High-pressure Processing of Salads and Ready Meals

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High-pressure processing (HPP) holds the potential for preserving foods by combining elevated pressures (up to 900 MPa or approximately 9000 atmospheres) and moderate temperatures (up to 120°C) over a short period. Though high-pressure processed salads are currently unavailable, HPP has been used as an alternative to heat pasteurization for processing ready meals in the USA and Europe. HPP effects on selected ready meals, salad dressings, dips and sauces are reviewed. Aspects covered include combined pressure-thermal effects on microbial and enzyme inactivation. Pressures up to 600 MPa can inactivate yeasts, moulds and most vegetative bacteria, including pathogens. While HPP leaves small molecules such as flavour compounds and vitamins intact, its influence on colour is product dependent. This varies between a full retention of the fresh colour and colour change similar to thermally processed foods.

1 Introduction

High-pressure processing retains food quality and natural freshness and extends shelf-life. During high-pressure processing (HPP) the food material is subjected to elevated pressures (up to 900 MPa or approximately 9000 atmospheres) with or without addition of heat to achieve microbial inactivation with minimal damage to the food. Physical compression of the product during the process increases the temperature of the product only during the treatment. This unique compression heating effect helps to reduce the severity of thermal effects encountered with conventional processing techniques such as retorting. Other advantages of the technology include uniform pressure application, minimal heat damage to food and potential for altering functional properties of foods. HPP breaks only hydrogen bonds and disrupts hydrophobic and electrostatic interactions. HPP does not affect covalent bonds and has little effect on chemical constituents associated with desirable food qualities such as flavour, colour or nutritional content. The possibility of extending shelf-life without heating

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Table 2.1 World-wide distribution of high-pressure processed salad and ready meal products

Product manufacturers	Category	Product
Avomex Incorporated, USA	Ready meals	Chicken fajita, chipotle chicken and chipotle beef dinners; smoothies
	Sauces and dips	Guacamole and salsa products
Avotec International, USA	Sauces and dips	Salsa and guacamole
Calavo Growers Inc., USA	Dips	Guacamole
Hannah International, USA	Dips	Hummus
Meidi-ya, Japan	Sauces and salad dressings	Salad dressings, fruit sauces
Espuna, Spain	Ready meats	Sliced ham
Hormel, USA	Ready meats	Ham
Perdue, USA	Ready meats	Seasoned chicken breasts
Orchard House, USA	Beverage	Smoothies and orange juice
Leahy Orchards, Canada	Snacks	Applesauce snacks
Winsoms of Walla Walla, USA	Ready meal ingredient	Chopped speciality onions

the food for prolonged periods greatly helps to satisfy consumer demand for fresher and higher quality heat-sensitive foods that are otherwise difficult to process using conventional food preservation methods. Currently there are no commercially available pressure-processed salads, but limited ready meals are commercially available (Table 2.1). In this chapter, the challenges and opportunities for high-pressure processing of ready meals and salads are highlighted.

2 Importance of salads and ready meals

Salads and ready meals comprise a popular and growing segment of the food industry. Sales of pre-cut salad mixes were at $1.94 billion in year 2001 and increased to $2.11 billion in 2003 (Hodge, 2003). In restaurants alone, salads/vegetables comprise 9 per cent of the main menu (Sloan, 2003). Estimated sales of ready meals in Europe alone were $10.4 billion in 2000 (Anon, 2003a). Total growth in the value of ready meals in the global market has increased by 4 per cent in the year 2002. Ready meals are very popular in the USA, the UK and Scandinavian countries. The consumer lifestyle tending towards smaller households and longer working hours has been cited as the main reason for the increase in the consumption of convenient ready meals. In Europe, it is projected that between 2002 and 2007, this segment of the market will record an 18 per cent increase in growth (Anon, 2003b).

Salads have evolved from traditional ones consisting of greens and potatoes to ones with widely varied compositions. Caesar salads, tossed green salads, potato salad and salads with pasta/macaroni are some of the most popular salads in USA (Sloan, 2003). Some of the ingredients in salads include but are not limited to lettuce, diced/cherry tomatoes, spinach, onions, shredded carrots, potatoes, coleslaw, cheese, chicken, tuna and mayonnaise.

