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The Effects of Non-Thermal Technologies on Phytochemicals

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1. Introduction

Phytochemicals are non-nutritive plant chemicals that possess protective roles in the human body, against disease. These phytochemicals are considered to be biologically active secondary metabolites that also provide color and flavor, and are commonly referred to as nutraceuticals (Kalt, 2001). There are thousands of known phytochemicals, which have been found to be derived mainly from phenylalanine and tyrosine, and which perform a variety of functions such as pigmentation, antioxidation, protection against UV light, etc. (Shahidi & Naczk, 2004). Evidences of the benefits to human-health associated with the consumption of plant-derived phytochemicals have caused an increase in the demand for fresh-like fruits and vegetables., where are present in different forms as alkaloids (eg., caffeine and threbromine), carotenoids (e.g. lycopene), flavonoids (e.g., flavon-3-ols), isoflavones (e.g. genistein), phenolic acids (e.g., capsaicin, gallic acid and tannic acid), etc., depending on plant species.

The health-promoting effects of many phytochemicals are attributed mainly to their antioxidant activity, although there could also be other modes of action. Fruit and vegetables are known to contain significant amounts of phytochemicals with free-radical and nonradical scavenging capacity towards reactive oxygen species. These deleterious substances have been identified as toxic against cell tissues, thus causing oxidative damage to proteins, membrane lipids and DNA, inhibiting enzymatic pathways, and inducing gene mutation. It is believed that these processes are underpinning several chronic human diseases such as diabetes, certain forms of cancer as well as some cardiovascular and degenerative diseases (Eberhardt et al., 2000; Arab & Steck, 2000).

Changes in the concentration of phytochemicals through processing and storage can greatly compromise the quality, and eventually the acceptance of a food. Despite most of these compounds, to some extent, may be affected by abusive temperatures, thermal processing remain the most commonly used technology for inactivating microorganisms and enzymes in processed food. However, during thermal processing, in addition to the inactivation of microorganism, sensory and nutritional compounds of plant-based foods are negative affected. The consumers demand towards products that keep their original nutritious values and, in this context, non-thermal technologies are preservation treatments that are effective at mild temperatures (up to 40 ºC), thereby minimising negative thermal effects on nutritional and quality of products. These non-thermal processes ensure microbial and enzyme inactivation with reduced effects on nutritional and quality parameters. Non-thermal technologies as, pulsed electric fields (PEF), high pressure (HP), irradiation or ultrasounds have been studied and developed during the last decades with the final aim of implementing them at an industrial level. The impact of non thermal technologies on microorganisms, enzymes and quality-related parameters has been extensively reviewed. However, many efforts in the last years have been made to evaluate the impact of PEF, HP, irradiation and ultrasounds on the stability of phytochemicals. The present review aims at reviewing the effects of non thermal technologies on health-related compounds in plant based products.

2. Non-thermal technologies to preserve phytochemicals

2.1 Non-thermal processing basics

Non-thermal technologies may allow obtaining safe and shelf-stable plant-based products with minor changes or increased content in phytochemicals. Most differences between non-thermal and thermal treatments can be explained through the temperatures reached through processing. In general, temperature during processing and storage are important factors affecting the phytochemical content of the processed product. PEF processing involves the application of a high intensity electric field (20–80 kV/cm) in the form of short pulses to a food placed between two electrodes. PEF technology provides fresh-like and safe foods while reducing quality losses that can be triggered after thermal processing (Morris et al., 2007). Application of continuous PEF processing is not suitable for solid food products that do not allow pumping and is restricted to low-conductive food products without air bubbles. Liquid food products are susceptible to be treated by PEF because they are primarily composed by water and nutrients, which are electrical conductors due of the presence of large concentrations of salts and dipolar molecules. Hence, PEF technology has been suggested for the pasteurization of foods such as juices, milk, yogurt, soups, and liquid eggs (Vega-Mercado et al., 1997; Evrendilek et al., 2004; Elez-Martínez et al., 2005; Monfort et al., 2010). In general, a continuous PEF treatment system is composed of treatment chambers, a pulse generator, a fluid-handling system, and monitoring systems (Elez-Martínez et al., 2006). Temperature- and pulse-monitoring systems are used to supervise the process. The effectiveness of PEF technology not only depends on the type of equipment used but also on the treatment parameters and media to be processed (Barbosa-Cánovas et al., 1999). In addition, processing parameters such as electric field strength, total treatment time, pulse shape, pulse frequency, pulse width, polarity and temperature are involved in the degradation or generation of phytochemicals.

HP processing uses water as a medium to transmit pressures from 300 to 700 MPa to foods resulting in a reduction on microbial loads and thus extending shelf life (Patras et al., 2009). Pressure is nearly transmitted uniformly throughout the food, thus leading to very different applications in many different food products. Although pressure results are uniform, the HP technique cannot completely avoid the well-known classical limitation of heat transfer especially during pressure build up and decompression. An increase or a decrease of pressure is associated with a proportional temperature (T) change of the vessel contents, respectively, due to adiabatic heating or cooling temperature gradient. The effectiveness of HP treatment on the overall food quality and safety is not only influenced by extrinsic (process) factors such as treatment time, pressurisation/decompression rate, pressure/ temperature levels and the number of pulses, but also by intrinsic factors of the treated food product such as food composition and the physiological states of microorganisms (Knorr, 2001; Smelt et al., 2002). Effects of pressure and temperature on food constituents are governed by activation volume and activation energy. Differences in sensitivity of reactions towards pressure (activation volume) and temperature (activation energy) lead to the possibility of retaining or even diminishing some desired natural food quality attributes such as vitamins, pigments and flavour or modifying the structure of food system and food functionality, while optimizing the microbial food safety or minimizing the undesired food quality related enzymes (Barbosa-Cánovas et al., 1997; Messens et al., 1997; Hendrickx et al., 1998).

