic compounds (TPC) were determined by the colorimetric method described by Singleton et al. (1999) using the Folin-Ciocalteu reagent. An aliquot of 0.5 mL of the beverage was mixed with 0.5 mL of Folin-Ciocalteu reagent and 10 mL of saturated Na_2CO_3 solution. Samples were kept at room temperature for 1 h. After this time, absorbance at 725 nm was measured using a CECIL 2021 spectrophotometer (Cecil Instruments Ltd., Cambridge, UK). Concentrations were determined by comparing the absorbance of the samples with a calibration curve built with solutions of 0, 100, 250, 500 and 1000 mg galic acid/100 mL (Scharlau Chemie, SA, Barcelona, Spain). Results were expressed as mg of galic acid per 100 mL of beverage.

Individual phenolic compounds

Individual phenolic compounds were extracted and quantified by HPLC, following a procedure validated by Hertog et al. (1992).

Extraction and hydrolysis

To perform the phenolic compounds extraction, untreated and treated beverages were first frozen at -30 °C and then, freeze-dried (Telstar Cryodos-80 Freeze-Dryer) at -44.6 ± 5 °C and vacuum at 0.131 mBar. Lyophilized samples were stored at room temperature until analysis. Twenty millilitres of 62.5% aqueous methanol with 2 g/L of *tert*-butylhydroquinone and 5 mL of hydrochloric acid 6M were carefully mixed with 0.5 g of freeze-dried beverage. After refluxing at 90 °C for 2 h with regular swirling, the extract was cooled and subsequently made up to 50 mL with methanol and sonicated for 5 min. The extract was then passed through a 0.45 μm filter prior to injection.

Chromatography conditions

An aliquot of 20 μL of the extracted samples was injected into the HPLC system, which was equipped with a 600 Controller, a 486 Absorbance Detector programmed to scan from 200 to 350 nm, a thermostatic column compartment, and a 717 Plus Auto Sampler with cooling system (Waters, Milford, MA). Phenolic compounds were separated following the procedure described by Morales-de la Peña et al. (2011) using a reverse-phase C18 Spherisorb ODS2 (5 μm) stainless steel column (4.6 mm x 250 mm) at room temperature with flow rate of 1 mL/min. A gradient elution was employed with a solvent mixture of 2.5% HCOOH in water (solvent A) and 2.5% HCOOH in methanol (solvent B) as follows: linear gradient from 5% to 13% B, 0-15 min; linear gradient from 13% to 15%B, 15-20 min; linear gradient from 15% to 30%, 28-32 min; isocratic elution 45% B, 32-35 min; linear gradient 45% to 90% B, 35-40 min; isocratic elution 90% B, 40-45 min; linear gradient to reach the initial conditions after 5 min; post-time 10 min before the next injection. Individual phenols were identified by comparison of their UV-vis spectral data and retention times with those of the reference standards (chlorogenic, caffeic, p-coumaric, ferulic, and sinapic acids; hesperidin, rutin, narirutin and quercetin). Quantification of phenolic compounds was carried out by integration of the peak areas. Data were compared to calibration curves of each phenolic compound and results were expressed as mg of phenolic compound per 100 mL of beverage.

Statistical analysis

Treatments were conducted in duplicate and two replicate analyses were carried out for each sample. Analysis of the variance (ANOVA) was performed 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 the Statgraphics Plus v.5.1 Windows package (Statistical Graphics Co., Rockville, Md).

RESULTS AND DISCUSSION

Total phenolic compounds

Initial concentration of TPC in FJ-SM and FJ-WM beverages varied from 57.0 to 58.8 mg of galic acid/100 mL. These values were within the range of those reported by Zulueta et al. (2007) in commercial beverages containing a blend of diverse fruit juices and milk (25.5 to 99.8 mg of galic acid/100 mL). Nonetheless, the dissimilarities in total phenolic content between different beverages could be mainly attributed to the ripening degree and environmental growing conditions of the fruits employed for the formulation (Fernández de Simon et al., 1992; Spanos and Wrolstad, 1992). Immediately after processing, no significant changes were observed among untreated FJ-SM (58.3 ± 3.25 mg of galic acid/100 mL) and FJ-WM (55.3 ± 0.96 mg of galic acid/100 mL); HIPEF treated FJ-SM (57.1 ± 2.59 mg of galic acid/100 mL) and FJ-WM (57.0 ± 1.15 mg of galic acid/100 mL); and heat treated FJ-SM (58.8 ± 2.62 mg of galic acid/100 mL) and FJ-WM (57.5 ± 0.58 mg of galic acid/100 mL) beverages. Similar results were reported by Morales-de la Peña et al. (2010), who observed that HIPEF or TP did not affect the concentration of TPC in a mixed beverage containing a blend of fruit juices and soymilk. Consistently, Odriozola-Serrano et al. (2008) and Quitão-Teixeira et al. (2009) did not find significant changes in TPC among fresh, HIPEF-treated and heat-pasteurized tomato and carrot juices, respectively.