Ready meals include any entrée or snack that requires minimal preparation before consumption. These would include dinner entrées with combinations of meat and rice or vegetables and snacks such as carrots. A processor may choose to process certain components of the ready meal using high pressure while other components may be thermally processed.

3 Pressure effects on microorganisms

The effect of high-pressure processing on the degree and mechanism of microbial inactivation has been extensively studied and reviewed (Cheftel, 1992, 1995; Smelt, 1998). Most vegetative cells can be inactivated at relatively low pressures, typically 200–400 MPa (Cheftel, 1995), while bacterial spores are more resistant and need a combination of higher pressures and temperatures (Rovere et al., 1996; Reddy et al., 1999; Balasubramaniam, 2003). High pressure can be used for both pasteurization and sterilization of food products. High-pressure pasteurization involves the application of moderate pressures (between 400 and 600 MPa) at temperatures up to 60°C. Like conventional thermal treatment, high-pressure pasteurization treatment inactivates harmful vegetative bacteria in foods effectively (Cheftel, 1995; Farkas and Hoover, 2000; Raghubeer et al., 2000) with minimal nutrient degradation. The products pasteurized by high pressure require refrigeration during storage and distribution. High-pressure sterilization is essentially a pressure-accelerated thermal process that requires a combination of elevated pressures (up to 800 MPa) and temperatures (up to 120°C) to produce a shelf-stable food free from harmful bacterial spores (Meyer et al., 2000; Balasubramaniam, 2003). At present, a number of food processors are actively investigating the feasibility of introducing high-pressure sterilized products in commercial markets.

3.1 Efficacy of microbial inactivation in HPP processed ready meals

Vegetables and meat are major ingredients in both salads and ready meals. When lettuce, tomato, asparagus, onions, cauliflower and spinach were high-pressure processed between 200 and 400 MPa for 30 min at 5°C, a 2–4 log reduction in mesophilic bacteria, yeast and mould was observed (Arroyo et al., 1999). Significant work has been done to evaluate the effect of high-pressure processing on the microbiological safety of meat products. High pressure has been used not only to reduce spoilage organisms and extend shelf-life, but also to reduce pathogens and spore formers. The physico-chemical properties of the food that contribute to the survival of microorganisms have also been studied.

Survival of microorganisms in food products depends not only on sensitivity of the microorganism to pressure, but also on the protective effect that a product offers during treatment and subsequent storage. Therefore, microbial reductions in media such as buffers cannot be extended to food systems. Hugas et al. (2002) reported that log reductions in meat model systems are lower compared to phosphate buffer. Furthermore, water activity of the food significantly affects survival under high pressure. When vacuum packaged marinated beef (a_w 0.99), cooked ham (a_w 0.98) and dry cured ham (a_w 0.89) were spiked with *Staphylococcus aureus* and lactic acid bacteria and processed at 600 MPa for 6 min at 31°C, the log reductions in the meat were lower at lower water activity. Other factors such as suboptimal pH and use of antimicrobials in food can enhance the effect of high pressure. It is therefore important to consider all these factors while evaluating the effect of high pressure on microbial reductions in food.

Despite the variable effects of food systems, in general, high-pressure treatment seems to be more effective in reducing bacterial numbers in a shorter time compared to thermal processing and it can significantly increase the shelf-life of the product. In tomato puree containing meat balls inoculated with *Bacillus stearothermophilus*, high-pressure processing the sauce mixture preheated to 80 or 90°C at 700 MPa for 30 s using single or double pulse, reduced the background microflora, which mainly consisted of spores, to undetectable levels. A single pulse at the above-mentioned conditions was sufficient to reduce six logs of the *B. stearothermophilus* populations to undetectable levels, while conventional sterilization processes reduced the levels of organism by less than two logs (Krebbers et al., 2003). Chicken breast inoculated with *Clostridium sporogenes* and pressure treated between 400 and 800 MPa for 1–60 min showed up to 3-log reduction in spore populations (Crawford et al., 1996). Hugas et al. (2002) reported that marinated beef loin and dry cured ham processed at 600 MPa for 6 min at 31°C, showed significant reduction in the number of spoilage microflora. Pressure-treated beef loin and cured ham both remained acceptable for 120 days, four times longer than untreated samples.