Exposure to radiation, either ionizing or non ionizing, is being regarded as one of the non thermal methods for food preservation with the best potential, especially for the decontamination of raw material for food production. The ionizing radiation source could be high-energy electrons, X-rays (machine generated), or gamma rays (from Cobalt-60 or cesium- 137), while the non-ionizing radiation is electromagnetic radiation that does not carry enough energy/quanta to ionize atoms or molecules, represented mainly by ultraviolet rays (UV-A, UV-B, and UV-C), visible light, microwaves, and infrared. Decontamination though ionizing radiation consists of the application of doses of 2-7 kGy. It has been proven that radiation can safely and effectively eliminate pathogenic nonsporeforming bacteria in foods (Alothman et al., 2009). However, radiation is not being widely used because of some misconceptions by consumers about its role in causing cancer. Radiation can influence the levels of phytochemicals and the capacity of a specific plant to produce them at different levels. Radiation treatments have been shown to either increase or decrease the antioxidant content of fresh plant produce, which is dependent on the dose delivered (usually low and medium doses have insignificant effects on antioxidants), exposure time, and the raw material used. The enhanced antioxidant capacity/activity of a plant after radiation is mainly attributed either to increased enzyme activity (e.g., phenylalanine ammonia-lyase (PAL) and peroxidase activity) or to the increased extractability from the tissues (Alothman et al., 2009).

Although ultrasound is unlike to become a commercial technology on its own, it can be applied in combination with other technologies with preservation purposes. Its lethal microbial effects have been related to cavitation, a phenomenon that generates high temperatures and pressures at a microscopic level that are responsible for the formation of highly reactive free radicals and for the mechanical damage of microorganisms (Raso et al., 1998). Ultrasound processing of juices is reported to have minimal effect on the degradation of key quality parameters such as colour and ascorbic acid in orange juice during storage at 10 ºC (Tiwari et al., 2009a). This positive effect of ultrasound is assumed to be due to the effective removal of occluded oxygen from the juice (Knorr et al., 2004).

2.2 Plant-based foods preserved by non-thermal technologies

2.2.1 Tomato

Vitamin C has received much attention when aiming at evaluating the effect of non thermal processing technologies on the phytochemicals. Vitamin C retention in PEF-treated juices depended on processing factors and thus, the lower the electric field strength, the treatment time, the pulse frequency or the pulse width, the higher the vitamin C retention in tomato juices (Odriozola-Serrano et al., 2007). According to these authors, maximal relative vitamin

C content (90.2%), was attained with PEF treatments of 1 µs pulse duration applied at 250 Hz in bipolar mode at 35 kV/cm for 1000 µs. Regarding the stability of vitamin C through the storage, the concentration of vitamin C decreased over time in both heat (90ºC for 60s) and PEF-treated (35 kV/cm for 1500 µs with 4 µs bipolar pulses at 100 Hz) tomato juices following an exponential trend. In addition, these works demonstrated vitamin C is better retained in PEF treated juices than in those thermally processed after 56 days at 4 ºC. Oxidation of ascorbic acid occurs mainly during the processing of juices and depends upon many factors such as oxygen presence, heat and light. Most differences between PEF and heat treatments can be explained through the temperatures reached through processing. Ascorbic acid is a heat-sensitive bioactive compound in the presence of oxygen. Thus, high temperatures during processing can greatly affect the rates of its degradation through an aerobic pathway (Odriozola-Serrano et al., 2008a). Studies in tomato juice showed that HP processing (300 and 500 MPa) could not preserve vitamin C and the depletion of vitamin C after HP treatment is dependent mainly on temperature intensity and treating time (Hsu et al., 2008). A long exposure (up to 6 h) to extreme pressure/temperature combinations (e.g., 850 MPa combined with temperatures from 65 to 80 ºC) degraded AA to a large extent (Oey et al., 2008). In tomato puree, a 40% and 30% decrease, respectively, in the content of ascorbic acid (AA) and total AA was observed after HP treatment of 400 MPa/25 ºC/15 min (Sánchez-Moreno et al., 2006).

Processing by using non thermal technologies may be advantageous regarding the amount and stability of carotenoids. For instance, lycopene concentrations in PEF-processed tomato juice have been found to be higher than those found in untreated juices (Odriozola-Serrano et al., 2008b). Consistently, Sánchez-Moreno, et al. (2005a) observed that the content of total carotenoids in a tomato-based cold soup 'gazpacho', increased roughly a 62% after applying bipolar 4-µs pulses of 35 kV/cm for 750 µs at 800 Hz. Odriozola-Serrano et al. (2009) have observed that β-carotene in treated tomato juice undergo a significant increase (31%-38%), whereas γ-carotene content is depleted (3%-6%) after a PEF treatment (35 kV/cm for 1500 µs with 4 µs bipolar pulses at 100 Hz). Authors suggested that a plausible explanation for this fact is that γ-carotene may undergo cyclization to form six membered rings at one end of the molecule, giving β-carotene as a product. During storage, PEF-processed tomato juices better maintained the individual carotenoid content (lycopene, neurosporene and γ-carotene) than thermally-treated and untreated juices for 54 days at 4 ºC (Odriozola-Serrano et al., 2009). Individual carotenoids with antioxidant activity (β-carotene, β-cryptoxanthin, zeaxanthin and lutein) appeared to be resistant to a HP treatment of 400 MPa at 40 °C for 1 min, thus resulting into a better preservation of the antioxidant activity of a tomato-based soup with respect to the thermally pasteurized (Sánchez-Moreno et al., 2005b). It seems that high pressure influences the extraction yield of carotenoids. A significant increase in the measured carotenoid content of pressurized (400 MPa/25 ºC/15 min) compared to either thermal treated or untreated tomato purée was observed by Sánchez-Moreno et al. (2006). Due to this potential, HP technology has also been studied to extract lycopene from tomato paste waste (Jun, 2006). In contrast, other studies have not reported major effects of HP treatments on tomato products. García et al. (2001) treated a tomato homogenate for 5 min with 500 and 800 MPa and did not find any influence on the total lycopene and β-carotene concentration. Barba et al. (2010) reported that total carotenoids are particularly affected by HP, having the unprocessed vegetable beverages made with tomato, green pepper, green celery, onion, carrot, lemon and olive oil, a higher total carotenoids content than HP-processed samples. The apparently inconsistent results may be explained through the combined effect of pressure and temperature.