As can be seen in Figure 1, HIPEF treated FJ-SM and FJ-WM beverages retained the initial content of TPC for a period of 56 days at 4 °C, although some fluctuations were observed during the storage. Different to our results, Zulueta et al. (2013) reported that storage time was a significant factor for TPC in an orange juice-milk beverage

processed by HIPEF and stored at refrigeration conditions. They found a significant increase on TPC on treated samples at the end of the storage. According to Prior et al. (2005) the Folin-Ciocalteu protocol for total phenols determination may also includes the contribution from ascorbic acid, reducing sugars, soluble proteins and other substances. This fact can explain the fluctuation and increase in TPC of HIPEF treated mixed beverages, mainly due to the formation of other compounds during the storage period that can react with Folin-Ciocalteu phenol reagent. Conversely, a significant decrease of the TPC concentration was observed in thermally treated FJ-SM (15%) and FJ-WM (5%) beverages during the storage period. In agreement to our data, Morales-de la Peña et al. (2010) reported that the concentration of TPC in heat treated fruit juice-soymilk beverages diminished after the third day of storage at 4 °C. Consistent with Kumar-Roy et al. (2007), TP affects in a considerable way, phenolic content in vegetables. Therefore, the elevated temperatures achieved during the conventional pasteurization process (90 °C, 60 s) might have affected some phenolic compounds in the mixed beverages, making them easily degradable over time.

Total flavonoid compounds

Initial content of TFC in untreated FJ-SM and FJ-WM beverages was 4.14 ± 0.18 and 4.33 ± 0.09 mg of quercetin/100 mL, respectively. No significant differences in TFC were observed between HIPEF and TP processed FJ-WM beverages just after processing with respect to the untreated sample (Figure 2, day 0). Nonetheless, HIPEF and thermally treated FJ-SM beverages showed a significant decrease in TFC reducing their concentration up to 3.68 ± 0.26 and 3.00 ± 0.35 mg of quercetin/100 mL, respectively (Figure 3, day 0). To the best of the authors' knowledge, there are no studies reporting the concentration TFC of mixed beverages containing fruit juices and whole or skim milk, neither the effects caused by TP nor HIPEF treatments in these

compounds contained in complex matrix. However, Gil-Izquierdo et al. (2002) reported that mild and standard pasteurization processes did not influence the flavanone content of an orange juice. But, when the juice was subjected to a concentration process the flavanone content slightly decreased with respect to the content before concentration. Otherwise, in a previous study, Gil-Izquierdo et al. (2001), suggested that orange juice flavones decreased as a result of the TP process. As can be seen in Figure 2 and 3, the TFC content of both untreated beverages tended to increase over time; whereas, no significant changes were observed in FJ-WM (Figure 2) and FJ-SM (Figure 3) independently of the treatment applied. The maintenance of TFC concentration throughout the time might be due to the inactivation of oxidative enzymes, such as peroxidase (POD) and polyphenols oxidase (PPO), responsible for flavonoid compounds degradation. Previous research have demonstrated that both TP and HIPEF processing are able inhibit POD and PPO in fruit juices and mixed beverages (Aguiló-Aguayo et al., 2010; Morales-de la Peña et al.; 2010).

Individual phenolic profile

The initial phenolic profile, including phenolic acids and flavonoids, of untreated and treated FJ-SM and FJ-WM beverages is shown in Figure 4. Five phenolic acids including caffeic, chlorogenic, coumaric, ferulic and sinapic; and four flavonoids, hesperidin, rutin, narirutin and quercetin, were identified in both beverages regardless of the treatment applied. As can be seen in Figure 4, chlorogenic was the main hydroxycinnamic acid derivative, obtained in concentrations of 38.34 to 51. 42 mg/100 mL, while hesperidin was the most abundant flavonoid (11.23 – 15.64 mg/100 mL) present in untreated and treated samples. In accordance to our results, Morales-de la Peña et al. (2011) found a similar phenolic profile in a mixed beverage containing fruit juices and soymilk. However, authors reported that coumaric acid and narirutin were the

major phenolic compounds present in the beverages contributing with 32 – 46% and 19.5 – 27.5% of the total content, respectively. Generally, phenolic concentration in fruits and vegetables is highly variable and is strongly influenced by the maturity stage, growing areas, variety and conditions of storage and processing.

