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Review

Does high pressure processing influence nutritional aspects of plant based food systems?

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High pressure (HP) technology could maintain food quality attributes such as colour, flavour and nutritional values due to its limited effects on covalent bonds. Under pressure, (bio)chemical reactions can also be induced and it could affect those quality attributes, e.g., nutrition value. In this article, the effects of HP on the stability and bioavailability of vitamins in plant based food systems especially in fruit and vegetables are briefly reviewed. Since HP treatment influences the vitamin stability and the extraction yield of some bioactive compounds, its impacts on antioxidant capacity are also further discussed. In this review, the degradation mechanisms of some vitamins during HP treatment are postulated based on current findings. In addition, possible impacts of conducting HP treatment at elevated temperature (such as HP sterilization) on vitamin stability are discussed.

Introduction

The potentials of High Pressure processing (HP) have been pointed out for industrial food applications, allowing high retention of food quality attributes such as colour, flavour and nutritional values. The limited effect of HP (at moderate temperatures) on covalent bonds represents a unique characteristic of this technology (Balny, Mozhaev, & Lange, 1997). Therefore, in theory, most of the natural food quality aspects, for example nutritional values, can be maintained during HP treatment. Many studies on vitamin stability under HP (at moderate temperatures) have shown that HP does not significantly affect or affects only slightly the vitamin content of fruit and vegetable products (Bignon, 1996; Donsì, Ferrari, & di Matteo, 1996; Sancho *et al*., 1999; Fernández Garcia, Butz, Bognàr & Tauscher, 2001; Fernández Garcia, Butz, & Tauscher, 2001; Butz *et al*., 2004), except at extreme pressure and temperature combinations (Van den Broeck, Ludikhuyze, Weemaes, Van Loey, & Hendrickx, 1998). Recently, HP applications at elevated temperatures (initial temperatures $> 70\,^{\circ}C$) mostly referred to ''High Pressure Sterilization (HPS)'', have been introduced and suggested as a food preservation technology for spore inactivation to reach commercially sterile conditions (Meyer, 2000; Meyer, Cooper, Knorr, & Lelieveld, 2000; Wilson & Baker, 2000, 2001; Van Schepdael, De Heij, & Hoogland, 2002). In the light of these new developments, the effect of HP on food quality particularly on stability of vitamins in fruit and vegetable based food products will be reviewed.

High pressure technology: short description

As compared to thermal treatment at atmospheric pressure, the great advantage of HP treatment is that pressure at a given position and time is the same in all directions, transmitted uniformly, immediately through the pressure transferring medium and independent of product size and geometry. 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

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food composition and the physiological states of microorganisms (Knorr, 2001; Smelt, Hellemons, & Patterson, 2002).

HPS takes the advantage of adiabatic heating during pressure build up either through single or multiple pressure cycles/pulses. For this application, pressurization takes place at high initial temperatures (>70 °C) to achieve final temperatures up to 110–120 °C during the associated adiabatic heating. The achieved final temperature is dependent on the initial temperature, the pressurization rate, the pressure level and the characteristics of both pressure medium and treated food product. In general, the treatment is conducted for a short duration (e.g., max 3 min).

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 destructing some desired natural food quality attributes such as vitamins (Van den Broeck *et al*., 1998), pigments (Van Loey *et al*., 1998) 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, Pothakamury, Palou, & Swanson, 1997; Messens, Van Camp, & Huygebaert, 1997; Hendrickx, Ludikhuyze, Van den Broeck, & Weemaes, 1998).

