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Effect of ultra-high hydrostatic pressure on hydrosoluble vitamins

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Abstract

The effect of ultra-high hydrostatic pressure on selected hydrosoluble vitamins (B1, B6 and C) is studied. Vitamin retention after pressurization has been compared to that induced by several *classic* food processing treatments, such as pasteurization or sterilization. Ascorbate, pyridoxal, and thiamin hydrochloride, included in a multivitamin model system (*mv*MS), are analyzed by high performance liquid chromatography (HPLC), and under current operating parameters for the above processes. Thereafter, these vitamins indicate the impact of ultra-high hydrostatic pressure on the nutritional quality of tested matrices, on the basis of *equivalent effects* induced by several industrial treatments. Minor variations are found among the vitamins after pressurization. Vitamins B1 and B6 undergo no significant losses after the treatments. Vitamin C levels, although significant, are not dependent on the intensity of the ultra-high hydrostatic pressure process. Naturally occurring vitamin C losses are analyzed in two representative foodstuffs (egg yolk and strawberry *coulis*) after several processes, in order to validate the model system results. A survey of ascorbate retention has been carried out on ultra-high pressurized strawberry *coulis*, over 30 days. Results have shown that ultra-high hydrostatic pressure plays only a minor role in degradation kinetics of vitamin C. This article will integrate a general and structured evaluation framework, including other precise nutritional data of food processing.

1. Introduction

Food processing under high pressure and low to moderate temperature (below 70°C) conditions has been recently introduced as an alternative to high temperature preservation. By avoiding the detrimental effects of elevated temperatures on various food quality attributes, high pressure processing can offer a distinct quality advantage, over traditional thermal processed foods (Hayashi, 1995; Knorr, 1994).

The use of high pressure as an alternative to conventional heat treatments for the preservation of foods (like acid fruit juice) has been demonstrated (Hayashi, 1989a; Mertens, 1995, Cheftel, 1992; Rovere, Carpi, Gola, Dall'Aglio & Maggi, 1996). The technique offers several advantages: the possibility of obtaining conserved products with characteristics very similar to those present before processing; homogeneity of treatment due to the fact that pressure is always the same at each point of the product; significant energy saving in comparison with thermal stabilization techniques (Rovere et al., 1996); products which are new from physical, functional and nutritional points of view (Balny, 1995; Tauscher, 1995); microbial reduction (Patterson, Quinn, Simpson & Gilmour, 1996) with better food quality parameters (nutrients, flavor, and sensorial preservation).

The aim of this work was to study degradation of hydrosoluble vitamins (B1, B6 and C) during high pressure and moderate temperature processing in a multivitamin model system and in food matrices, representative of animal and vegetable foodstuffs (egg yolk and strawberry *coulis*).

Vitamins B1, B6, and C have been selected for this survey. Vitamin B6 occurs naturally in foodstuffs, mainly as pyridoxal, pyridoxol, its amine form pyridoxamine and their corresponding phosphates. The major part of B6 vitamin found in many foodstuffs (e.g. milk) is present as the aldehyde (pyridoxal) form (Gregory, 1975).

Vitamin C is present in most vegetables and fruits. Ascorbate is a powerful reducing agent which plays a key

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role in human nutrition, acting as an electron donor in O_2 depending reducing reactions, and promoting iron and copper in their reduced states by its antioxidant function.

Thiamin is widely present in bakery products, meats, and milk products. This vitamin reacts readily with proteins (specially with cystine and cysteine residues), sugars, and aldehydes. Thiamin breakdown in food processing yields volatile sulphur compounds, like furanes or thiazoles, which are important contributors to food aroma and overall quality.

Food vitamins are inevitably and irrevocably damaged during heat treatment (Noble & Gomez, 1962; Priestley, 1979; Sancho, Grelier, Ribera, Berbille & Narbonne, 1996; Sanco, Ribera & Narbonne, 1997), and vitamin contents in foodstuffs are closely related to their nutritional quality (Hermus, 1993). The reason for the choice of hydrosoluble vitamins is their sensitivity to physical factors, like temperature, which limits their use in conventional industrial processing. There is substantial literature on vitamin losses during food processing (Baldwin, Korschgen, Russell & Mabesa, 1976; Bowers et al., 1974; Bowman et al., 1975; Karmas & Harris, 1977; Jenkins et al., 1989; Raab et al., 1973; Watier, 1988). High pressure treatment is a cold treatment, and, is very interesting for the nutrients of this study (Rovere et al., 1996). In order to compare high pressure treatments, data were also obtained for classical preservation processes, such as pasteurization or sterilization.