Tomato juices have been found to be a rich source of flavonoids, containing as the main flavonols quercetin and kaempferol, and minor phenolic acids such as ferulic, *p*-coumaric, caffeic acid, etc. PEF processing (35 kV/cm for 1500 µs with 4 µs bipolar pulses at 100 Hz) and thermal treatments (90 ºC 30 s and 90 ºC 60 s) did not affect phenolic content of tomato juices. Both PEF- and heat-treated tomato juices undergo a substantial loss of phenolic acids (chlorogenic and ferulic) and flavonols (quercetin and kaempferol) during 56 days of storage at 4ºC. Caffeic acid content was slightly enhanced over time, regardless the kind of processing, whereas PEF and heat treated tomato juices underwent a substantial depletion of *p*-coumaric acid during storage (Table 1). The increase of caffeic acid in tomato juices after 28 days of storage could be directly associated with residual hydroxylase activities, which convert coumaric acid in caffeic acid. Total phenolics in tomato based beverages and tomato purées appeared to be relatively resistant to the effect of HP (Patras et al., 2009; Barba et al., 2010).The effect of ionizing radiation on the phenolic content of tomatoes has also been studied. The gamma-radiation treatment (2, 4, and 6 kGy) markedly reduced the concentration of the phenolic compounds (*p*-hydroxybenzaldehyde, *p*-coumaric acid, ferulic acid, rutin and naringenin) in tomatoes (Schindler et al., 2005).

	Phenolic compound	Process	Phenolic retention (%) after 56 days of storage at 4 ºC
Phenolic acids	Chlorogenic	HIPEF	86
		TT	79
	Ferulic	HIPEF	67
		TT	69
	p-coumaric	HIPEF	53
		TT	53
	Caffeic	HIPEF	132
		TT	118
Flavonols	Quercetin	HIPEF	80
		TT	64
	Kaempferol	HIPEF	82
		TT	75

HIPEF: High intensity pulsed electric fields treatment at 35 kV/cm for 1000 µs; bipolar 4-µs pulses at 100 Hz; TT: thermal treatment at 90 ºC for 60 s

Table 1. Phenolic acid and flavonols retention during storage for 56 days at 4 ºC of tomato juices stabilized by heat or HIPEF treatments. Adapted from Odriozola-Serrano et al. (2009)

2.2.2 Orange

Ascorbic acid is a heat-sensitive bioactive compound in the presence of oxygen. Thus, high temperatures during processing can greatly affect the rates of its degradation through an aerobic pathway. Storage conditions such as storage temperature or oxygen concentration may have a significant influence on the rates of vitamin C degradation. Vitamin C is usually degraded by oxidative processes which are stimulated in the presence of light, oxygen, heat peroxides and enzymes (especially ascorbate oxidase and peroxidase). Many authors have reported that vitamin C in different fruit and vegetable products is not significantly affected by HP processing (Sánchez-Moreno et al., 2009). Sánchez-Moreno et al. (2006) reported 91% retention of ascorbic acid in orange juice after HP processing at 400 MPa/40 ºC/1min. In addition, HP orange juices (400 MPa/ 40 ºC/ 1 min) maintained better the vitamin C during more days of refrigerated storage than low pasteurized treated juice (70 ºC/30 s) (Plaza et al., 2006). However, differences in vitamin C pressure stability during storage could be explained by the initial oxygen content and possible endogenous pro-oxidative enzyme activity. The effects of ultrasonication on the vitamin C content of orange juice have also been studied. Degradation of vitamin C in sonicated orange juices was observed and the degradation level depended on the wave amplitude and treatment time (Tiwari et al., 2009a). Increased shelf life based on ascorbic acid retention was found for sonicated orange juice compared to thermal processed samples at 98 ºC for 21 s due to higher processing temperature (Tiwari et al., 2009a).

Some studies have demonstrated that carotenoid content is increased significantly after PEF processing compared to the untreated orange juices. Cortés et al. (2006) observed that the carotenoid concentration in orange juice rose slightly after applying intense PEF treatments of 35 and 40 kV/cm for 30-240 μs. Carotenoid concentration rose as treatment time increased when HIPEF treatments of 25 or 30 kV/cm were applied to orange-carrot juice (Torregrosa et al., 2005). It has been reported that thermal treatment may imply an increase in some individual carotenoids owing to greater stability, enzymatic degradation, and unaccounted losses of moisture, which concentrate the sample (Rodriguez-Amaya, 1997). However, carotenoids are highly unsaturated compounds with an extensive conjugated double-bonds system and they are susceptible to oxidation, isomerisation and other chemical changes during processing and storage. Cortés et al. (2006) reported a significant decrease in total carotenoids of orange juice when applying bipolar treatments of 30 kV/cm for 100 μs. This decrease in provitamin A carotenoids could be correlated with a significant decrease in vitamin A by 7.52% in high-intensity PEF-treated orange juice (30 kV/cm, 100 μs) and by 15.62% in a pasteurized orange juice (90ºC, 20 s). Moreover, PEF processing (35 kV/cm, 750 μs) or thermal treatments (70 ºC, 30 s and 90 ºC, 30 s) did not exert any effect on vitamin A content of an orange juice (Sánchez-Moreno et al., 2005a). Research efforts have been made to obtain fruit and vegetable juices by HP processing without the quality and nutritional damage caused by heat treatments (Sánchez-Moreno et al., 2009). Individual carotenoids with antioxidant activity (β-carotene, β-cryptoxanthin, zeaxanthin and lutein) appeared to be resistant to a HP treatment of 400 MPa at 40 °C for 1 min, thus resulting into a better preservation of the antioxidant activity of orange juices with respect to those thermally pasteurized (Sánchez-Moreno et al. 2005a). Interestingly, an orange juice treated at 350 MPa/30ºC/5 min exhibited a higher carotenoid content (α-carotene, 60%; β-carotene, 50%; α-cryptoxanthin, 63%; β-cryptoxanthin, 42%) than a freshly squeezed juice (De Ancos et al., 2002), which was attributed to the desnaturation of the carotenoid-binding protein induced by pressure. Regarding the stability of carotenoids through storage of HP-pasteurized juices, non significant changes have been reported for at least 10 days of refrigerated storage in an orange juice treated at 100MPa/60ºC/5 min, whereas substantial losses were found at the end of the storage period of samples processed at 350MPa/30ºC/2.5 min or 400MPa/40ºC/1 min (20.56% and 9.16%, respectively). Plaza et al. (2010) reported that HP-treated orange juice showed a higher content in carotenoids than heat pasteurized juice during refrigerated storage at 4 ºC. In consequence, vitamin A values showed an increase above 40% the value of the untreated sample. The inactivation of enzymes that caused losses of carotenoids during storage and the improvement of the extraction caused by HP treatments are the reasons exposed by some authors to explain that results (De Ancos et al., 2002).