Pressure effect can be enhanced by combining it with mild to moderate heat, as observed in study by Krebbers et al. (2003). This effect was also demonstrated when ground pork patties inoculated with *Listeria monocytogenes* were pressure treated at 414 MPa for 6 min at a moderate temperature of 50°C (Murano et al., 1999). About a 10-log reduction in the pathogen population was observed by increasing the processing temperature. Furthermore, under refrigerated storage, the shelf-life of the product was increased to 28 days compared to 5 days for the untreated sample. Similarly, minced pork meat inoculated with *Streptococcus faecalis*, *Bacillus subtilis* or *Bacillus stearothermophilus* and treated with pressures between 50 and 400 MPa for 1–60 min between 20 and 80°C, showed synergistic effects of pressure and temperature with higher log reductions observed at 400 MPa pressure at 80°C (Moerman et al., 2001).

Pressure application in food processing can be continuous or pulsed. When mechanically recovered poultry meat (MRPM) was subjected to an alternate low (60 MPa) and high pressure (450 MPa) pulse or continuous high pressure at 450 MPa for 15 min at ambient temperature, no decrease in survival of mesophiles was observed because of pulsing. Approximately 3.2 to 3.8 log reduction in mesophiles was observed with both methods. However, a higher reduction in psychrotrophs of up to 5.3 logs was observed with pulsing (Yuste et al., 2001).

All the above studies suggest that high-pressure processing can be successfully used for meat products to reduce spoilage and prolong shelf-life. It can also be used for obtaining significant reductions in pathogenic microflora. However, as with thermal processing, some studies showed that the elimination of microorganisms is not complete and some portion of the population seems to be pressure resistant. Increasing the pressure levels does not affect this portion of microorganisms (Crawford et al., 1996) and high pressure may have to be combined with other methods such as use of antimicrobials to increase the process lethality.

3.2 Efficacy of microbial inactivation in HPP-processed dips, sauces and salad dressings

Commercially processed mayonnaise and salad dressings may have to undergo some form of processing because of the recent emergence of acid-tolerant pathogens and some spoilage microflora. For such products, high-pressure processing may be considered as an attractive alternative processing method. There are a limited number of studies on survival of microorganisms in HPP salad dressings and sauces. In addition, a few studies discuss the safety of high-pressure processed salad or sauce ingredients such as cheeses. In salad dressings (ranch, French and slaw) inoculated with 10^4 cfu/g of *Lactobacillus fructivorans* and *Zygosachharomyces bailli* and treated for 10 min between 500 and 800 MPa at 25 or 50°C, the spoilage organisms were reduced to undetectable levels at the lowest pressure and temperature used (Neinaber et al., 2001). In three types of cheeses inoculated with *L. monocytogenes* and subjected to pressures of up to 500 MPa for 15 min at room temperature, an approximately 6-log reduction of pathogen was observed (Szczawinski et al., 1997).

4 Pressure effects on enzyme activity

Enzyme activity is an important parameter affecting quality, particularly of cut fruits and vegetables. In whole fruits and vegetables, the enzymes are usually confined to compartments. However, in cut products, this compartmentalization is destroyed and the substrates and enzymes mix freely causing undesirable changes in the products. Of particular importance in fruits and vegetables are pectin methyl esterase (PME) and polygalacturonase (PG), which are involved in cell wall breakdown and thus cause reduction in viscosity and changes in colour and other organoleptic properties. Other enzymes such as peroxidase; and polyphenol oxidases and lipoxygenase affect colour and lipid breakdown, respectively.

The effect of high pressure on enzyme activity seems to be variable. Pressure affects weak bonds such as hydrophobic interactions, which are primarily responsible for maintaining the tertiary structures of proteins. Since enzymes are proteins, it is expected that application of pressure changes the structural conformation and may sometimes lead to loss of activity. However, it has been observed that though there is a partial or complete inactivation of enzymes in some cases, activation of enzymes has been reported in others. It can be concluded that in general, pressure alone, in most vegetables, is insufficient to inactivate enzymes and hence needs to be combined with heat.