Immediately after HIPEF or TP processing, the concentration of most individual phenolic acids and flavonoids identified in both beverages increased or remained with no significant changes (Tables 1 and 2). Interestingly, HIPEF process better maintained the initial content of most phenolic compounds in both beverages than TP. Similarly, Vallverdú-Queralt et al. (2012) reported that HIPEF treated tomato juices retained higher concentration of polyphenols than those thermally treated. The lower processing temperatures (< 40 °C) reached during HIPEF treatment would explain the higher retention of phenolic acids and flavonoids in the HIPEF-treated beverages compared to those processed by heat. Other studies have been focused on the variation in phenolic compounds of an orange juice thermally treated and a HIPEF-processed tomato juice (Sentandreu et al., 2007; Odriozola-Serrano et al., 2009). Results from both studies agreed that neither TP nor HIPEF caused significant effects on the phenolic concentration identified in both juices. On the other hand, Dawes and Kenee (1991) observed that just after processing, an ultra-pasteurized kiwi juice contained higher levels of phenolic acids in comparison to the fresh juice. In the same way, Morales-de la Peña et al. (2011) reported that the concentration of most individual phenolic compounds of a fruit juice-soymilk beverage increased immediately after HIPEF or TP treatments.

According to Kelebek et al. (2009), individual phenolic concentration in processed fruit juices mainly depends on the preservation treatments, storage conditions and food matrix. Different kind of stress such as extreme temperatures may provoke changes and

reactions on the fruit phenolic content (Zobel et al., 1997). It has been reported that during processing, different reactions such as hydroxylation, methylation, isoprenylation, dimerization, and/or glycosylation, which induce modifications between the different phenolic compounds, can occur at various levels (Rice-Evans et al., 1997). Moreover, the presence of some enzymes such as phenylanine ammonialyase (PAL), which is the key enzyme in phenolic biosynthesis, can alter phenolic composition of fruits (Macheix et al., 1990). Hence, the changes observed in the concentration of individual phenolic compounds of the FJ-SM and FJ-WM immediately after HIPEF or TP can be attributed to biochemical reactions between them, such as hydroxylation or methylation, among others, that might occurred during processing. Likewise, it may be possible that HIPEF treatment induced favorable conditions to increase PAL activity, resulting in an enhancement of phenolic concentration in the beverages.

During the storage, the concentration of the individual phenolic acids and flavonoids underwent different effects in the untreated and treated FJ-SM and FJ-WM beverages (Tables 1 and 2). Regarding individual phenolic acids, HIPEF treated beverages had lower concentration of most compounds at the end of the storage (56 days) compared to those untreated and thermally treated. It was observed that the content of caffeic, chlorogenic, coumaric and ferulic acids was reduced in 7.6%, 8.4%, 29% and 17%, respectively, in the FJ-SM beverage; while just 8.3% of caffeic and 15% of ferulic acids concentration was diminished in FJ-WM beverage. Coumaric and ferulic acids content in heat treated beverages was also reduced in 5% and 18%, respectively, as storage time increased. However, the rest of the phenolic acids in both thermally treated beverages remained with no significant changes or increased indeed (chlorogenic – 6% and sinapic 22%) with respect to their initial values. On the other hand, the concentration of most flavonoids identified in the FJ-SM and FJ-WM beverages processed with HIPEF or TP

diminished with time, except hesperidin. Interestingly, the concentration of this flavonoid increased about 19 to 61% in both treated beverages at 56 days.