Effect of high pressure on stability of water soluble vitamins

Ascorbic acid

Numerous researchers have investigated the effect of HP on the stability of ascorbic acid (AA) in controlled model/buffer system and in food products. The stability of AA in pressure treated food product during subsequent storage has also been studied. Oxygen plays an important role in AA degradation both at atmospheric pressure and at elevated pressure. The AA degradation is limited by lowering the initial oxygen concentration. In other words, pressure stability of AA is dependent on the molar ratio of vitamin and oxygen concentrations (Taoukis *et al*., 1998; Oey, Verlinde, Hendrickx, & Van Loey, 2006a). In buffer solution, a tremendous AA degradation through aerobic pathway has been observed during adiabatic heating/pressure build up and already occurred at relatively low pressure level, i.e., approximately 100 MPa. When the major part of soluble oxygen concentration is already eliminated during pressure build up due to the occurrence of aerobic degradation, increasing pressure level and/or prolonging pressure treatment has no/little effect on the ascorbic acid degradation (only valid in moderate pressure and temperature combinations for short time). This can be explained by the fact that the rate of anaerobic degradation is much lower than that of aerobic degradation (Oey *et al*., 2006a). Under isobaric–isothermal conditions for example at 850 MPa and 50 °C for 1 h, ascorbic acid did not show any further decrease after pressure build up (the AA content during isobaric–isothermal condition compared to the AA content after adiabatic heating). However, a long exposure (up to 6 h) to extreme pressure/temperature combinations (e.g., 850 MPa combined with temperatures from 65 to 80 °C) degraded AA to a large extent and the degradation could be described by first order reaction kinetics. Based on the estimated kinetic parameters, it was clear that the degradation rate of AA was enhanced by increasing pressure and temperature combinations. Furthermore, the temperature dependence of ascorbic acid degradation rate constants (i.e., activation energy/Ea value) was independent of pressure level (Van den Broeck *et al*., 1998). Similar observation was also found by Taoukis *et al*. (1998). The latter investigators reported that sugar addition, e.g., 10% sucrose, resulted in a protective effect on ascorbic acid degradation as the concentration of soluble oxygen was decreased by the presence of sugar. Furthermore, they found a higher vitamin C loss in fruit juice compared to in buffer solutions because of the existence of endogenous pro-oxidants in fruit juice such as metal ions and enzymes. In addition, food matrix influences the AA stability. For example, AA in tomato juice was more stable (the degradation rate constants are lower) than in orange juice (Van den Broeck *et al*., 1998).

Among food products, AA in vegetable based food products has been reported to be less stable compared to that in fruit based food products, for example, (i) 77% decrease in the initial content of AA in sprouted alfalfa seed in citric acid pickle after pressure treatment of 500 MPa/room temperature/10 min (Gabrovska *et al*., 2005); (ii) 40% and 30% decrease, respectively, in the content of AA and total AA in tomato puree after HP treatment of 400 MPa/25 °C/15 min (Sánchez-Moreno, Plaza, De Ancos, & Cano, 2006a) or (iii) 10–28% and 9–41% decrease in the total vitamin C content, respectively, of 4 and 6 days germinated cowpeas after HP treatments at 300–500 MPa/room temperature/15 min (Doblado, Frías, & Vidal-Valverde, 2007). As compared to fruit based products, a higher residual AA concentration after HP treatment is mostly found, for example, (i) less than 9% loss of vitamin C in orange juice after HP at 400 MPa/40 °C/1 min (Sánchez-Moreno *et al*., 2005); (ii) 88.68% of the initial content of AA in strawberry coulis and in strawberry nectar retained after HP treatment of 400 MPa/20 °C/30 min. (Yen & Lin, 1996); (iii) a high retention of AA in strawberry nectar after HP treatment at 500 MPa/room temperature/3 min (Rovere, Carpi, Gola, Dall'Aglio, & Maggy, 1996); (iv) no effect on the initial (total and dehydro) AA concentrations in citrus juices after HP treatments of 200–500 MPa/30 °C/1 min (Donsì *et al*., 1996) or in guava puree after HP treatments at 400 and 600 MPa/15 min (Yen & Lin, 1996).

Evolution of vitamin C content in pressure treated food products during storage has been followed. It is suggested

that further vitamin C degradation after HP processing could take place during storage and it could be eliminated by lowering storage temperature, for example, (i) in pressurized (500 MPa/room temperature/3 min) strawberry nectar (Rovere *et al*., 1996); (ii) in pressurized (500 and 800 MPa/25and 50 °C/1 min) orange juice (Nienaber & Shellhammer, 2001); (iii) in pressurized (500 MPa, 35 °C, 5 min) reconstituted orange juice (Polydera, Stoforos, & Taoukis, 2003); (iv) in pressurized (two pulses of 75 °C/1000 MPa/80 s with interval time of 30 sec/1 atm) peas (Krebbers, Matser, Koets, Bartels, & Van den Berg, 2002) or (iv) in pressurized (500 MPa/room temperature/10 min) sprouted alfalfa seed in citric acid pickle (Gabrovska *et al*., 2005). Furthermore, it has been reported that different HP combinations had different influences on the stability of vitamin C in guava puree during storage (Yen & Lin, 1996). The ascorbic acid content of untreated and pressurized (400 MPa/room temperature/15 min) guava puree started to decline after 10 and 20 days, respectively, whereas it remained constant in thermal (88–90 °C/24 s) and in higher pressure (600 MPa/room temperature/15 min) treated guava puree during 30 and 40 days, respectively. The latter could be caused by the inactivation of endogenous pro-oxidative enzyme during HP treatment at high pressure level. A kinetic study on degradation of vitamin C in pressure treated strawberry coulis during storage has shown that a pressure treatment neither accelerated nor slowed down the kinetic degradation of ascorbic acid during subsequent storage, for example, identical kinetics of vitamin C degradation in pressurized (400 MPa/20 °C/30 min) and untreated coulis during storage at 4 °C (Sancho *et al*., 1999).