4.1 Effect of high pressure on enzyme activity of fruits and vegetables

Most work related to the effect of high-pressure processing on enzyme activity has been done with respect to PE and PG in intact, cut, and pureed vegetables or juices. Fachin et al. (2002) investigated the suitability of high-pressure processing for tomato products. They studied the inactivation kinetics of the crude polygalacturonase (PG) extract under isothermal and isobaric-isothermal conditions. The authors (Fachin et al., 2002) reported that the pressure–temperature ranges that inactivate tomato PG are between 300 and 600 MPa and 5 and 50°C. Stoforos et al. (2002) studied the inactivation kinetics of tomato pectin methylesterase (PME) under a combination of temperature and high pressure. PME inactivation rates increased with increasing processing temperature. While pressure $<$700 MPa did not inactivate the enzyme successfully, at pressures $>$700 MPa higher inactivation was observed. In tomato puree that was preheated to 90°C and high-pressure processed at 700 MPa for 30 s using single or double pulse, pectin methyl esterase and polygalacturonase activity were reduced to undetectable levels (Krebbers et al., 2003). Enzyme inactivation can be best described by a first order kinetic model taking thermal, pressure and their combination effect into consideration. Diced tomatoes processed at 400, 600 and 800 MPa for 1, 3 or 5 min at 25°C or 45°C showed loss of polygalacturonase activity (PG) at pressures above 400 MPa irrespective of temperatures (Shook et al., 2001). However, pectin esterase (PE) activity markedly increased with application of 400 MPa pressure at 45°C. In the same study a loss of lipoxygenase activity was observed with application of pressure $>$600 MPa at all temperatures. Whole cherry tomatoes processed under high pressure showed a similar trend in PE and PG activity (Tangwongchai et al., 2000). In general, tomato polygalacturonase seems to be sensitive to pressure while tomato pectin methyl esterase seems to be insensitive to pressure.

The effect of high pressure on peroxidase activity has also been documented. Among pressure-treated tomato, lettuce, spinach, onion, asparagus and cauliflower (100–400 MPa for 30 min at 5°C), the only vegetable that showed slight browning at higher pressures was cauliflower and this was attributed to incomplete inactivation of peroxidase. The other vegetables did not show a significant change in sensory properties (Arroyo et al., 1999). In green peas processed under high pressure (400–900 MPa) for 5 or 10 min at 20°C, peroxidase activity was significantly reduced because of the pressure treatment. Greatest reduction in enzyme activity (about 88 per cent) was obtained at 900 MPa for 10 min and this reduction, according to the authors (Quaglia et al., 1996), was comparable to blanching.

The effect of high pressure on polyphenoloxidase (PPO) activity was studied by Gomes and Ledward (1996). In general, crude extracts of potatoes and mushrooms showed considerable reduction in polyphenoloxidase activity when treated with pressures ranging between 200 and 800 MPa for 10 min, with the exception of mushroom extract at 400 MPa, which showed a marked increase in PPO activity. However, whole tissues subjected to similar pressures showed considerable browning. Potatoes treated with 800 MPa alone showed acceptable colour but had a cooked appearance.

4.2 Effect of high pressure on enzyme activity in meats

Loss of colour in meat is an important quality aspect affecting its salability. The mechanism of colour loss is an issue of debate. Some researchers attribute it to certain enzyme systems in the meat, while others attribute this change to denaturation of globin proteins at higher pressures. Colour changes typically occur above 150 MPa, resulting in products that resemble cooked meats (Hugas et al., 2002). In fresh beef, formation of metmyoglobin is responsible for loss of red colour and a decrease in perceived quality. Fresh beef processed for up to 2 days with pressures between 80 and 120 MPa for 20 min showed a reduction in formation of this pigment. However, the effect of pressure processing was quickly lost if the time between slaughter and processing increased to more than 7 days (Cheah and Ledward, 1996).