Flavonoids are the most common and widely distributed group of plant phenolics. Among them, flavones, flavonols, flavanols, flavanones, anthocyanins, and isoflavones are particularly common in fruits. In this way, Sánchez-Moreno et al. (2005a) evaluated the effect of a PEF treatment at 35 kV/cm for 750 µs with 4-µs bipolar pulses at 800 Hz on the flavanone content of orange juice. No changes in the total flavanones were observed, nor in the individual flavanone glycosides and their aglycons hesperetin and naringenin. Recent results show that HP processed orange juice (400 MPa, 40 ºC, 1 min) presented a significant increased on the extractability of each individual flavanone with regard untreated juice and hence on total flavanone content whereas mild pasteurization (70 ºC, 30 s) treatments retained similar levels to those found in untreated juices (Plaza et al., 2010). Regarding the main flavanones identified in orange juice, HP treatments (400 MPa/40 ºC/1 min) increased the content of naringenin by 20% and by 40% the content of hesperetin in comparison with an untreated orange juice (Sánchez-Moreno et al., 2005a). The increase in the extractability of flavanones by Plaza et al. (2010) in HP orange juice happened at beginning due to treatment. Thus, during refrigerated storage at 4 ºC, flavanone content in HP juice decreased around 50% during the first 20 days of storage at 4 ºC. The degradation of phenolic compounds during storage has been mainly related to the residual activity of polyphenol oxidase (PPO) and peroxidase (POD) (Odriozola-Serrano et al., 2009).

2.2.3 Berries

Different studies have proven the effectiveness of PEF in achieving higher vitamin C content in comparison with heat treatments in berries. Odriozola-Serrano et al. (2008a) reported that vitamin C retention just after treatment in heat-processed (90ºC, 60s) strawberry juice was significantly lower (94%) than that found in a juice treated at 35 kV/cm for 1700 µs in bipolar 4 µs pulses at 100 Hz (98%). Low processing temperatures reached through PEF-processing (T<40 ºC) would explain the higher retention of vitamin C in HIPEF-treated strawberry juice compared to the thermally processed samples. The concentration of vitamin C in thermally and PEF-processed juices decreased gradually with storage time. Although during 21 days of storage the concentration of vitamin C was similar among processed strawberry juices, beyond this day, juices subjected to thermal treatment at 90 ºC for 60 s exhibited lower vitamin C content compared to PEF-treated juices (Odriozola et al., 2008c). Recommended daily intake (RDI) of vitamin C is currently revised but should be never below 60 mg, as established by the U.S. Food and Drug Administration (FDA, 1999). According to this recommendation, a strawberry juice 250 mL serving size should contain 24 mg/100 mL in order to contribute to the 100% of the RDI. Vitamin C content of juice processed with either PEF or heat at 90 ºC for 30 s felt below the RDI at 35 days of storage. The concentration of vitamin C in juices treated at 90 ºC for 60 s was reduced below 24 mg/mL after 28 days of storage at 4 ºC. As compared to fruit based products, a high residual ascorbic acid concentration after HP treatment is mostly found. In berries, a high retention of AA in strawberry nectar was observed after HP treatment at 500 MPa/room temperature/3 min (Rovere et al., 1996). Changes of vitamin C content in pressure treated food products during storage have been followed. It is suggested that further vitamin C degradation after HP processing during storage could be reduced by lowering storage temperature, for example, in pressurized (500 MPa/room temperature/3 min) strawberry nectar (Rovere et al., 1996). A kinetic study on degradation of vitamin C in pressure treated strawberry coulis has shown that a pressure treatment neither accelerated nor slowed down the kinetic degradation of ascorbic acid during subsequent storage. Sancho et al. (1999), observed identical kinetics of vitamin C degradation in pressurized (400 MPa/20 ºC/30 min) and untreated coulis during storage at 4 ºC. In general, it can be concluded that ascorbic acid is unstable at high pressure levels combined with high temperatures (above 65 ºC) and the major degradation is caused by oxidation especially during adiabatic heating. Therefore, eliminating the oxygen content in packaging can decrease the ascorbic acid degradation during processing and subsequent storage (Oey et al., 2008). The effects of ultrasonication on the vitamin C content of juices have been also studied. Degradation of vitamin C in sonicated strawberry juices was observed and the degradation level depended on the wave amplitude and treatment time (Tiwari et al, 2009b). Ascorbic acid degradation during sonication may be due to free radical formation and production of oxidative products on the surface of bubbles (Tiwari et al., 2009a). During refrigerated storage, similar vitamin C depletion in strawberry juice was observed in both sonicated and untreated juices (Tiwari et al., 2009b).