At elevated temperatures, pressure treatment could degrade vitamin C to a large extent for long treatment time, e.g., pressurization up to 600 MPa at 75 °C for 40 min. resulting in 70% and 50% losses of vitamin C, respectively, in pineapple and grapefruit juice (Taoukis *et al*., 1998). At constant pressure, increasing temperature enhanced the vitamin C degradation, for example loss 20–25% at 40 °C; 45–50% at 60 °C and 60–70% at 75 °C at 600 MPa for 40 min.) in pineapple juice (Taoukis *et al*., 1998). However, at elevated temperatures, pressure treatment for short time could eliminate the AA degradation such as during HPS, e.g., two pulses of 75 °C/1000 MPa/80 s with interval time of 30 s/1 atm retained 76% of AA content in green peas (Krebbers *et al*., 2002).

In general, it can be concluded that AA is unstable at high pressure levels combined with high temperatures (above 65 °C) and the major degradation is caused by oxidation especially during adiabatic heating. Therefore, eliminating the oxygen content in packaging can decrease the AA degradation during processing and subsequent storage. Moreover, the possible contradictory finding in literature concerning AA pressure stability (even in the same food matrices) could be explained by differences in molar ratio between ascorbic acid and the initial oxygen content and possible existence of other anti or pro-oxidants.

Folates and other B vitamins

Numerous studies have shown that vitamin B is stable during HP treatment at room temperature, for example no change in contents of vitamin B1, B2, B6 and niacin in pressurized (200–500 MPa/30 °C/1 min) red orange juice (Donsì *et al*., 1996); vitamin B1 (thiamine) and B6 (pyridoxal) in food model systems (200, 400, 600 MPa/20 °C/30 min) (Sancho *et al*., 1999; Butz, Bognar, Dieterich, & Tauscher, 2007); riboflavin, niacin and pantothenic acid in sprouted alfalfa seed in citric acid pickle after HP treatment at 500 MPa/room temperature/10 min (Gabrovska *et al*., 2005). In the latter study, the contents of niacin and pantothenic acid in pressurized sprouted seed decreased during storage down to 60% at the third day.

In the past 6 years, the effect of HP processing on natural folate (vitamin B9) stability has been intensively explored. In buffer solution, Oey, Verlinde, Hendrickx, and Van Loey (2006b) have observed that (i) folate degradation during HP treatment was primarily caused by oxidation and (ii) the cleavage of covalent bonds could occur during HP treatments especially at high temperatures due to pressure enhanced oxidation reactions. In addition, (non oxidative) chemical conversion such as the conversion of 5-formyltetrahydrofolic acid to 5,10-methenyltetrahydrofolic acid can be induced during HP processing particularly at elevated temperatures. In presence of antioxidant such as ascorbic acid, on the one hand, the (oxidative) folate degradation during HP treatment is retarded but on the other hand, the reduction of 5-formyltetrahydrofolic acid to 5,10-methenyltetrahydrofolic acid is enhanced (unpublished data). It can be explained by the fact that volume reductions (i.e., formation of charged molecules and cyclization by covalent bond formation) accelerate chemical reactions under pressure according to the *Le Chatelier's* principle (Butz *et al*., 2004).

Detailed kinetic studies on HP stability of natural folate derivates such as 5-formyl and 5-methyltetrahydrofolic acid in buffer solution have been performed. Based on the estimated kinetic parameters, it is clear that (i) different folate derivates have different pressure and temperature stability as illustrated in Fig. 1 and (ii) folate degradation is enhanced by increasing pressure at constant temperature (above 40 °C) and increasing temperature at constant pressure (Nguyen, Indrawati, & Hendrickx, 2003; Indrawati, Van Loey, & Hendrickx, 2005; Nguyen, Oey, Hendrickx, & Van Loey, 2006).

In fruit and vegetable based food products, temperature and pressure stability of 5-methyltetrahydrofolic acid (i.e., reduced forms of folic acid, abundant in fruit and vegetables) has been studied (Indrawati, Arroqui, *et al*., 2004). In orange juice and kiwi puree, 5-methyltetrahydrofolic acid was relatively temperature (up to 120 °C) and pressure (up to 500 MPa/60 °C) stable in contrast to that in carrot