Colour changes are most significant in raw products. In sausages containing various levels of mechanically recovered poultry meat and minced pork meat, and cooked with high pressure (500 MPa for 30 min) at 50, 60, 70, or 75°C, a significant loss of redness and an increase in lightness were observed (Yuste et al., 1999). In products that have already been cooked, the application of high pressure has no additional effect.

Secondary pasteurization is routinely performed for meat products where casings have been removed to destroy any microbes that have been transferred during post-process handling. High pressure treatment of cooked sausages at 500 MPa for 5 or 15 min at 65°C did not significantly affect the colour and therefore high pressure has been suggested as a substitute treatment for pasteurization for this product (Mor-Mur and Yuste, 2003). Lack of colour change due to high-pressure processing has also been observed in cooked ham (Goutefangea et al., 1995).

5 Pressure effects on texture

5.1 Textural changes in pressure treated ready meals

The texture of vegetables is an important quality attribute for commercial sale. The effect of high-pressure processing on texture varies with commodity and applied pressure. For example, processing green peas between 400 and 900 MPa for 5–10 min at 20°C did not significantly affect the texture (Quaglia et al., 1996). On the other hand, for carrot, celery and red and green peppers, a 'dual effect' of pressure was reported, where an initial loss of texture was seen immediately after pressurization, followed by regaining of texture during a hold time. This effect depended on the type of vegetable and duration and level of pressure applied. The immediate loss of texture on application of pressure was described as 'instantaneous pressure softening (IPS)'. For carrot, celery and green pepper, application of 100 MPa with extended hold times up to 60 min resulted in a higher firmness compared to untreated products. However, at higher pressures the firmness decreased. At high pressures celery was the most sensitive while red peppers were least sensitive (Basak and Ramaswamy, 1998).

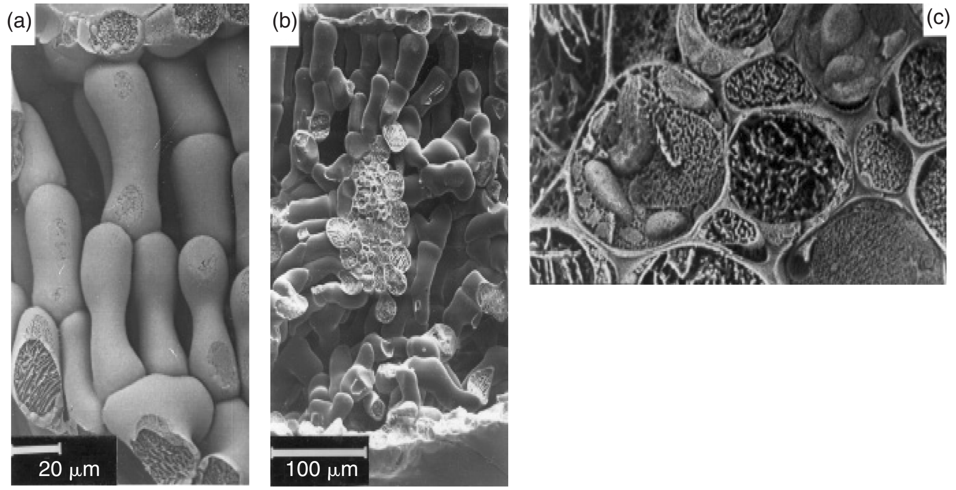

Figure 2.1 Freeze fracture scanning electron micrographs (SEM) of raw spinach leaf before high-pressure processing; (a) parenchyma cells, (b) vascular tissue, (c) magnified vascular tissue with organelles. (Source: Préstamo and Arroyo, 1998 *Journal of Food Science*.)

In tomatoes and lettuce subjected to pressures ranging between 100 and 600 MPa for 10 or 20 min at 10 or 20°C, changes in sensory qualities were observed above pressures of 300 MPa. In tomatoes, loosening of skin was observed, whereas in lettuce browning was observed, though flavour in both vegetables was unaffected (Arroyo et al., 1997). Cherry tomatoes processed under pressures of 200–600 MPa for 20 min at 20°C showed different degrees of texture loss and cell damage. Between 200 and 400 MPa, higher losses of water from cell tissue and a higher degree of cell rupture were observed, which resulted in a product with less firmness compared to those processed between 500 and 600 MPa. Light microscopy of samples revealed that processing tomatoes at lower pressures resulted in entrapment of bubbles, which seemed to have been expelled under high pressures. Samples processed at 500–600 MPa were visually similar to their unprocessed counterparts and hence were considered more acceptable (Tangwongchai et al., 2000).