Flavonoids are the most common and widely distributed group of plant phenolics. Among them, flavones, flavonols, flavanols, flavanones, anthocyanins, and isoflavones are particularly common in fruits. In strawberry juices, *p*-hydroxybenzoic content was enhanced slightly but significantly after PEF processing (35 kV/cm for 1700 μs in bipolar 4-μs pulses at 100 Hz) compared to the untreated juice, whereas ellagic acid was substantially reduced when the heat treatment was conducted at 90ºC for 60 s. No significant differences in flavonol (kaempferol, quercetin and myricetin) content were obtained between fresh and treated strawberry juices; thus, these phenolic compounds were not affected by processing (Odriozola-Serrano et al., 2008c). It is well known that anthocyanins are unstable pigments and can be decolorized and degraded by many factors such as temperature, pH, oxygen, enzymes, light, the presence of copigments and metallic ions, ascorbic acid, sulphur dioxide and sugars. Numerous studies have evaluated the stability of anthocyanins to different kinds of nonthermal processing. Zhang et al. (2007) observed that after processing a cyanidin-3-glucoside methanolic solution by PEF (300 pulses of 1.2-3 kV/cm, $T^a \leq 47$ ºC), the anthocyanin was degraded and the formation of the colorless anthocyanin species, particularly chalcones took place. In real foods such as strawberry juice, it was suggested that the lower the treatment time and the higher the electric field strength, the greater the anthocyanin retention in strawberry juice (Odriozola-Serrrano et al., 2008b). Contrarily, Zhang et al. (2007) reported that the degradation of cyanidin-3-glucoside in blackberry increased as the electric field strength rose. Jin and Zhang (1999) indicated that the losses of anthocyanins in PEF-treated cranberry juice increased during the storage period at 4 ºC. However, the content over time was higher that of thermally-treated samples, which contained the lowest amount of anthocyanins after two weeks of storage. Pelargonidin-3-glucoside and pelargonidine-3-rutinoside content and, in turn, total anthocyanins of strawberry juices, depleted with storage time after PEF (35 kV/cm for 1,700 μs in bipolar 4-μs pulses at 100 Hz) and thermal treatments (90ºC for 60 or 30 s) (Odriozola-Serrano et al., 2008b) (Figure 1). Changes in anthocyanins content throughout storage of PEF-treated juices

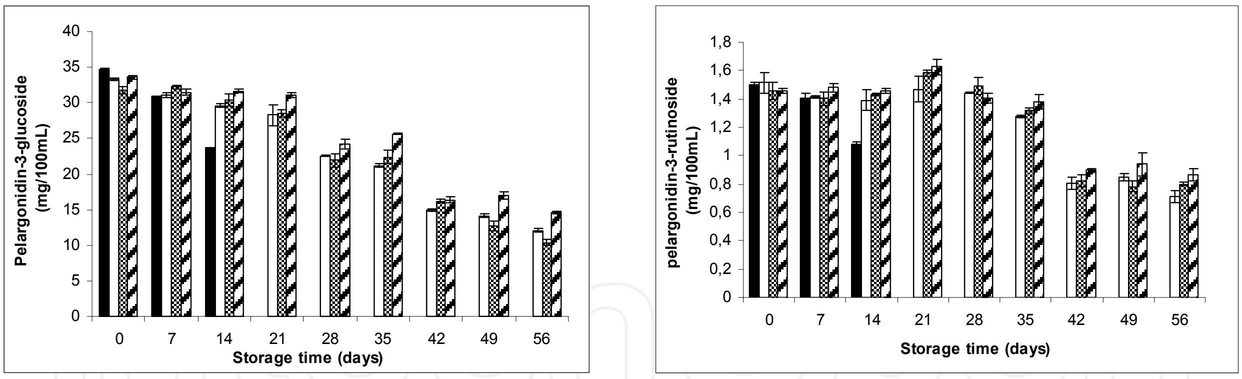

Fig. 1. Effect of HIPEF and heat processing on pelargonidin-3-glucoside (a) and cyaniding-3-glucoside of strawberry juices throughout storage at 4 ºC. Strawberry juices: (■) untreated, (□) heat treated at 90 ºC for 30 s, (▩) heat treated at 90 ºC for 60 s and (◪) HIPEF treated at 35 kV/cm for 1700 µs in bipolar 4-µs pulses and 100 Hz. Data shown are mean ± standard deviation. Adapted from Odriozola-Serrano et al. (2008c)

were associated to the presence of residual enzyme activities such as β-glucosidase (Aguiló-Aguayo et al., 2008). HP treatment at ambient temperature has been reported to have minimal effects on the anthocyanins content of various food products (Oey et al., 2008). Anthocyanins have been reported to be stable to HP treatments in different products such as strawberry juice (Zabetakis et al., 2000), blackcurrant juice (Kouniaki et al., 2004) and raspberry juice (Suthanthangjai et al., 2004). No significant changes in anthocyanin content of strawberry and blackberry purées after 15 min treatments at 500-600 MPa was also reported by Patras et al. (2009). Similarly, cyanidin-3-glucoside, a predominant anthocyanin in berries, was found to be stable in a model solution at 600 MPa for 30 min at 20 ºC. However, 30 min application of 600 MPa at 70 ºC reduced cyanidin-3-glucoside by 25%, whereas only 5% was lost after 30 min heating at the same temperature and ambient pressure (Corrales et al., 2008). Combined pressure and temperature treatment of blueberry pasteurized juice led to a slightly faster degradation of total anthocyanins during storage compared to heat treatments at ambient pressure (Buckow et al., 2010). Thus, pressure seems to accelerate anthocyanin degradation at elevated temperatures. This could be related to condensation reactions involving covalent association of anthocyanins with other flavanols present in fruit juices. During refrigerated storage, the stability of anthocyanins in HP-treated fruit juices at moderate temperatures has been related to the residual PPO and POD activities, a sufficient activity for rapid oxidation of anthocyanins and other polyphenols in the presence of oxygen. Ultrasonication may be also considered a potential technology for processing of red juices because of its minimal effect on anthocyanins. Tiwari et al. (2009b) reported a slight increase (1-2%) in the pelargonidin- 3-glucoside content of the juice at low acoustic energy density (0,33 W/mL) and treatment time (3 min) which may be due to the extraction of bound anthocyanins from the suspended pulp. Some authors have also studied the effects of non ionizing radiation in phenolic content of berries. UV-C doses at 0.25 and 1.0 kJ/m^2 increased anthocyanins concentrations in the fresh strawberries (Baka et al., 1999). Also, UV-C treatment for different durations (1, 5, and 10 min) increased the antioxidant capacity and the concentrations of anthocyanins and phenolic compounds of strawberries (Erkan et al., 2008). Related to ionizing radiation, gamma-radiation (1-10 kGy) led to the degradation of cinnamic, *p*-coumaric, gallic, and hydroxybenzoic acids

(Breitfellner et al., 2002a). The hydroxylation (decomposition) of these phenolic acids has been attributed to the formation of free hydroxyl (OH·) radicals during the treatment. Catechin and kaempferol components also diminished noticeably due to gamma-radiation (1-6 kGy), whereas ellagic acid derivatives and quercetin concentrations were not affected by the treatment in strawberries (Breitfellner et al., 2002b).