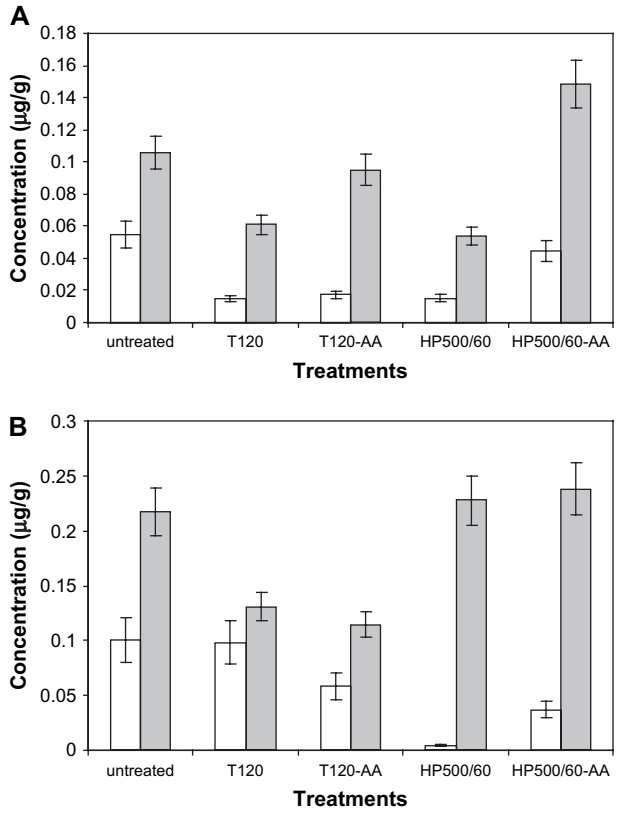

Fig. 1. Effect of ascorbic acid (0.5 mg/g) on temperature and pressure stability of free (white column) and total (shaded column) 5-methyltetrahydrofolates in carrot juice (A) and asparagus (B). T120 and T120-AA represent thermal treatments at 120 °C for 40 min, respectively, in absence and presence of ascorbic acid. HP500/60 and HP500/60-AA represent pressure treatments at 500 MPa and 60 °C for 40 min, respectively, in absence and presence of ascorbic acid. Error bars represent standard deviation of the measurements (reprinted from Indrawati *et al.* (2004a) with permission from ACS Publisher).

juice and in asparagus. Addition of ascorbic acid (0.5 mg/g) in carrot juice resulted in a remarkable protective effect against pressure (500 MPa/60 °C/40 min) and temperature degradation (120 °C/40 min) of 5-methyltetrahydrofolic acid as illustrated in Fig. 1. Another study carried out by Oey *et al.* (2006b) shows a high correlation between the stability of various endogenous folates and the stability of endogenous antioxidants, particularly L-ascorbic acid, in intact vegetables such as carrots, Brussels sprouts and asparagus.

HP stability of various natural folates (i.e., tetrahydrofolic acid, 5-formyltetrahydrofolic acid and 5-methyltetrahydrofolic acid) in orange juice and in model media has also been investigated under conditions corresponding to high pressure pasteurization (600 MPa/25 °C/5 min) and sterilization (600 MPa/80 °C/5 min) (Butz *et al.*, 2004). In this study, the influences of matrix components, pH and excess of ascorbic acid on folate stability have been investigated. Again, it has been shown that excess ascorbate strongly protects folates against pressure and heat. The order of pressure and temperature stabilities of folates in orange juice is 5-methyltetrahydrofolic acid > 5-formyltetrahydrofolic acid -> tetrahydrofolic acid. Moreover, pressure enhanced folate thermal degradation (e.g., 600 MPa/80 °C/5 min compared to 80 °C/0.1 MPa/5 min). At elevated temperature (80 °C), pressure induced conversion of 5-formyltetrahydrofolic acid to 5,10-methenyltetrahydrofolic acid was also observed in plant based food model system similar to orange juice (Fig. 2).

Effect of high pressure on stability of fat soluble vitamins

In literature, information about HP effect on stability of fat soluble vitamins is less abundant than that on water soluble vitamins. A study on stability of vitamin K in buffer solutions has shown that pressure (650 MPa/70 °C/3 h) induced chemical reaction resulting in small quantities of *meta* and *para* isomeric Diels–Alder products of vitamin K1 degradation (Tauscher, 1999). Moreover, pressure induced isomerization can also take place during HP treatment. In literature, it has been recently reported that HP treatments at 500 and 600 MPa and room temperature for 12 min decreased the total content of *all-trans* lycopene in hexane due to *trans–cis* isomerization of lycopene but this phenomenon was not observed in food matrices such as in tomato puree (Qiu, Jiang, Wang, & Gao, 2006).