In spinach leaves, loss of texture was reported after processing at 400 MPa for 30 min at 5°C (Prestamo and Arroyo, 1998). At microscopic level, loss of parenchyma structure, formation of cavities and loss of intercellular space was observed (Figures 2.1 and 2.2). Compared to cauliflower processed under these same conditions, marked changes and degradation were observed in spinach and the researchers (Prestamo and Arroyo, 1998) attributed this to the softer nature of the spinach tissue. It was concluded that spinach is unsuitable for high-pressure processing.

High-pressure processing can lead to reversible or irreversible changes in textural properties of meat products. Similar to colour, changes to the texture of meat seem to depend on the time lag between meat slaughter and processing. In fresh meat where rigor had not set in, pressurization leads to shortened muscles accompanied by severe damage to the muscle structure. However, the processed muscle was found to be tender on cooking (MacFarlane, 1973). Hence, it can be inferred that pressure treatment of pre-rigor muscle can increase tenderization.

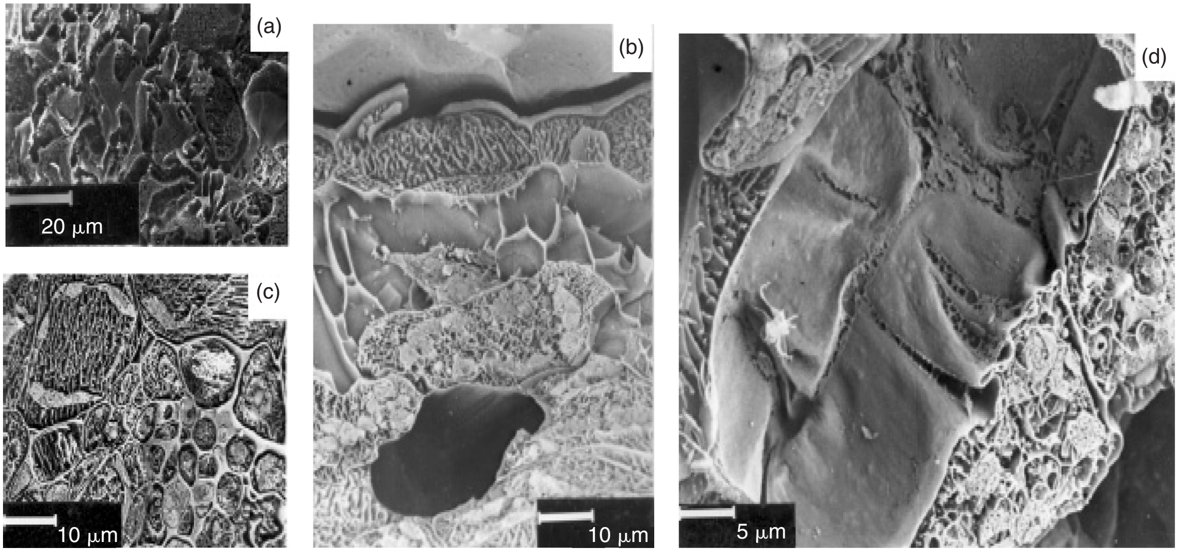

Figure 2.2 Effect of high-pressure processing (400 MPa, for 30 min at 5 °C) on spinach tissues as observed after freeze fracture SEM; (a) collapsed parenchyma cells after HPP, (b) cavity formation, (c) preservation of vascular tissue structure, (d) folding of collapsed membrane after HPP. (Source: Préstamo and Arroyo, 1998 *Journal of Food Science*.)

High-pressure treatment of cooked sausages at 500 MPa for 5 or 15 min at 65°C did not significantly affect the colour. However, high pressure treated sausages were less firm than their heat pasteurized counterparts. A taste test on both products concluded that for attributes that were significantly different for the two products, high pressure processed sausages were preferred over heat pasteurized samples (Mor-Mur and Yuste, 2003).

