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High Hydrostatic Pressure (HHP) Processing on Food Bioactives

Ozlem TOKUSOGLU 1,2

Abstract

 High Hydrostatic Pressure (HHP) is an excellent food processing technology that has the potential to retain the bioactive constituents with health properties in fruits, cereals, and other foods. HPP-treated foods retain more of their fresh-like features and can be marketed at a premium over their thermally processed counterparts. HPP can have an effect on food yield and on sensory qualities such as food color and texture. High pressures can also be used to enhance extraction of compounds from foods. Recent studies have shown that high pressure extraction (HPE) can shorten processing times, and provide higher extraction yields while having less negative effects on the structure and antioxidant activity of bioactive constituents. The use of HPE enhances mass transfer rates, increases cell permeability, and increases diffusion of secondary metabolites. Also, HPP increased the capacity to extract phenolic constituents, and HPP-treated samples retain higher levels of bioactive compounds and bioactive phenolics.

Keywords: High Hydrostatic Pressure (HHP), Processing, Bioactives, Foods

Ozlem Tokusoglu 1,2

¹Celal Bayar University, Engineering Faculty, Department of Food Engineering, Manisa, Turkey

2 Dokuz Eylül University Technology Development Zone, DEPARK Technopark, SPİL INNOVA Ltd.Şti, İzmir, Turkey E-mail: proftokusogluozlem@gmail.com

Introduction

In recent years, there has been increasing interest in moving from conventional methods of processing for food preservation toward the use of novel and emerging nonthermal food processing technologies, to control or eliminate microbes, enzymes, or chemical reactions and deliver more fresh-like, nutritious, value- added, and safe high-quality food products to satisfy consumer demand for less processed foods with an extended shelf-life and that are free from additives. High pressure processing (HPP), irradiation, pulsed electric field (PEF), ultraviolet light (UVL), and other nonthermal processing methods are becoming increasingly popular to treat foods, capable of eliminating harmful microorganisms in foods, while *minimizing* thermal degradation reactions in foods compared to thermal processing [2, 3, 16]. More information on various nonther- mal processing technologies is available from the Nonthermal Processing Division of the Institute of Food Technologists (www.ift.org/divisions/nonthermal/). The major aims of utilizing these methods are to improve food safety and food quality concomitantly, and thereby facilitate the development of innovative high value products and the creation of new opportunities for expanding markets.

High hydrostatic pressure or ultrahighpressure processing or HPP is one technology that has begun to fulfill its potential to satisfy both consumer and scientific requirements, and it is a leading alternative in replacing thermal processing in some food applications in the drive to meet increasing consumer demand for foods featuring improved organoleptic qualities and higher acceptance [24]. The technology is especially beneficial for heat sensitive products [3]. HPP can be conducted at ambient or moderate temperatures, thereby eliminating thermally induced cooked off- flavors. Compared to thermal processing, the HPP of foods results in products with a fresher taste, better appearance, and texture.

In the 1990s, the HPP technology began gaining prominence in the food industry because of its advantages for inactivating microorganisms and enzymes at ambient or relatively low temperatures with less adverse affect on the flavor, color, and nutritional constituents of foods compared to conventional thermal processes [19, 20, 4].

Companies began marketing commercial HPP-treated products, such as jam, fruit juice, sauces, rice wine, and rice cake [17]. In recent years, HPP has been successfully implemented in food industries worldwide (United States, Europe, and Japan) to extend shelf-life or improve safety of fruit products (avocado, guacamole, salsa, applesauce, fruits juices, etc.), ready-to-eat (RTE) meats, and fresh oysters. Among the most successfully commercialized HPPtreated food products are sliced, cooked ham and a range of tapas products in Spain [15]. Tapas products are convenient heat-and serve mini-pork sausages made with Spanish paprika and marinated diced pork. The benefits of HPP for increasing the retention of food organoleptic attributes and other more freshlike characteristics combined with increased convenience and extended shelf-life will no doubt continue to increase for the market [8].

The HPP provides an alternative means of killing vegetative bacteria, spoilage organisms (yeasts and molds), viruses, and bacterial spores that can cause food spoilage or food-borne diseases without compromising food sensory quality attributes or food nutrients. In many cases with vegetative pathogens and bacterial spores, the survival curves for organisms subjected to HPP exhibit nonlinear inactivation kinetics "shoulders" [10, 14] or "tailing" [11]. Predictive microbiology models provide convenient tools to assess whether a process will ensure the safe preservation of foods. Two examples are the quasi-chemical model [30, 29] and the Weibull distribution model, both of which are nonlinear models that can accurately describe the nonlinear inactivation kinetic models of vegetative pathogens (*Escherichia coli*, *Listeria monocytogenes*) in foods treated with HPP [11, 12]. An enhanced version of the

quasi-chemical model is being developed to account for unique features of the inactivation kinetics of bacterial spores of *Bacillus amyloliquefaciens* by HPP, including the presence of a subpopulation of increased baro resistance.