Even if we assume that a case-by-case evaluation remains indispensable, a general approach is nevertheless attractive, and should be intrinsically relevant.

2. Materials and methods

2.1. Sample composition

Multivitamin model system: Hydrosoluble vitamins were diluted in 10 ml of Clarks and Lubs buffer (Dawson et al., 1986), adjusted to a pH of 6.70 at concentrations of 2.085, 3.725, 1.475 mg/10 ml, respectively for ascorbate, thiamin and pyridoxal, all values being in the approximate range found in major foodstuffs (Gregory, 1975).

Strawberry "coulis": This preparation, a common sauce in French desserts, is made from ground "Garriguette du Périgord" strawberries. In order to obtain the juice, the solid particles are removed.

Egg yolk: White and yolk of 30 fresh hen eggs ("Domaine de Turcoing" variety, 50/55 sizing, category A, and "Saint Guénault" variety, 55/60 sizing, category A) were separated and independently mixed for each analysis.

2.2. Extraction

Strawberry "coulis" and egg yolk: Each sample was weighed, all values being between 1 and 2 g. Extraction was performed by adding 1 ml of n-Hexane and 4 ml of bi-distilled water, mixing and centrifuging at 5000 rpm for 40 min, in order to eliminate the protein fraction and to conveniently separate the phases. The aqueous phase was filtered and injected into the HPLC apparatus. Injection volumes were between 5 and 50 μl.

2.3. Sample preparation

All samples were homogenized and filtered (45 μm) prior to HPLC analysis.

2.4. Analysis

Vitamin analysis: The analytical system consisted of a Hewlett Packard Series 1050 HPLC, a Shimazdu SP10 A spectrometer, a Hewlett Packard fluorescence programmable 1046 detector, and a Hewlett Packard Vectra® computer system for integration and main system control. The analytical column was an Alltech Alltima™ C18, 5 μm, 250×4.6 mm. The mobile phase was an isocratic solvent system consisting of 95% of 50 mM dipotassium phosphate and 5% acetonitrile, the flow rate being 1 ml/min. The analyses were conducted at ambient temperature. The detection was carried out at 254 nm in absorbance mode. Retention times (average of 10 standard analyses) were 4.724 min (vitamin B6), 7.208 min (vitamin B1), and 2.248 min (vitamin C), for the above mentioned parameters. All reagents were glass-distilled, HPLC grade, obtained from Sigma (Saint Quentin Fallavier, France).

pH *measurement*: pH of the samples was measured by means of an Accumet 15 (OSI, Maurepas, France) pH-meter. Each data point was the average of three measures.

Water activity: Water activity was calculated with a FwM AwMeter (GBX Scientific Instruments, Romans-Sur-Isère, France). Calibration was performed with potassium sulfate (K_2SO_4, $Aw = 0.755$), and potassium chloride (KCl, $Aw = 0.432$). Results are presented in Table 1, they were the average of two consecutive determinations at the mentioned sample temperature.

Microbiological analysis: One milliliter of untreated and pressure treated samples was analyzed after dispersion in two petri dishes. Total mesophilic aerobic flora (TMAF) were enumerated using a spread plate method on plate count agar (Korano Industries,

Table 1
Aw values for selected foodstuffs

Sample	Aw	T (°C)	pH
Vitamin model system	0.987	26.48	6.71
Egg yolk homogenate	0.979	26.93	6.28
Strawberry *coulis*	0.975	20.01	3.64

La Balme-les-Grottes, France). Incubation was performed at 30°C for 72 h. Each test was performed in duplicate and results were expressed as colony forming units (CFU) per milliliter.

2.5. Food processes

Ultra-high hydrostatic pressure: Samples were packed in Cryovac™ Nop-120 non-porous polyethylene hermetic bags and hydrostatically pressurized in a 3 l sample compartment, by means of a hyperbar experimental apparatus, designed by NFM-Technologies (Le Creusot, France) and FRAMATOME (Paris, France), marketed by CLEXTRAL (Firminy, France) to attain a maximum pressure of 800 MPa. The pressures applied were 200, 400 and 600 MPa, over 30 min, at ambient temperature, and with a pressure climbing rate of 375 MPa per minute. Pressure and temperature were constantly monitored and recorded during the process.

Pasteurization: Samples were pasteurized in test tubes using a laboratory water bath, under constant agitation. "High" pasteurization process (20 s at 76°C) was applied.

Sterilization: The samples were placed in sealed tubes and sterilized in a water–steam vertical autoclave, Apave model, 40×70 type, for 20 min and 120°C, reaching a final pressure of 1.5 atmospheres.