In food products, HP treatment does not (or only slightly) affect the carotene stability. At room temperature,

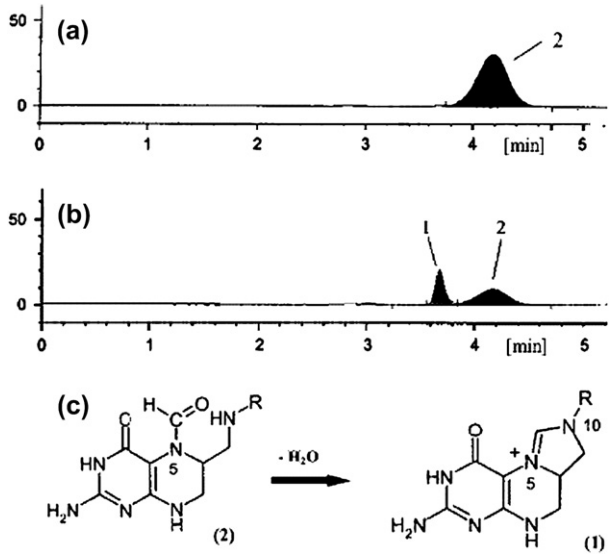

Fig. 2. Pressure induced conversion of 5-formyltetrahydrofolic acid (assigned as 2) in model juice to 5,10-methenyltetrahydrofolic acid (assigned as 1) at elevated temperature. (a) HPLC chromatogram of 5-formyltetrahydrofolic acid in model juice before treatment. (b) HPLC chromatogram of 5-formyltetrahydrofolic acid in model juice after HP (600 MPa/80 °C/3 min) treatment. (c) Degradation mechanism of 5-formyltetrahydrofolic (reprinted from Butz *et al.*, (2004) with permission from Blackwell Publishing).

HP treatment gave no or minor effect on carotenoid stability, e.g., in orange–lemon–carrot mixed juice (500 and 800 MPa/room temperature/5 min) or in tomato puree (also the case observed for lycopene). During storage at 4 °C, the carotenoid content of the pressure treated juices and puree mostly remained constant for 21 days (Fernández Garcia, Butz, Bognàr, *et al*., 2001; Fernández Garcia, Butz, & Tauscher, 2001). During HP treatment at elevated temperature (e.g., 600 MPa/75 °C/40 min), carotene loss in carrot based products was very low (max 5%) (Tauscher, 1998; De Ancos, Gonzalez, & Pilar Cano, 2000).

Pressure influences the extraction yield of carotenoids. Numerous researchers have found an increase in extraction yield of carotenoids due to HP treatment, e.g., more than 40% increase in the measured carotenoid content of pressurized carrot homogenate (600 MPa/25 °C/10 min) (De Ancos *et al*., 2000); 20–43% increase in the measured carotenoid content of orange juice at 360 MPa in the temperature range between 30 and 60 °C for 2.5, 5 and 15 min (De Ancos, Sgroppo, Plaza, & Cano, 2002); 53.88% increase in the measured carotenoid content of orange juice after treatment of 400 MPa/40 °C/1 min (Sánchez-Moreno *et al*., 2005) and a significant increase in the measured carotenoid content of pressurized (400 MPa/25 °C/15 min) tomato puree compared to either thermal treated or untreated puree (Sánchez-Moreno *et al*., 2006a). Due to this potential, HP technology has also been studied to extract lycopene from tomato paste waste (Jun, 2006).

The effect of HP on the extraction yield of carotenoids is influenced by fruit cultivars and pressure levels but not by pressure holding time. For example, carotene extraction in persimmon fruit purees of Rojo Brillante cultivars (9%; 50 and 300 MPa/25 °C/15 min) was lower than that of Sharon cultivars (27%; 50 and 400 MPa/25 °C/15 min) (De Ancos *et al*., 2000). Different pressure level at constant temperature gave different release of various carotenes depending on their chemical properties and chromoplast location. For example, screening studies from 50 up to 400 MPa at 25 °C for 15 min showed that release of lutein, lycopene, lycopene epoxide, gamma- and beta carotene in tomato puree under 200 MPa was different from pressure above 200 MPa (Sánchez-Moreno, Plaza, De Ancos, & Cano, 2004a). It was also observed in pressurized Mediterranean vegetable soup (gazpacho) that HP treatment at low pressure (e.g., 150 MPa) combined with high temperature (60 °C) for short time (15 min) resulted in higher carotene retention than at high pressure (e.g., 350 MPa) (Plaza, Sánchez-Moreno, De Ancos, & Cano, 2006). Prolonging treatment time (above 5 min up to 15 min) for different HP combinations (30–60 °C; 50–350 MPa) induces no additional increase in carotene (beta carotene, alpha carotene, zeaxanthin, lutein, beta-cryptoxanthin) yield of orange juice (De Ancos *et al*., 2002).