As indicated above, the HPP pasteurization safely inactivates vegetative cells, and some enzymes, while retaining nutritive content, sensory attributes, and a fresh-like character of foods. HPP tends to affect cell membranes, enzymes, and large molecules. Macromolecules such as proteins and starches can undergo changes in their native structure during HPP treatments (and during thermal treatments) that can be used to influence texture. Doona et al. [9] studied the retrogradation kinetics, water dynam- ics, and thermometric characteristics of HPP-treated wheat starch [1,12,14,24]. In contradistinction, HPP generally has little effect on the primary structure of low molecular weight food individual components such as flavors, vitamins, pigments, peptides, lipids, and saccharides. In general, HPP tends not to destroy the covalent bonds between atoms of the constituent molecules. The energy used during HPP treatment is relatively low and covalent bonds tend to have low compressibility below 2000 MPa, whereas the process affects hydrogen bonds and ionic and hydrophobic interactions in macromolecules. HPP protects nutraceuticals, functional food ingredients, and so on, whose functionality can be compromised by the use of heat. HPP is an innovative, emerging technology with potential for optimizing intake of nutrient and nonnutrient phytochemicals in foods [8].

High Pressure Processing (HPP)

HPP conditions in the range of 300– 700 MPa at moderate initial temperatures (around ambient) are generally sufficient to inactivate vegetative pathogens for pasteurization processes, some enzymes, or spoilage organisms to extend shelf-life. For example, HPP is used to inactivate spoilage organisms and extend shelf-life (and provide extra assurance against pathogens) at conditions of 400 MPa and 15°C (in a 320 L

unit) for sliced cooked ham, and 600 MPa and 15°C (in a 218 L unit) for dry-cured ham and tapas products [15]. To inactivate bacterial spores such as *Clostridum botulinum* for the production of ambient shelf-stable, low-acid foods requires high pressure and high temperature combina- tions. Such processes typically involve high pressures in the range of 600–800 MPa and higher initial temperatures around 80-90°C. During pressurization, rapid adiabating heating generates temperatures above 121°C. This process, achieving commercial sterility in low-acid foods, is called "pressure-assisted thermal sterilization" and has several technical advantages over conventional thermal sterilization methods (shorter processing times, improved food quality, and increased energy efficiency).

HPP: Principles of Operation

A typical HPP process (Ohio State University 2009) involves treating packaged food products (usually in flexible plastic pouch material or plastic bottles) by loading them into a high pressure vessel filled with an incompressible transmission fluid (usually water) then closing the vessel. Using one or more pumps, fluid is pumped into the vessel to increase the pressure to the intended end-level and then the pumping is stopped. The packaged food products are subjected to these combinations of high hydrostatic pressure and temperature for a sufficient time to induce inactivation of the target organisms or enzymes and then the pressure is released. Since pressure is transmitted uniformly throughout the package and product, the food retains its original shape. This works particularly well for unstructured foods containing water, whereas foods with internal air pockets (strawberries, marshmallows, some bakery items) tend to col- lapse, and dry solids tend not to have enough moisture to allow efficient microbial destruction. When the product is removed from the high pressure vessel, the package is covered with water. In the case of RTE meats, for example, some companies use cold drying equipment to remove the water and prepare the package for labeling and packing. Cold drying helps

maintain product quality by reducing the potential effects of using heat.

Large-Scale and research HPP equipment

There are a number of high pressure equipment manufacturers worldwide making HPP equipment for food preservation (Ohio State University 2009). Systems cost in the range of \$0.5–2.5 million, depend- ing on the size of the vessel, extent of automation, and other design features. Units can range in sizes of 420 L, 350 L, or 150 L, and systems run in batch or semicontinuous modes of operation for food industry purposes. Figure 1 demonstrates large-scale HPP equipment, including a 420 liter unit (Figure 1a) and a 350 liter unit (Figure 1b). Both units are in a horizontal configuration. The illustration in Figure 1c depicts the semicontinuous mode of operation, in which carriers full of packaged food products enter on a conveyor belt from the left and are loaded into the pressure vessel. After the HPP treatment, the carriers are removed from the pressure vessel, and exit the area to the right on the conveyor belt. The treated products are removed from the carrier, dried, labeled, and packed for shipping and distribution. Figure 1d shows a smaller vertical configuration HPP unit used to process oysters. A 215 L batch system has the capacity to produce about 10 million pounds of food per year and products may cost about \$0.03–0.10 more per pound than thermally processed counterparts.