2.2.4 Fruit juice-milk beverage

Fruit juice and milk beverage is a product in which the antioxidant capacity of fruit constituents can be delivered in combination with the health benefits of milk. Morales et al. (2010a) did not found significant differences in vitamin C retention (87–90%) between (35 kV/cm, 4 µs bipolar pulses at 200 Hz for 800 or 1400 µs) and thermally treated (90 ºC, 60 s) blend fruit juice-soymilk beverages. In addition, the vitamin C content of the beverages decreased gradually during storage, regardless of the treatment applied. However, throughout the first 31 days, vitamin C was better maintained in the 800 µs-PEF treated fruit juice-soymilk beverages (46.4%) than in those treated for 1400 µs (22.6%); whereas, those that where thermally treated showed the lowest retention of vitamin C (6.7%) (Morales et al., 2011). These results showed that the shorter the PEF treatment time, the higher the vitamin C retention, as previously found in other studies focused on individual fruit juices treated by PEF. The higher retention of vitamin C of PEF treated fruit juice-soymilk beverages compared to those thermally treated might be due to the lower processing temperatures achieved during PEF treatments (<32 ºC). Currently, few studies have been carried out about the effect of HP processing on phytochemicals of fruit juice–milk beverages. High ascorbic acid retention (91%) in the orange juice-milk beverage after HP (100-400 MPa/120-540 s) treatment was reported by Barba et al. (2011).

Initial degradation of ascorbic acid was less in orange juice-milk beverages treated by PEF (25 kV/cm and 280 µs) than in a heat-treated (90 ºC, 20 s) juice (Zulueta et al., 2010a). During storage, total carotenoid content of untreated and treated blend of fruit juice-soymilk beverage tended to decrease as the storage time increased (Morales et al., 2011). Moreover, thermally treated juices showed higher rate of degradation than those PEF-treated. Although the pathways of carotenoids degradation have not been well established, oxidation is the main cause of carotenoid loss, which is a spontaneous free-radical chain reaction in the presence of oxygen (Sánchez-Moreno et al., 2003). During autooxidation of carotenoids, alkylperoxyl radicals are formed and these radicals attack the double bounds resulting in formation of epoxides (Odriozola-Serrano et al., 2009). Total carotenoid content was significantly enhanced in orange juice-milk beverage treated by HP (100-400 MPa) when treatment time was 420 and 540 s in comparison with the unprocessed samples (Barba et al., 2011). According to these authors, this may be because when pressures of 100 MPa are applied, they are sufficient to cause breakage of the intracellular vacuoles and the cell walls of the plant or they also suggested that an increase in free carotenoids in juices after HP might be because there is probably an alteration in the structure of the proteins that are linked to the carotenoids. With regard to individual carotenoid concentrations, there were no significant differences for any of them (Neoxanthin + 9-cis-violaxanthin, Mutatoxanthin, Lutein, Zeaxanthin, β-Cryptoxanthin, α-Carotene, Phytoene+phytofluene, β-Carotene, ζ-Carotene, 15-cis-β-Carotene), and only in the case of the electric field strength of 35 kV/cm there was significant increase in ζ-carotene in comparison with the untreated beverage after

60 µs of treatment. Although the reductions in carotenoids with provitamin A activity are very small after pasteurization, the decreases in the concentrations of lutein (22.8%) and zeaxanthin (22.5%) after pasteurizing are considerable and must be taken into account, because these two xanthophylls play a fundamental part in sight, prevent degenerative eye diseases, and are antioxidant compounds that give quality to food products (Zulueta et al., 2010b).

Coumaric acid, narirutin and hesperidin were the most abundant phenolic compounds in a blend fruit juice-soymilk beverage. Immediately after PEF (35 kV/cm with 4 µs bipolar pulses at 200 Hz for 800 or 1400 µs) or thermal (90 ºC, 60 s) treatments, hesperidin content of the beverage showed a huge rise, resulting in a significant increase on the total phenolic concentration. In addition, total phenolic concentration seemed to be highly stable during refrigerated storage (Morales et al., 2011). According to these authors, changes observed on the phenolic content of the fruit juice-soymilk beverage after PEF or thermal treatments could be due to some of the followed reasons: (i) biochemical reactions could have occurred during the PEF or heat processing, which led to the formation of new phenolic compounds; (ii) PEF or thermal processing might have caused significant effects on cell membranes or in phenolic complexes with other compounds, releasing some free phenolic acids or flavonoids; (iii) PEF and thermal process may inactivate PPO, preventing further loss of phenolic compounds; and (iv) PEF treatment might have induced favorable conditions to increase PAL activity, resulting in an enhancement of phenolic concentration in the beverage. Levels of total phenolic compounds also increased significantly by HP in processed orange juice–milk; reaching a maximum at 100 MPa/420 s, when there was a significant increase of 22% in comparison with unprocessed samples (Barba et al., 2011). As it was mentioned before, the increase in total phenolic content may be related to an increased extractability of some of the antioxidant components following high-pressure processing.