Up to now, studies on pressure stability of retinol and vitamin A are limited and mostly carried out in buffer systems. It has been reported that pressure treatment lowered the content of vitamin A. For example, pressure up to 400–600 MPa induced a significant degradation of retinol in 100% ethanol solution. HP treatment at elevated temperatures (600 MPa/40, 60 and 75 °C for 5 min) resulted in retinol degradation up to 45% and the kinetics followed a second order reaction (Tauscher, 1999). Another study on vitamin A acetate has showed that degradation of vitamin A acetate (in 100% ethanol solution) was more pronounced with increasing pressure and temperature. About half of the vitamin A acetate concentration was retained by HP treatment at different pressure/temperature/time combinations, i.e., 650 MPa/70 °C/15 min and 600 MPa/25 °C/40 min. With regard to HPS, a complete degradation was observed after pressurization up to 600 MPa at 90 °C for 2–16 min. The mechanism of the vitamin A degradation under pressure is still unknown but some studies have observed that oxygen does not affect retinol and vitamin A acetate degradation (Butz & Tauscher, 1997; Kübel, Ludwig, & Tauscher, 1997; Tauscher, 1999).

Studies in food products have showed that vitamin A and E in pressurized orange juice were maintained after HP treatment at room temperature (Bignon, 1996). Furthermore, it seemed that the vitamin A content of orange juice was increased by 38.74% after HP treatment of 400 MPa/40 °C/1 min (Sánchez-Moreno *et al*., 2005) or maximally by 45% in the pressure range of 50 and 350 MPa combined with temperatures between 30 and 60 °C for longer treatment time intervals, i.e., 2.5, 5 and 15 min (De Ancos *et al*., 2002). This could be explained by enhanced extraction due to pressure.

Effect of high pressure on antioxidant capacity

It is obvious that HP treatment influences the vitamin stability and the extraction yield of some bioactive compounds. For example, the content of flavanones (naringenin and hesperetin) in orange juice was increased due to pressure treatment of 400 MPa/40 °C/1 min, respectively, by 20.16%) and 39.88% (Sánchez-Moreno *et al*., 2005). As a consequence, changes in antioxidant capacity could also occur during HP treatment.

The effect of pressure on antioxidant capacity is not the same among the food products. The TEAC (*Trolox Equivalent Antioxidant capacity*) index of orange juice decreased after HP treatment in the pressure range of 100 and 800 MPa combined with temperatures from 30 up to 65 °C) as a function of treatment time (up to 90 min). At all temperatures studied, the antioxidant capacity of orange juice decreased faster when the pressure level of the HP/T treatment was increased (Indrawati, Van Loey, & Hendrickx, 2004). The decrease of antioxidant capacity in orange juice during HP processing is mainly caused by ascorbic acid degradation. However, for short treatment time, no change in antioxidant capacity of orange juice and tomato puree was found after HP treatments, e.g., 500 and 800 MPa/20 °C/5 min., described as TEAC index

(Fernández Garcia, Butz & Tauscher, 2001; Fernández Garcia, Butz, Bognàr, *et al*., 2001) or 400 MPa/40 °C/1 min, determined as total scavenging activity using DPPH in aqueous and organic portions (Sánchez-Moreno *et al*., 2005).

In contrast, the TEAC value of carrot juice was increased by HP treatment (100 up to 800 MPa/from 30 up to 65 °C/max. 90 min) but the increase in antioxidant capacity of carrot juice was slowed down by increasing pressure at temperatures above 40 °C (Indrawati, Van Loey, *et al*., 2004). Regarding HP treatment at high temperature, pressure treatment of 600 MPa/60 °C/30 min only slightly affected antioxidant capacity (determined as TEAC value) of apple juice (Fernández Garcia, Butz, & Tauscher, 2000).

In tomato puree, total scavenging activity (DPPH) in aqueous and organic fractions was not changed by a HP treatment of 400 MPa/25 °C/15 min (Sánchez-Moreno *et al*., 2006a). Effect of additives (NaCl 0–0.8% w/w and citric acid 0–2% w/w) on total scavenging activity (DPPH) in aqueous and organic fractions of tomato puree has been studied at different pressure levels (50 up to 400 MPa) at 25 °C for 15 min. Pressure increased antioxidant activity of aqueous fraction of tomato puree in absence of additives. However, combined treatments of pressure between 300–400 MPa and high citric acid concentration (1.2–2%, w/w) decreased the antioxidant activity of tomato puree, while the opposite effect was observed at low pressures (50–150 MPa) and high citric acid concentrations (1.2–2%, w/w). NaCl (0–0.8% w/w) addition lowered the antioxidant activity. The latter effect was more pronounced at low pressures (50–150 MPa) than at high pressures (300–400 MPa). In contrast, citric acid addition (1–2% w/w) increased the (organic portion) antioxidant capacity of tomato puree. A slight increase in antioxidant activity occurred at pressures of ~200 MPa in absence of additives, although the highest antioxidant capacity was found when HP treatment (up to 400 MPa) was combined with NaCl addition (Sánchez-Moreno *et al*., 2004a).