Significantly smaller laboratory-scale units are also manufactured to operate on the same general basic principles but for research purposes, and they are available at research facilities and universities worldwide. Figure 2 depicts one such unit that operates at pressures of $100,000$ psi with sample sizes of $10-30$ mL. Figure 2a shows the front view of the high pressure research unit with the accompany- ing workstation to the left and the bath cover emanating from the top. In addition to containing heat in the bath where the high pressure chamber vessel cylinder is located, the bath cover also acts as a potential safety shield. Figure 2b details the arrangement of the pressure unit's components, with the chamber

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vessel cylinder located in the covered bath and connected to the pump and an assortment of valves for regulating and releasing high pressures. Figure 3a shows the actual interior of the high pressure unit, with the components labeled in accordance with the schematic of the interior side view in Figure 3b. Figure 4a shows the top view of the bath with the bath cover removed and the bath fluid drained to reveal the chamber vessel cylinder (with the cap removed). The corresponding crosssectional side view of the bath (Figure 4b) shows the chamber vessel cylinder with the cap in place and a thermocouple inserted into the sample chamber.

HPP on Bioactive Components

Consumer perception of food quality depends not only on microbial quality, but also on other food factors such as biochemical and enzymatic reactions and structural changes [4, 23]. In this context, HPP can have an effect on food yield and on sensory qualities such as food color and texture [18]. High pressures can also be used to enhance extraction of compounds from foods. Recent studies have shown that high pressure extraction (HPE) can shorten processing times, and provide higher extraction yields while having less negative effects on the structure and antioxidant activity of bioactive constituents. The use of HPE enhances mass transfer rates, increases cell perme- ability, and increases diffusion of secondary metabolites [13, 28]. Also, HPP increased the capacity to extract phenolic constituents, and HPP-treated samples retain higher levels of bioactive compounds $[1, 31, 1]$ 32, 33, 34, 36, 37].

HPP effects on antioxidant Phenolics and antioxidant activity

The study of Patras et al*.* (2009) was undertaken to assess the effect of HPP treatments and conventional thermal processing on antioxidant activity, levels of bioactive antioxidant compounds (polyphenols, ascorbic acid, and anthocyanins), and the color of strawberry and blackberry purées [21]. It was reported that key antioxidants (cyanidin-3-glycoside, pelargonidin-3-glucoside, and ascorbic acid)

in strawberry and blackberry purées and the antioxidant activity of these purées were quantified after various HPP treatments (400, 500, 600 MPa/15 min/10–30°C) and thermal treatments (70°C/2 min). Table 1 shows the antioxidant indices of HPP-treated and thermally processed strawberry and blackberry purées [21]. The three different pressure treatments did not cause any significant changes in ascorbic acid levels. Following thermal processing ($P_{70} \ge 2$ min), the ascorbic acid content degraded by 21% compared to the unprocessed purée. Similarly, no significant changes in anthocy- anin compounds were observed in HPP-treated and unprocessed purées, while conventional thermal treatments significantly reduced the anthocyanin levels (Patras et al. 2009). Patras et al. [21] reported that antioxidant activity of HPP-treated strawberry and blackberry purées were significantly higher than in thermally processed purées [21].

Qui et al. (2006) studied the stability and isomerization of lycopene by HPP. Standard lycopene and tomato purée were pressurized at 100, 200, 300, 400, 500, or 600 MPa for 12 minutes and at controlled temperature (20 \pm 1°C), then stored at refrigerator temperature $(4 \pm 1^{\circ}C)$ and ambient laboratory tem- perature $(24 \pm 1^{\circ}C)$ under lightproof conditions. Afterward, HPP-treated and controlled lycopene and its *cis*-isomers in tomato purée samples were measured [27] by HPLC and IR spectral analysis after 2, 4, 8, and 16 days of storage (Table 2). It was found that 500 and 600 MPa led to the highest reduction of lycopene, while 400 MPa could retain the maximal stability of lycopene [27]. The highest stability of lycopene in tomato purée was found when pressurized at 500 MPa and stored at 4 ± 1 °C in the study of Qui et al. [27] which retained most of the total lycopene content in tomato purée $(6.25 \pm 0.23 \text{ mg}/100 \text{ g})$; see Table 2). It was established that HHP is an alternative preservation method for producing ambient-stable tomato products in terms of lycopene conservation [27].