2.6. Statistical analysis

The data were statistically analyzed using the analysis of variance (ANOVA) and the Student's *t*-test (Snecdor & Cochran, 1967). The differences were considered significant when $P<0.05$.

3. Results and discussion

Initial TMAF of strawberry *coulis* was 1.94×10^3 CFU/ml. After a treatment at 400 MPa for 30 min, and at 20°C, TMAF was reduced to 2.35. This data proved the efficiency of the high pressure treatment. To inactivate bacteria on fruits and vegetables, a pressurization of 400 MPa for 15 min at 30°C is usually sufficient (Butz & Tauscher, 1997). Previous tests performed on strawberry *coulis* and egg yolk are presented in Table 2. These results demonstrate the extent of the inactivation of TMAF. A pressurization with 400 MPa for 30 min suffices to stabilize the microbial population. This process time can be long for microbial inactivation but it is good for checking vitamin losses.

Table 2
Pressure inactivation of TMAF in selected foodstuffs

TMAF (CFU/ml)	Untreated	400 MPa/30 min
Strawberry *coulis*	$1.94\times10^3\pm9.22\times10^2$	2.35±0.58
Egg yolk homogenate	$5.5\times10^1\pm4.76$	4.25±0.78

3.1. Tests using a multivitamin model food system

Ultra-high hydrostatic pressure treatments have no significant effect on the retention of pyridoxal and thiamin vitamins, when included in a model system (Table 3). The untreated model system had a vitamin B1 concentration of 1.475 μg/ml, and after pressurization (600 MPa, for 30 min at 20°C), 1.468 μg/ml (99.57% retention). Thiamin (vitamin B1) presented no significant losses when pressurized for 18 h at 600 MPa and 20°C, in both model solutions and a food matrix like rehydrated pork (Butz & Tauscher, 1997). In the same way, initial vitamin B6 concentration in our model system was 3.725 μg/ml, while a concentration 3.794 μg/ml (101.84%) was registered after a pressurization at 600 MPa for 30 min and at 20°C.

However, the pressure process had a significant effect on vitamin C levels in the multivitamin model system (Table 3). However, the intensity of the treatment had no effect on the destruction of ascorbate. In fact, concentrations after a high pressure treatment at 200 MPa for 30 min and at 20°C were 1.832 μg/ml (87.83%), and 1.847 μg/ml (88.58%) after a 600 MPa treatment for 30 min, and at 20°C. The results of Taoukis et al. (1997) were indicative of the detrimental effect of pressure on vitamin C in a model system (1000 mg/l ascorbic acid and 10% sucrose in 0.1 N sodium acetate buffer, with an acid pH of 3.5–4.0). Only 76.1% of vitamin C from the

Table 3
Effect of ultra-high pressure treatment on hydrosoluble vitamin retention in the model system

Parameters	Quantity (mg/10 ml)	%	Significance*
Ascorbate			
Untreated	2.085±0.00030	100	
200 MPa/30 min	1.832±0.00026	87.83	S
400 MPa/30 min	1.876±0.00130	89.94	S
600 MPa/30 min	1.847±0.00078	88.58	S
Thiamin			
Untreated	3.725±0.0628	100	
200 MPa/30 min	3.814±0.0496	102.39	NS
400 MPa/30 min	3.804±0.0349	102.11	NS
600 MPa/30 min	3.794±0.0240	101.84	NS
Pyridoxal			
Untreated	1.475±0.0014	100	
200 MPa/30 min	1.473±0.0128	99.85	NS
400 MPa/30 min	1.465±0.0138	99.36	NS
600 MPa/30 min	1.468±0.0091	99.57	NS

* Significance: S = Significant for $p<0.05$; NS = Not Significant for $p<0.05$. Students *t*-test.

model system was retained after a high pressure treatment (450 MPa, 40 min, 40°C).

The inherent nature of the hydrostatic pressure allows nutrients such as vitamins to remain in their natural state. Applied pressure had no effect on covalent bonds (Hayashi, 1989a) and affects only non-covalent bonds, i.e, hydrogen, ionic, and hydrophobic bonds (Heremans, 1985; Weber, 1986). For example, raw fish or meat, fresh fruit purees or juice, and various flavor extracts can be processed without any alteration of their raw or fresh taste and flavor characteristics (Cheftel, 1992; Shinada et al., 1990).