In (raw and germinated) legume seeds, HP treatment at room temperature slightly affects the antioxidant capacity. A slight decrease in TEAC value of raw and germinated cowpeas (*Vigna sinensis* var. *carilla*) was noticed after HP treatments (300–500 MPa/room temperature/15 min.). In the pressure range studied, the antioxidant capacity of raw and germinated cowpeas was lowered by 10–15% and 3–20%, respectively (Doblado *et al*., 2007).

During subsequent storage at 4 °C, total antioxidant capacity of pressurized (500 and 800 MPa/20 °C/5 min) orange juice was slightly decreased by approximately 15% after 21 days. As compared to thermal treated orange juice, the decrease of antioxidant capacity in pressurized juice was higher, but not significant (Fernández Garcia, Butz, & Tauscher, 2001). On the contrary, Polydera, Stoforos and Taokis (2004, 2005) reported that pressurized (600 MPa/40 °C/4 min) orange juice had higher retention in antioxidant capacity during storage at 0 up to 30 °C compared to pasteurized orange juice. This decrease could be explained by vitamin C degradation during storage.

Effect of high pressure on bioavailability of vitamins

In fruit and vegetables, pressure instability of vitamins is not solely due to chemical reaction but also due to enzymatic conversion reactions. For example, natural folates (reduced forms of folic acid) exist as monoglutamate and polyglutamate forms. In human intestine, polyglutamate folates must be hydrolyzed by γ-glutamyl hydrolase resulting in monoglutamate folates (having a higher bioaccessibility) before absorption. A study conducted by Melse-Boonstra *et al*. (2002) has shown that HP treatment of 200 MPa/room temperature/5 min increased the proportion of folates in the monoglutamate forms in leeks (74%), cauliflower (12%) and green beans (82%). However, the resulting monoglutamate forms after treatment were degraded during frozen and refrigerated storage. Increase in monoglutamate folates after HP (150 and 200 MPa) treatment is also noticed in orange juice (Fig. 3). In broccoli tissue, pressure induced enzymatic depolymerization of polyglutamate folates to folates with shorter polyglutamate chains was observed not only at pressure levels up to 300 MPa and room temperature but also at pressure levels up to 600 MPa combined with temperatures up to 40 °C (unpublished data). This implies that HP processing can result in vegetables with higher folate bioaccessibility.

Human studies have shown that vitamins in pressure processed food products still posses their bioavailability, for instance in pressurized orange juice and vegetable soup (gazpacho) (Fig. 4). Consumption of pressure (400 MPa/40 °C/1 min.) processed orange juice increased plasma vitamin C and decreased 8-*epi*$PGF_{2\alpha}$ and PGE_2 (inflammatory biomarkers) levels in humans, which may help reduce the risk of chronic diseases (Sánchez-Moreno *et al*., 2003a).

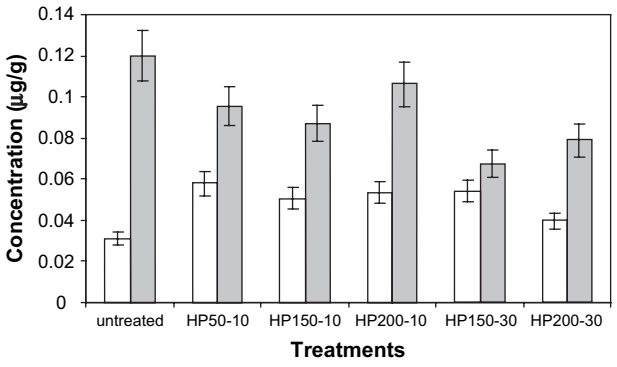

Fig. 3. Influence of different pressure levels (50–200 MPa) at 25 °C on the stability of free (white column) and total (shaded column) 5-methyltetrahydrofolic acid in orange juice. Error bars represent standard deviation of the measurements. Treatments were symbolized as 'HP(pressure level)–(treatment time)', e.g., HP150–10 (pressure treatment at 150 MPa for 10 min). (reprinted from Indrawati *et al*. (2004a) with permission from ACS Publisher).