Considering different results reported in literature, the content of individual phenolic compounds may increase, decrease, or remain stable along time depending on the food matrix under study, treatment and storage conditions. Namely, Odriozola-Serrano et al. (2009) and Valverdú-Queralt et al. (2012) agreed that chlorogenic acid content in tomato juices treated by HIPEF or TP significant decreased with time. On the other hand, Morales-de la Peña et al. (2011) reported that the concentration of chlorogenic and sinapic acids diminished throughout the storage in HIPEF or thermally treated fruit juice-soymilk beverages. Del Caro et al. (2004), reported that minimally processed citrus fruits had a higher concentration of hesperidin at the end of the storage; nonetheless, some juices, such as orange juice, showed a significant diminution on its flavonoid content after a certain period of time. Therefore, it could be said that the variation observed on the content of phenolic acids and flavonoids in FJ-SM and FJ-WM beverages throughout the time are highly influenced by the treatment applied, the type of reactions that take place within the products along the storage and the enzymatic activity which can induce the degradation or synthesis of each compound.

CONCLUSIONS

The concentration of TPC and TFC, regardless of the treatment applied, did not present significant changes in FJ-SM and FJ-WM beverages just after treatments. TPC determined by the Folin-Ciocalteu protocol presented some fluctuation in both treated samples throughout time. HIPEF treated FJ-SM and FJ-WM beverages showed higher retention of TPC at the end of the storage than those thermally pasteurized. No significant changes were observed along the storage period in the concentration of TFC in FJ-WM and FJ-SM, independently of the treatment applied. Five phenolic acids and

four flavonoids were identified in both beverages. Chlorogenic acid was the phenolic compound present at highest concentration followed by hesperidin. HIPEF and thermal treatments, led to mixed beverages with higher amount of individual phenolic acids and flavonoids. However, this increase was better in HIPEF processed mixed beverages than in those heat treated. The concentration of individual phenolic acids and flavonoids in fresh and treated FJ-SM and FJ-WM beverages remained with no significant changes or tended to decrease during storage, with the exception of hesperidin content, which significantly increased after 56 days. The application of HIPEF processed in mixed beverages containing fruit juices and milk, could be a good alternative to thermal pasteurization. Nonetheless, there is a need for focused studies to better understand the different reactions occurred among phenolic compounds in mixed beverages throughout storage period at refrigerated conditions.

ACKNOWLEDGMENTS

This study was supported by the Ministerio de Ciencia e Innovación (Spain) throughout the project AGL2006-12758-C02-02. L. Salvia-Trujillo and M. Morales-de la Peña thanks the Agència de Gestió d'Ajuts Universitaris i de Recerca (AGAUR) (Catalonia, Spain), Consejo Nacional de Ciencia y Tecnología (CONACYT) (México) and Tecnologico de Monterrey (México) for the predoctoral and posdoctoral Research Funds. ICREA Academia Award is also acknowledged by Olga Martín-Belloso.

REFERENCES

BOSKOU, D., GEROTHANASSIS, P.I. AND KEFALAS, P. 2006. *Natural Antioxidant Phenols. Sources, Structure-Activity Relationship. Current Trends in Analysis and Characterization*, pp. 242 Kerala, India.

DAE-OK, K., SEUNG, W.J. AND CHANG, Y.L. 2003. Antioxidant capacity of phenolic phytochemicals from various cultivars of plums. Food Chem. 81, 321 – 326.

ERLUND, I. 2004. Review of the flavonoids quercetin, hesperetin, and naringenin. Dietary sources, bioactivties, bioavailability, and epidemiology. Nutr Res. 24, 851 – 874.

DAWES, H.M. AND KENEE, J.B. 1991. Phenolic composition of kiwifruit juice. J. Agr Food Chem. 47, 2398 – 2403.

DEL CARO, A., PIGA, A., VACCA, V. AND AGABBIO, M. 2004. Changes of flavonoids, vitamin C and antioxidant capacity in minimally processed citrus segments and juices during storage. Food Chem. 84, 99 – 105.

ELEZ-MARTINEZ, P. AND MARTÍN-BELLOSO, O. 2007. Impact of pulsed electric fields on food enzymes and shelf-life. In *Food Preservation by Pulsed Electric Fields*, (H.L.M. Lieved, S. Nontermans and S.W.H. de Haan, eds.). CRC-Press.

FERNÁNDEZ DE SIMÓN, B., HERNÁNDEZ, T., ESTRELLA, I. AND GÓMEZ-CORDOVÉS, C. 1992. Variation in phenol content in grapes during ripening: low-molecular-weight-phenols. Z Lebensm Unters Forsch. 194, 351 – 354.

FITZPATRICK, D.F., HISRSCHFIELD, S.L. AND COFFEY, R.G. 1993. Endothelium dependent vasorelaxing activity of wine and other grape products. Am J Phys. 256, H774 – H778.