3.2. Tests in foodstuffs

Hermus (1993) noted that vitamins should be determined depending on whether their presence in the food is likely to reduce or enhance nutritional adequacy significantly. For a food product like strawberry "*coulis*", which has a high content of vitamin C, high pressure treatment had no significant effect on its retention. In strawberry *coulis*, ultra-high pressure treatment of 400 MPa, for 30 min at 20°C, was responsible for 88.68% non-significant retention (Table 4). In a strawberry nectar (50% juice, 49.5% sucrose syrup, 0.2% ascorbic acid and 0.3% citric acid), ascorbic acid remains practically the same during high pressure processing. After treatment, the measured quantity of ascorbic acid was 1129 ppm for an initial count of 1100 ppm. The concentration of ascorbic acid in the citrus juice did not indicate any change after high pressure treatments (Ogawa et al., 1992). The concentration for fresh citrus juice was 27.2 mg/100 g and after treatment at 500 MPa for 10 min at 20°C, it became 27.1 mg/100 g.

We have also measured vitamin C concentrations in strawberry *coulis* when treated with other processes such as pasteurization (0.1 MPa, 20 s at 72°C). We obtained a 91.52% ascorbate retention percentage. These non-significant data indicate that this vitamin is conserved in the pasteurization process. There was thus no significant effect on the destruction of the vitamin C of the strawberry *coulis*.

On the other hand, sterilization process (0.1 MPa, 20 min at 120°C). had a more noticeable effect on vitamin C concentrations in strawberry "*coulis*". Only 67.1% of the initial vitamin C was conserved after sterilization.

In a matrix like egg yolk, results are almost identical, high pressure treatment having no significant effect on vitamin C concentrations (Table 4). No variations in its concentrations was found after high-pressure treatments ranging from 400 to 1000 MPa, while it tended to decrease with increased boiling time (Hayashi, 1989a).

A small increase in vitamin C levels in egg yolk is found after pressurization. Quite similar results are obtained when analyzing egg yolk ascorbate. When this foodstuff is pressurized for 30 min, and at 20°C, at 200, 400, or 600 MPa, ascorbate variations remain non-significant (92.58%, 101.34% and 102.66%, respectively). These high percentages can be attributed to the extractive effect of the process. Hayashi et al. (1989b) made similar observations when studying thiamin retention in egg yolk. They had noted an increase of 6.2% and 2.8% in thiamin retention after, respectively, high pressure

Table 4
Change in vitamin C content after different food processes

	Quantity (mg/100 g)	%	Significance*
Strawberry coulis			
Untreated	33.133 ± 0.521	100	
High-pressurized (400 MPa/30 min)	29.384 ± 1.628	88.68	NS
Untreated	26.0255 ± 0.3955	100	
Pasteurized (72°C/20 sec)	23.8173 ± 0.9109	91.52	NS
Untreated	26.0255 ± 0.3955	100	
Sterilized (120°C/20 min)	17.4653 ± 0.6340	67.11	S
Egg yolk			
Untreated	4.2223 ± 0.1530	100	
High-pressurized (200 MPa/30 min)	3.9088 ± 0.1379	92.58	NS
Untreated	4.2223 ± 0.1530	100	
High-pressurized (400 MPa/30 min)	4.2787 ± 0.0233	101.34	NS
Untreated	4.2223 ± 0.1530	100	
High-pressurized (600 MPa/30 min)	4.3348 ± 0.0813	102.66	NS

* Significance: S = Significant for $p < 0.05$; NS = Not Significant for $p < 0.05$. Students t-test.

processing at 600 and 800 MPa, for 30 min at 20°C. There is an inexplicable extractive effect of the pressurization process to which higher percentage of vitamin compared to the control can be attributed.

Ascorbate retention of untreated and pressurized (400 MPa, 30 min, 20°C) strawberry *coulis* decreases with time. Samples were stored at 4°C and without light. Initial concentration of vitamin C was 33.13 mg/100 g, and was reduced to 29.38 mg/100 g (88.68%) after ultra-high hydrostatic pressure treatment (Table 4). After 28 days of storage, it was 26 mg/100 g (78.48%) for untreated strawberry *coulis* and 22.8 mg/100 g (68.82%) for pressurized *coulis* (Fig. 1). These percentages were acceptable during the one month preservation period. The profiles of decrease of vitamin C in the pressurized and untreated *coulis* are nearly identical. Therefore, the pressure neither accelerates nor slows the kinetic degradation of ascorbic acid. Oxidation is increased in the presence of light, air and heat, so it is important to preserve the samples in darkness, at low temperatures (4°C).

The vitamin C content of untreated orange juice did not vary in a significant manner during the first 18 days of storage, but declined between the 18th and 22nd day. The processing by high pressure allowed to preserve a profile of diminution of the content in vitamin C quite similar to the untreated sample (Lamballerie-Anton et al., 1997).