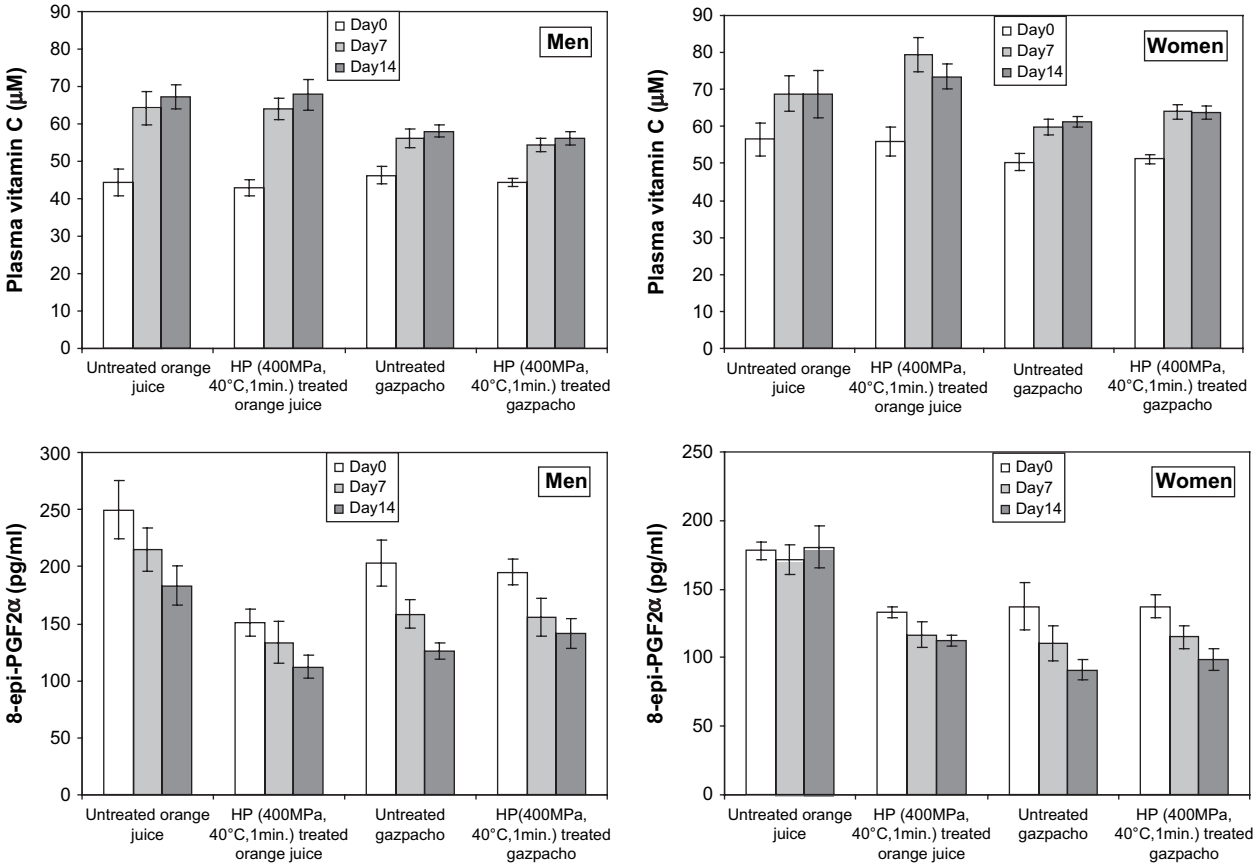

Fig. 4. Effects of daily consuming HP treated food products on plasma vitamin C and 8-*epi*-prostaglandin $F_{2\alpha}$ (biomarkers of oxidant status) in humans. The figures are depicted based on the data of Sánchez-Moreno *et al.* (2003a, 2003b, 2004b, 2006b).

Similar findings are also reported for pressurized (400 MPa/40 °C/1 min.) vegetable soups gazpacho. The consumption of the pressurized soup decreased oxidative stress and inflammatory biomarkers (Sánchez-Moreno *et al.*, 2004b). Compared to untreated orange juice and vegetable soups gazpacho, increase in human plasma vitamin C and decrease in 8-*epi*-$PGF_{2\alpha}$ due to daily consumptions of HP treated orange juice and gazpacho show similar tendencies as illustrated in Fig. 4.

General conclusions

Hitherto, information on pressure stability of some vitamins such as niacin, cobalamin, vitamin A, calciferol (vitamin D), tocopherol (vitamin E) and phyllochinon (vitamin K) are still limited. Therefore, it calls further researches on those topics. Based on current knowledge, it can be concluded that in general HP treatment at moderate temperatures can maintain the vitamin content of fruit and vegetable based food products, however, mostly not at high temperatures (e.g., HPS applications). Vitamin stability is highly influenced by chemical reaction which can be enhanced by increasing pressure and temperature during HP treatment. As a consequence, HP treatment at extreme pressure and temperature combinations could result in vitamin degradation. Since the mechanism of chemical reaction occurring at atmospheric pressure cannot be extrapolated directly for HP treatment especially when combined with high temperatures, it is possible that HP processing particularly at high temperatures could induce some (un)expected and (un)desired chemical reactions which (in)directly influence food quality and safety. Hereto, it calls further investigations on this issue to obtain better understanding in mechanism and kinetics of vitamin stability especially at extreme pressure and temperature combinations. This information is indispensable in the future especially for process optimization and food regulation of pressure-sterilized food products and a comparison with pasteurization and sterilization at atmospheric pressure.

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