Prasad et al. [25]. determined that HPE has tremendous potential for use in flavonoid extraction. After 30 minutes of HPE of Litchi (*Litchi chinensis* Sonn.) fruit pericarp (LFP), the extract yield, total phenolic level, 1,1 diphenyl-2-picrylhydrazyl radical scavenging activity (DPPH), and superoxide anion scavenging ability were determined by Prasad et al. [25]. The extraction yield by treatments of 400 MPa HPE for 30 minutes was 30%, while that by conventional extraction (CE, control) was 1.83%. There was no significant difference in the total phenolic content (as mg/g DW [dry weight]**)** among the two extraction methods (HPE and CE). It was found that the DPPH radical scavenging activity obtained by HPE (400 MPa) was the highest (74%), while that of ascorbic acid was the lowest (44%) , when using a 10 mg/mL concentration. Additionally, HPE could provide a more effective alternative than CE, because HPE requires less organic solvents and a shorter extraction time [25]. Table 3 describes the quantification of the individual flavonoids epicatechin (EC), epicatechin gallate (ECG), catechin (C), and procyanidin $B₂$ and total flavonoids from LFP tissues by conventional extrac- tion (CE), ultrasonic extraction (UE), and HPE. Both EC and ECG were identified and quantified as the major flavonoids, while C and procyanidin $B₂$ were identified as the minor compounds [25]. The total flavonoid content detected was 0.65, 0.75, 0.29, and 0.07 mg/g dry weight by HPE at 200 and 400 MPs, UE, and CE, respectively. The HPE increased the flavonoid extraction yield up to 2.6 times in comparison with UE, and up to 10 times compared with CE.

Patras et al. (2009) reported of the effect of thermal and HPP on antioxidant activity and the color sta- bility of tomato and carrot purées. High pressure processed purées had significantly higher antioxidant capacities when compared to thermally treated samples. High pressure treatments at 600 MPa retained more than 93% of ascorbic acid (vitamin C) as compared to thermally processed tomato purées (Table 4; see [21].

Yen and Lin [35] reported that the level of retention of ascorbic acid in guava purée proceeded according to the following decreasing order: $(400 \text{ MPa}$ for $15 \text{ min}) > (88 -$ 90 °C for 24 s) > (600 MPa for 15 min). In the

study given by Patras et al. (2009), ascorbic acid levels were in the order (600 MPa) > (water immersed purées) $> (400 \text{ MPa})$ $> (500 \text{ MPa})$ MPa).

Zhang et al. [37] reported a higher extractability of flavonoids from propolis by HPE. Similar results were reported in the extraction of anthocyanins from grape byproducts (Corrales et al. 2008), and flavones and salidroside from *Rhodiola sachalinensis* using HPE [37].

Prasad et al. [26] indicated that effects of HPE on the extraction yield, total phenolic content, and the antioxidant activity of longan fruit (*Dimpcarpus longan* Lour.) pericarp. The different solvent effects, solvent concentration $(25-100\%$, $v/v)$, solid-to-liquid ratio $(1:25-1:100)$, w/v) were individually determined using these optimum extraction conditions. With utilizing the various pressures of HPP $(200-500 \text{ MPa})$, durations $(2.5-30)$ min), and temperatures $(30-70^{\circ}C)$, the extraction yield, total pheno- lics, and scavenging activities of superoxide anion radical and 1,1-dipheny l-2-picrylhydrazyl (DPPH) radical by HPE were determined and compared with those from a conventional extraction. The HPE pro- vided a higher extraction yield and required a shorter extraction time compared to CE. In addition, the total phenolics and the antioxidant activities of HPE were higher than those produced by CE. Table 4 shows the effect of thermal (TP) and high pressure treatments on anti-radical power, total phenols, total carotenoid content in tomato purées.

Tokusoğlu et al. [31] reported that the total phenolics of table olives increased (2.1–

2.5)-fold after HPP (as mg gallic acid equivalent/100 g). Phenolic hydroxytyrosol in olives increased on aver- age (0.8–2.0)-fold, whereas oleuropein decreased on average (1– 1.2)-fold after HPP (as mg/kg dwt). Antioxidant activity values varied from 17.238–29.344 mmol Fe²⁺/100 g for control samples, and 18.579–32.998 mmol Fe²⁺/100 g for HPP-treated samples. In the HPP application of olives, total mold was reduced 90% at 25°C, and it was reduced 100% at 4°C based on the use of the Rose- Bengal Chloramphenicol Agar (RBCA). Total aerobic-mesofilic bacteria load was reduced 35–76% at 35 ± 2 °C based on the use of plate count agar (PCA). Citrinin load was reduced 64–100% at 35 ± 2 °C. Citrinin contamination (CITcont) at concentrations of 2.5 ppb and less in table olives degraded by 56%, whereas concentrations of 1 ppb CITcont in table olives degraded 100% [31].