Rovere et al. (1996), found that the content of ascorbic acid of strawberry nectar remained practically the same during processing but decreased during storage, reaching 75% after 60 days at 3°C.

Significant losses in processed foods are associated with chemical degradation, usually associated with non-enzymatic browning reactions, as well as aerobic oxidation which produces L-dehydroascorbic acid (Davidek et al., 1990). This molecule undergoes hydrolysis by alkaline nucleophilic reagents, finally yielding a wide variety of chemical products (mainly reductones in alkaline solutions like the model system and furan derivatives in neutral and acid solutions such as strawberry *coulis*). Tamakoa et al. (1991) concluded that the high pressure process suppresses browning reactions. We propose that this is caused by the previously cited aerobic oxidation.

Simultaneously, in order to give a more convincing analysis, bacterial flora of *coulis* were determined during storage at 4°C after 28 days (Fig. 2). TMAF were nearly the same for the pressurized *coulis* after 28 days of storage at 4°C and the untreated *coulis* after 7 days of storage. Thus, the shelf life of the product is increased by the high pressure treatment. Orange juice stabilized by high pressure had a shelf life of at least 2 months at 8°C, as proved by periodical microbiological analysis. The level of vitamins was fully maintained (Donsi et al., 1996). Thus, it is possible to stabilize some acid products such as fruit juices or jams over a long time after high pressure pressure treatment (300 MPa, 30 min, 20°C) without modifications of color, flavor and vitamins (Cheftel,1991).

4. Conclusions

Hydrosoluble vitamins i.e., ascorbate, thiamin and pyridoxal, contained in model system, did not show the same extent of variation after the treatments. While thiamin and pyridoxal were not significantly affected by the food process tested, ascorbate retention data were always of significance, but independent of the intensity of the ultra-high hydrostatic pressure applied. These percentages of vitamin loss were small, never higher than 12%.

Ascorbate levels in strawberry *coulis* decreased with both processes, high pasteurization, and sterilization, as well as ultra-high pressure process. However, retention percentages were only significant, and the most severe, in sterilization treatment. Obtained retention percentage had an analogous value than previously (*mv*MS) obtained, which validates the proposed model system.

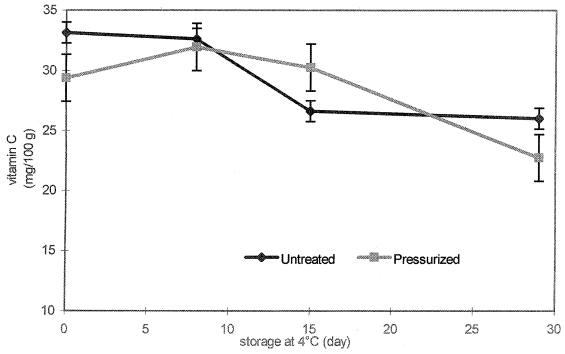

Fig.1. Change in ascorbate content during storage at 4°C of a pressurized and untreated strawberry *coulis* (400 MPa/30 min/20°C).

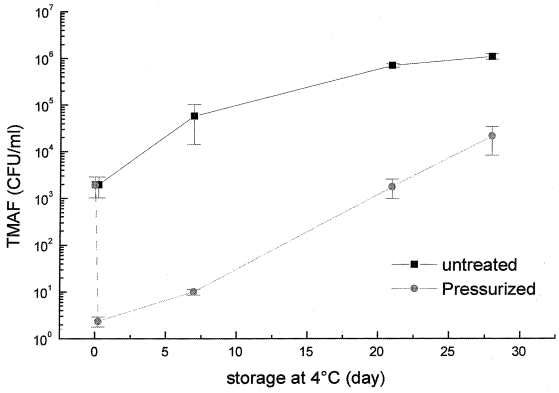

Fig.2. Change in TMAF during storage at 4°C of a pressurized and untreated strawberry *coulis* (400 MPa/30 min/20°C).

When studying ascorbate retention in egg yolk after high pressure treatment, it appears than none of the process parameters applied, induced significant results. Also, vitamin retention percentages registered are independent from pressure intensity, as previously observed in multivitamin model system, and in agreement with previously discussed literature.

Results presented also demonstrate that ultra-high hydrostatic pressure has a minor importance on the degradation kinetics of vitamin C, while insuring a good microbial stability.

According to several authors (Hayashi, 1989a; Kimura et al., 1994), ultra-high hydrostatic pressure was the technological process which less affected tested hydrosoluble vitamins, thus contributing to preserve their nutritional quality in foodstuffs.

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