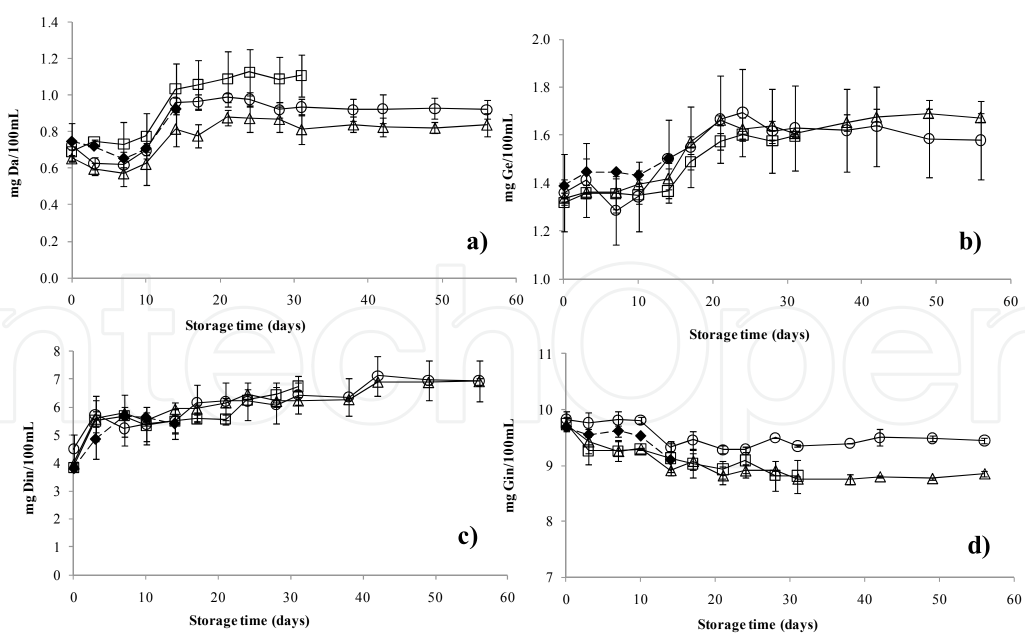

Fig. 2. Individual isoflavone profile: a) daidzein, b) genistein, c) daidzin and d) genisitin of untreated (dotted line, ♦), high intensity pulsed electric field (35 kV/cm with 4 µs bipolar pulses at 200 Hz for 800 (continue line, □) or 1400 µs (continue line, △)) and thermal (continue line, ○) (90 ºC, 60 s) treated fruit juice-soymilk beverages throughout storage at 4ºC (Morales et al., 2010b)

During the last years, soy beverages consumption has gradually increased due to their significant concentration of health-promoting compounds, such as isoflavones. PEF seems to be a good technology in order to obtain fruit juice-soymilk beverage with a high content of isoflavones and fresh-like characteristics. In a blend fruit juice-soymilk beverage, PEF treatment (35 kV/cm with 4 μs bipolar pulses at 200 Hz for 800 or 1400 μs) did not caused significant changes on the total isoflavone content and, during the storage period, total isoflavone content tended to increase throughout the time. Genistein, daidzein and daidzin content increased; while genistin showed a slight decrease, irrespective of the treatment applied (Figure 2) (Morales et al., 2010b). These authors suggested that the concentration of some isoflavones in the fruit juice-soymilk beverage might increase during storage from the flavonoids (mainly naringenin from orange) present in the fruits used for the elaboration of the beverage. Nevertheless, there is a need for more in-depth research to provide biochemical evidence of the observed changes.

2.2.5 Broccoli

Isothiocyanates are organosulfur compounds formed by enzyme-catalysed hydrolysis of glucosinolates, which are largely found in vegetables of the Brassicaceae family. Some isothiocyanates have shown anticarcinogenic potential (Conaway et al., 2002). Knowledge about the impact of non thermal technologies on the stability of these compounds is really scarce. Van Eylen et al. (2007) studied the pressure (600-800 MPa) and temperature (30-60ºC) stabilities of sulforaphane and phenylethyl isothiocyanate in broccoli juice. Authors concluded that isothiocyanates are relatively thermolabile and pressure stable. At the same time, mild pressure treatments where suggested as the most advantageous, because myrosinase activity is stabilized, thus leading to products with increased isothiocyanate content. In a subsequent study, Van Eylen en al. (2008) observed that the composition of glucosinolate hydrolysis products may greatly differ between different HP process conditions. Upon this base, HP treatments can be selected to optimise the health beneficial properties of plant foods.

The non ionizing radiation treatments have been shown to increase the antioxidant capacity of broccoli, which could be useful from the nutritional point of view. UV-C (4-14 kJm^{-2}) treated broccoli florets displayed lower total phenolic and flavonoid content along with higher antioxidant capacity compared to the control samples (Costa et al., 2006). On the other hand, exposure to UV-C (8 kJm^{-2}) increased total phenolic and ascorbic acid contents, as well as the antioxidant capacity of minimally processed broccoli (Lemoine et al., 2007) These authors related, an increment in the activity of PAL after treatment with UV-C to an increase in the content of phenolic compounds in treated samples since PAL is one of the key enzymes in phenolic synthesis.