FUHRMAN, B., LAVY, A. AND AVIRAM, M. 1995. Consumption of red wine with meals reduces the susceotibility of human plasma and low density-lipoproteins to lipid-peroxidation. Am J Clin Nutr. 61, 549 – 554.

GORDON, M.H. 1996. Dietary antioxidants in disease prevention. Nat Prod Rep. 265 – 273.

HAN, X., SHEN, T. AND LOU, H. 2007. Dietary polyphenols and their biological significance. Int J Mol Sci. 8, 950 – 988.

HERTOG, M., HOLLMAN, P. AND VENEMA, D. 1992. Optimization of a quantitative HPLC determination of potentially anticarcinogenic flavonoids in vegetables and fruits. J Agr Food Chem. 40 (9), 1591 – 1598.

IGNAT, I.; VOLF, I.; AND POPA, V.I. 2011. A critical review methods for characterisation of polyphenolic compounds in fruit and vegetables. Food Chem. 126 (4), 1821 – 1835.

KELEBEK, H., SELLI, S., CANBAS, A. AND CABAROGLU, T. 2009. HPLC determination of organic acids, sugar, phenolic composition and antioxidant capacity of orange juice and orange wine made from a Turkish cv. Kozan. Microchem J. 91, 187 – 192.

KUMAR-ROY, M., TAKENAKA, M., ISOBE. S. AND TSUSHIDA, T. 2007. Antioxidant potential, anti-proliferative activities and phenolic content in water soluble fractions of some commonly consumed vegetables: effects of thermal treatments. Food Chem. 103, 106 – 114.

MACHEIX, J.J., FLEURELIET, A., AND BILLIOT, J. 1990. Fruit phenolics. CRC Press Inc. Boca Raton, FL.

MEDA, A., LAMIEN, C.E., ROMITO, M., MILLOGO, J. AND NACOULMA, O.G. 2005. Determination of the total phenolic, flavonoid and proline contents in Burkina Fasan honey, as well as their radical scavenging activity. Food Chem. 91, 571 – 577.

MORALES-DE LA PEÑA, M., SALVIA-TRUJILLO L., ROJAS-GRAU, M.A. AND MARTÍN-BELLOSO, O. 2010. Impact of high intensity pulsed electric field on antioxidant properties and quality parameters of a fruit juice-soymilk beverage in chilled storage. LWT Food Sci Tech. 43, 872 – 881.

MORALES-DE LA PEÑA, M., SALVIA-TRUJILLO L., ROJAS-GRAU, 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 beverage during refrigerated storage. Food Chem. 129, 982 – 990.

NAGY, S.; CHEN, C.S. AND SHAW, P.E. 1993. In *Fruit Juice Processing Technology* (S. Nagy, C.S. Chen, and P.E. Shaw, eds.). Agscience: Auburndale, FL.

ODRIOZOLA-SERRANO, I., SOLIVA-FORTUNY, R. AND MARTÍN-BELLOSO, O. 2008) Changes of health-related compounds throughout cold storage of tomato juice stabilized by thermal or high intensity pulsed electric field treatments. Innov Food Sci Emer Tech. 9, 272 – 279.

ODRIOZOLA-SERRANO, I., SOLIVA-FORTUNY, R., HERNÁNDEZ-JOVER, T. AND MARTÍN-BELLOSO, O. 2009. Carotenoid and phenolic profile of tomato juices processed by high intensity pulsed electric fields compared with conventional thermal treatments. Food Chem. 112, 258 – 266.

PLAZA, L., SÁNCHEZ-MORENO, C., ELEZ-MARTÍNEZ, P., DE ANCOS, B., MARTÍN-BELLOSO, O. AND CANO, M. P. 2006. Effect of refrigerated storage on vitamin C and antioxidant activity of orange juice processed by high-pressure or pulsed electric fields with regard to low pasteurization. Eur Food Res Technol. 223,487–493.

PRIOR, L., WU, X.L. AND SCHAICH, K. 2005. Standarized methods for the determination of antioxidant capacity and phenolics in foods and dietary supplements. J Agr Food Chem. 53, 4290 – 4302.

QUITÃO-TEIXEIRA, L., ODRIOZOLA-SERRANO, I., SOLIVA-FORTUNY, R., MOTA-RAMOS, A. AND MARTÍN-BELLOSO, O. 2009. Comparative study on antioxidant properties of carrot juice stabilised by high-intensity pulsed electric fields or heat treatments. J Sci Food Agr. 89, 2636 – 2642.