5.2 Textural changes in pressure-treated dips, sauces and salad dressings

French, ranch and slaw dressings treated with high pressures between 500 and 800 MPa for 10 min at 25 or 50°C differed significantly in their viscoelastic behaviour, which was attributed to compositional differences. French dressing containing egg yolk as the stabilizer was found to be least stable, while ranch dressing containing proteins and xanthan gum was found to be most stable (Neinaber et al., 2001). However, it was concluded that pressure treatment does not significantly change the rheological properties of salad dressings. Further studies on acidified emulsions containing soy lecithin, polysorbate 60 and whey protein isolate as emulsifiers and xanthan gum as stabilizer confirmed that pressure does not significantly affect the rheology of emulsions. Differences in the flow behaviour, viscoelasticity, particle size and stability of the emulsions were attributed to the lipid content and emulsifier used. It was observed that soy lecithin-based emulsions were unstable from the point they were prepared and this instability was increased with the application of high pressure. Emulsions containing whey protein and polysorbate 60 were stable to pressure. Whenever xanthan gum was added as stabilizer to polysorbate 60 emulsions, the stability of the emulsion was further enhanced by the application of pressure (Arora et al., 2003).

Process temperature, pressure holding time and nature of the surfactant seem to be the important factors affecting the physical stability of emulsion systems. In model emulsion at neutral pH containing sodium caseinate (50 g/kg) and peanut oil (300 g/kg), application of 450 MPa of pressure for 30 min at room temperature did not affect particle size distribution or viscosity. However, when sodium caseinate was substituted with β-lactoglobulin, application of pressure increased emulsion viscosity and promoted gelation at 40°C (Dumay et al., 1996). Dickinson and James (1998) further observed that β-lactoglobulin-stabilized emulsions showed increases in average droplet diameter when they were subjected to 800 MPa pressure for up to 60 min.

6 Pressure effects on nutrients

It has been generally known that high pressure has very little effect on low molecular weight compounds such as flavour compounds, vitamins and pigments compared to thermal processes. This effect is particularly important in salads, as most vegetables are rich sources of antioxidant compounds, pigments and vitamins. Butz et al. (2002) studied the effect of 600 MPa pressure in combination with elevated temperatures on the pigment and vitamin content of three vegetables. Broccoli treated with pressure of 600 MPa for 40 min at 75°C showed no loss of chlorophyll a or b compared to the untreated samples. Similarly, tomatoes treated at a combination of 600 MPa pressure and 25°C temperature for 60 min did not show a change in either lycopene or carotenoid content and were similar to the heat-treated sample (95°C, 60 min). In addition, the loss in antioxidant capacity of a water-soluble portion of pressure treated carrot and tomato (500 and 800 MPa for 5 min) was very little compared to the untreated samples. High retention of ascorbic acid (82 per cent) was observed in green peas treated with 900 MPa pressure for 5–10 min at 20°C (Quaglia et al., 1996).

Model multivitamin systems containing varied levels of water-soluble vitamins such as ascorbic acid, thiamin and vitamin B6 (pyridoxal) and food systems containing naturally occurring levels of vitamin C were subjected to pressures ranging between 200 and 600 MPa for 30 min to determine the effect on vitamin retention. In the model systems observed, ascorbic losses were close to 12 per cent while in food material, these losses were insignificant. Compared to conventional sterilization processes, high-pressure treatments retained the vitamins better. Thiamin and pyridoxal in the model system were unaffected by high-pressure processing (Sancho et al., 1999). These findings confirm the fact that high pressure has minimal effect on nutrients in foods.

7 Conclusions

High-pressure processing of salads and ready meals is an emerging niche market that provides unique opportunities and challenges to the food industry. HPP lends itself for processing a variety of novel, convenient, minimally processed ready meals with long shelf-life, fresh-like attributes and natural colours. Identification of commercially viable applications is the likely challenge faced by the food processor. Furthermore, combination processes are likely to reduce the severity of process requirements.

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Reference to commercial product or trade names is made with the understanding that no endorsement or discrimination by The Ohio State University is implied.

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