2.2.6 Others

As it was mentioned before, PEF processing may allow obtaining juices with higher antioxidant potential and extended shelf-life, thus becoming a feasible alternative to heat processing. PEF processing may help to achieve fresh-like carrot juices with increased amounts of health-related phytochemicals. PEF processing (35 kV/cm for 1500 μs with 6-μs bipolar pulses at 200 Hz) resulted into a carrot juices with significantly greater vitamin C retention of 95.1% than thermal processing (90 ºC, 30 s and 90 ºC, 60 s), which exhibited a retention of 86.6–89.0% (Quitao-Teixeira et al., 2009). Watermelon juice was subjected to high-intensity pulsed electric fields (HIPEF). The effects of process parameters including electric field strength (30–35 kV/cm), pulse frequency (50–250 Hz), treatment time (50–2050 μs), pulse width (1–7 μs) and pulse polarity (monopolar/bipolar) on lycopene, vitamin C and antioxidant capacity were studied using a response surface methodology (Oms-Oliu et al., 2009). Watermelon juices treated at 25 kV/cm for 50 μs at 50 Hz using mono- or bipolar 1-μs pulses exhibited the highest vitamin C retention (96.4–99.9%). On the other hand, vitamin C loss was higher than 50% when PEF treatment was set up at 35 kV/cm for 2050 (s at 250 Hz applying mono- or bipolar 7-μs pulses. Such severe conditions seem to greater affect vitamin C retention in watermelon juice than in other juices such as orange, orange–carrot or strawberry juices, which exhibited retention of vitamin C above 80%, because more acidic conditions are known to stabilise vitamin C (Oms-Oliu et al., 2009). During storage, vitamin C was better retained in the PEF-treated carrot juice than in the thermally processed juices for 56 days at 4 ºC. Differences in vitamin C reduction between PEF and heat treated juices throughout the storage might be due to the activity of enzymes such as ascorbate oxidase. A first-order kinetic model adequately fitted vitamin C depletion (R^2 = 0.9680–0.838; Af = 1.039–1.068) as a function of the storage time (Quitao-Teixeira et al., 2009). Storage conditions such as temperature or the oxygen concentration may also have a significant influence on the rates of vitamin C degradation. Sonication also showed to increase vitamin C content in sonicated (ficar les condicions) guava juice compared to untreated sample, the most likely reason being the elimination of dissolved oxygen that is essential for ascorbic acid degradation during cavitation (Cheng et al., 2007). Research reporting on the impact of ionizing radiation on vitamins of plant-derived products is restricted to the effect of gamma-radiation on vitamin C. In general, most fresh-cut vegetables (iceberg, romaine, green and red leaf lettuce, spinach, tomato, cilantro, parsley, green onion, carrot, broccoli, red cabbage, and celery) can tolerate up to 1 kGy radiation without significant losses in vitamin C content (Fan and Sokorai, 2008). In the case of minimally processed irradiated cucumber and carrot, no significant differences between the vitamin C content of control and treated samples were reported through refrigerated storage (Khamat et al., 2005; Hajare et al., 2006). Minor vitamin C losses were reported for minimally processed refrigerated capsicum after gamma-radiation (1-3 kGy) during storage (Ramamurthy et al., 2004). Vitamin C content of irradiated fresh-cut celery (0.5-1.5 kGy) or lettuce (1 kGy) during refrigerated storage was higher than in non-irradiated products (Lu et al., 2005; Zhang et al., 2006). Regarding fresh-cut fruits, Fan et al. (2006), reported that vitamin C of cantaloupe was not substantially affected by treatment with non ionizing radiation. No differences have been reported between treated and untreated products regarding the stability of vitamin C during storage. On the other hand, light treatments applied as short pulses with a total fluence of 4.8 and 12 J cm^{-2} maintained amounts of vitamin C similar to those found in untreated fresh-cut mushrooms during 7 days of refrigerated storage under modified atmosphere packaging (Oms-Oliu et al., 2010).

An increase in lycopene (114%) was observed in watermelon juice treated with 7-μs bipolar pulses for 1050 μs at 35 kV/cm and frequencies ranging from 200 to 250 Hz (Oms-Oliu et al., 2009). The increase in lycopene has been related to the conversion of some carotenoids to lycopene as a result of an intense PEF treatment. β-Carotene concentration substantially increased in processed carrot juices compared to the untreated juice and thermally treated juices. PEF-treated carrot juice maintained β-carotene content better than heat treatments during 56 days of storage at 4 ◦C. The major cause of carotenoid losses in vegetable products is the oxidation of the highly unsaturated carotenoid structure. The severity of oxidation depends on the structure of carotenoids and the environmental conditions, and the compounds being formed may vary upon the oxidation process and the carotenoids structure (Quitao-Teixeira et al., 2009). Therefore, the higher depletion of β-carotene throughout the storage in thermally treated carrot juice compared to those PEF-processed might be due to the greater changes in carotenes structure as a consequence of high temperature. In addition, a better retention of carotenoids in HP treated carrot pureés compared to thermally processed samples was observed (Patras et al., 2009). Such effect has been well documented elsewhere and would appear to be related to an increase in extractability of antioxidant components following high pressure treatment rather than an absolute increase.

No significant differences in the amount of total phenolics were observed between untreated and PEF-treated products such as spinach puree (Yin et al., 2007) and carrot juice (Quitão-Teixeira et al., 2009) just after processing. It has been also reported that radiation treatments can generate free radicals, thus leading to an induction of stress responses in plant foods, which in turn may lead to an increase in the antioxidant synthesis. Results by Song et al. (2006) are consistent with this idea. These authors observed that total phenolic content of carrot and kale juices was substantially increased by applying a radiation treatment. However, reductions in the total phenolic content have been reported for treatments of more than 10 kGy in some irradiated products (Villavicencio et al., 2000; Ahn et al., 2005).

3. Conclusion

Non thermal technologies may allow obtaining safe and shelf-stable plant-based products with minor changes or increased content in health-related phytochemicals. Most differences between non thermal and heat treatments can be explained through the temperatures reached through processing. In general, temperature during processing and storage are important factors affecting the phytochemicals of the processed product. The stability of these compounds through storage is dependent in each case on the residual amounts of enzymes involved in their degradation. In addition, processing parameters are involved in the degradation or generation of bioactive compounds. In-depth research is needed in order to elucidate the mechanisms involved in the destruction or generation of these compounds in a food matrix processed by these novel technologies.

Few studies assessing the impact of non thermal technologies on the bioavailability of bioactive compounds reported an increase of plasma vitamin C and a decrease of the oxidative stress and inflammation biomarkers in healthy humans. Thus, new applications of non thermal processing technologies should be further explored not only to stabilize the content of health-related phytochemicals but also their bioavailability and biological activity in humans.

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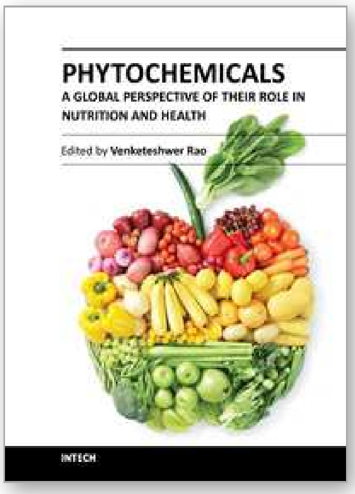

Phytochemicals - A Global Perspective of Their Role in Nutrition and Health

Edited by Dr Venketeshwer Rao

ISBN 978-953-51-0296-0

Hard cover, 538 pages

Publisher InTech

Published online 21, March, 2012

Published in print edition March, 2012

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