RENAUD, S. AND DELORGERIL, M. 1992. Wine, alcohol, platelets, and the French paradox for coronary heart-disease. Lancet. 339, 1523 – 1526.

RICE-EVANS, C.A., MILLER, N.J. AND PAGANGA, G. 1997. Antioxidant properties of phenolic compounds. Trends Plant Sci. 2 (4), 152 – 159.

RIVAS, A., RODRIGO, D., COMPANY, B., SAMPEDRO, F. AND RODRIGO, M. 2007. Effects of pulsed electric fields on water-soluble vitamins and ACE inhibitory peptides added to a mixed orange juice and milk beverage. Food Chem. 2007, 104, 1550 – 1559.

SALVIA-TRUJILLO L., MORALES-DE LA PEÑA, M., ROJAS-GRAU, M.A. AND MARTÍN-BELLOSO, O. 2011a. Microbial and enzymatic stability of fruit juice-milk beverages treated by high intensity pulsed electric fields or heat during refrigerated storage. Food Control. 22, 1639 – 1646.

SALVIA-TRUJILLO L., MORALES-DE LA PEÑA, M., ROJAS-GRAU, M.A. AND MARTÍN-BELLOSO, O. 2011b. Changes in water soluble-vitamins and antioxidant capacity of fruit juice-milk beverages as affected by High-Intensity Pulsed Electric Fields (HIPEF) or heat during chilled storage. Journal of Agricultural and Food Chem. 59, 10034 – 10043.

SENTANDREU, E., NAVARRO, J.L. AND SENDRA, J.M. 2007. Effect of technological processes and storage on flavonoids content and total, cumulative fast-kinetics and cumulative slow-kinetics antiradical activities of citrus juices. European Food Res Tech. 225, 905 – 912.

SINGLETON, V.L., ORTHOFER, R.M. AND LAMUELA-RAVENTOS, R.M. 1999. Analysis of total phenols and other oxidation substrates and antioxidants by means of Folic-Ciocalteu reagent. Method Enzymol. 299, 152 – 178.

SOLIVA-FORTUNY, R., BALASA, A., KNORR, D. AND MARTÍN-BELLOSO, O. 2009. Effects of pulsed electric fields on bioactive compounds in foods: a review. Trends Food Sci Tech. 20 (11 – 12), 544 – 556.

SPANOS, G.A. AND WROLSTAD, R.E. 1992. Phenolic of apple, pear and white grape juices and their changes with processing and storage – a review. J Agr Food Chem. 40, 1478 – 1487.

VALLVERDÚ-QUERALT, A., ODRIOZOLA-SERRANO, I., OMS-OLIU, G., LAMUELA-RAVENTÓS, R.M., ELEZ-MÁRTINEZ, P. AND MARTÍN-BELLOSO, O. 2012. Changes in the polyphenol profile of tomato juices processed by pulsed electric fields. J Agr Food Chem. 60, 9667 – 9672.

VINSON, J.A. AND DABBAGH, Y.A. 1998. Effect of green and black tea supplementation on lipids, lipid oxidation and fibrinogen in the hamster: mechanisms for the epidemiological benefits of tea drink. FEBS Lett. 433, 44 – 46.

ZOBEL, A.M., LYNCH, J.M. AND JEFFREY, M. 1997. Extrusion of UV absorbing phenolics in *Acer spp*. in response to UV and freezing temperature I. UV-A absorbing compounds on the surface of *Acer saccharum* and *Acer platanoides* autumn leaves. Alleopathy J. 4, 276 – 279.

ZULUETA, A., ESTEVE, M.J., FRASQUET, I. AND FRÍGOLA, A. 2007. Vitamin C, vitamin A, phenolic compounds and total antioxidant capacity of new fruit juice and skim milk mixture beverages marketed in Spain. Food Chem. 103, 1365 – 1374.

ZULUETA, A.; ESTEVE, M.J. AND FRÍGOLA, A. 2010. Ascorbic acid in orange juice-milk beverage treated by high intensity pulsed electric fields and its stability during storage. Innov Food Sci EmerTech. 11, 84 – 90.

ZULUETA, A., BARBA, F.J., ESTEVE, M.J. AND FRÍGOLA, A. 2013. Changes in quality and nutritional parameters during refrigerated storage of an orange juice-milk beverage treated by equivalent thermal and non-thermal process for mild pasteurization. Food Bioprocess Tech. 6, 2018 – 2030.