Corrales et al. [7] examined the extraction capacity of anthocyanins from grape by-products (Figure 5) assisted by HPP and other techniques. The HPP at 600 MPa showed feasibility and selec- tivity for extraction purposes. After 1 hour of extraction, the total phenolic levels of grape by-product samples subjected to this novel HHP technology was 50% higher than in the control samples [7].

From a nutritional prospective, HPP is an excellent food processing technology that has the potential to retain the bioactive constituents with health properties in fruits, cereals, and other foods. HPP-treated foods retain more of their fresh-like features and can be marketed at a premium over their thermally processed counterparts.

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FIGURE 1 Large-scale HPP equipment: horizontal configuration units, including (a) a 420 liter unit (Courtesy of NC Hyperbaric.), (b) a 350 liter unit (Courtesy of Avure Technologies Inc.), and (c) an illustration depicting the semicontinuous mode (Courtesy of Avure Technologies Inc.), in which carriers enter the pressure vessel area on a conveyor belt from the left. After HPP treatment, the carrier is removed from the vessel and exits to the right on the conveyor belt. For comparison, the vertical configuration unit shown in (d) is used to process oysters (Courtesy of Avure Technologies Inc.)

1

The Antioxidant Indices of HPP-Treated and Thermally Processed Strawberry and Blackberry Purées

Source: Adapted from Patras, A., Brunton, N. P., Pieve, S. D., and Butler, F., Innov. Food Sci. Emerg. Technol., 10, 308-13, 2009b.

Notes: Values are mean ± standar deviation, $n = 3$, mean values in a column with different letters are significantly different at $p < .05$; nd = not detected.

^a Dry weight.

^b Expressed as mg/100g DW pelargonidin-3-glucoside

^c Expressed as mg/100g DW cyanidin-3-glucoside.

2

Total Lycopene Losses in Lycopene Standard (as Percentage) and Total Lycopene Content in Tomato Puree (as mg/100g) as a Function of Storage Time at 4 ± 1 °C, at Six Different HHP Conditions

Source: Adapted from Qiu, W., Jiang, H., Wang, H., and Gao, Y., Food Chem., 97, 516-23, 2006.

FIGURE 3 (a) Actual and (b) schematic diagram of the interior of the HPP unit viewed from the side

3

The Quantification of Individual Flavonoids from Litchi Fruit Pericarp Tissues by Conventional Extraction, Ultrasonic Extraction and High Pressured-Extraction

Flavonoids $(mg/g\,DW)^*$	Extraction Methods			
	CЕ	UE	HPE at 200 MPa	HPE at 400 MPa
Epicatechin	$0.0414 + 0.001$	0.16 ± 0.04	0.32 ± 0.002	0.348 ± 0.06
Epicatechin gallate	0.0121 ± 0.003	0.06 ± 0.01	0.019 ± 0.04	$0.2527 + 0.04$
Catechin	0.0002 ± 0.0	0.0020 ± 0.0005	0.0016 ± 0.001	0.0160 ± 0.07
Procyanidin B_2	0.0175 ± 0.0003	0.0731 ± 0.0011	0.14 ± 0.03	0.1346 ± 0.03
Total flavonoids	0.0712 ± 0.004	0.2951 ± 0.051	0.6516 ± 0.07	0.7513 ± 0.2

Source: Adapted from Prasad, K. N., Yang, B., Zhao, M., Ruenroengklin, N., and Jiang, Y., Journal of Food Process Engineering, 32, 828-43, 2009a.

Notes: Values reported are means of triplicate determinations $(n = 3) \pm SD$.

 $DW^* =$ dry weight; CE = conventional extraction; UE = ultrasonic extraction; HPE = high-pressure extraction.

4

Source: Adapted from Patras, A., Brunton, N., Da Pieve, S., Butler, F., and Downey, G., Innov. Food Sci. Emerg. Technol., 10, 16-22, 2009a.

Notes: Values reported are means of triplicate determinations $(n = 3) \pm SD$; expressed on dry weight basis. *Least significant difference ($p = 5\%$).

FIGURE 5 Anthocyanins in grape by-products. (Adapted Tauscher, B., Innov. Food Sci. Emerg. Technol., 9, 85–91, 2008.) Anthocyanins in grape by-products. (Adapted from Corrales, M., Toepfl, S., Butz, P., Knorr, D., and

