GUACAMOLE AND AVOCADO PRODUCTS

INTRODUCTION

High Pressure Processing (HPP) is a non-thermal food processing technology that allows preserving nutrients, fresh taste and appearance of avocado and guacamole, with a longer and safer shelf-life. On this sector of vegetable-derived products, the pressure used is generally 600 MPa (6000 bar / 87000 psi) applied typically over 3 minutes at refrigerated temperature.

Regarding to a physico-chemical effect on food, the HPP technology is softer than a thermal treatment: it does not break or create covalent bonds, and does not create new compounds by molecule degradation, such as in a conventional thermal process. However, HPP is able to break, or create, weak bonds (hydrophobic and electrostatic interactions), only present on macromolecules (Cheftel, 1992). It allows microorganism inactivation without modifying the food nutritional quality and without significantly altering enzymatic activities. To minimize the residual microorganism growth, the enzymatic reactions and changes in sensory attributes, avocado products and guacamole must be stored at chilled temperature.

There are many reasons that make the HPP technology beneficial:

- Safer food products with a longer shelf-life are produced, thanks to the inactivation of vegetative microorganisms (bacteria, yeasts and molds).
- Sensory food quality is maintained, keeping the fresh-like taste of home-made product.
- Nutritional quality is preserved.
- Suitable for “clean label” and organic food products.

FOOD SAFETY AND LONGER SHELF-LIFE

Consumers have a keen interest in preservative-free food products. HPP technology is an effective method to reduce the microbiota without adversely affecting the sensory attributes and decreases the dependence on additives (Waite et al., 2009).

Guacamole is a minimally processed avocado product, providing conditions that are ideal for the growth of spoilage bacteria. Since these food products are ready to eat, microbial growth must be strictly controlled.

Thermal treatments and chemical preservatives are the traditional technologies used in preservation of these products. These treatments reduce microbial load but lead to an unacceptable sensory quality such as the generation of bitter off-flavors (Jacobo-Velázquez and Hernández-Brenes, 2010; López-Malo, et al., 1999).

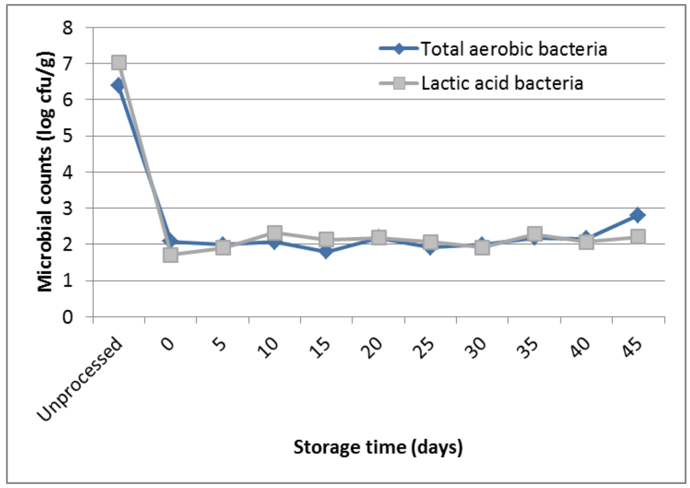

Figure 1. *Total aerobic and lactic acid bacteria in HPP guacamole (600 MPa, 3 min) stored at 4 ºC (Jacobo-Velázquez and Hernández-Brenes, 2010).*

Shelf- life increase

Guacamole spoils within the first 5 days, even when it is stored at 5 ºC (41 ºF) (López-Malo *et al.*, 1999). Lactic acid bacteria, molds and yeasts are the main groups of microorganisms responsible of the quick spoilage of these product, thus their control by HPP should be a priority.

At 600 MPa (87,000 psi) held at for 3 min, significant instantaneous reduction in microbial load of guacamole (pH 6.35) was achieved without any previous acidification (Jacobo-Velázquez and Hernández-Brenes, 2010). Levels of mesophilic aerobic and lactic acid bacteria, two of the spoilage indicators, remained constant during the first 40 days of storage at 4 ºC (39.2 ºF) in HPP guacamole, around 2 log cfu/g **(Figure 1).**

Molds and yeasts were well controlled (<10 cfu/g) in HPP avocado puree and guacamole (689 MPa / 100,000 psi; 5 min; pH 4.3) for 30 days of storage at 5, 15 and 25 ºC (41, 59 and 77 ºF, respectively) (Palou *et al*., 2000).

Challenge tests

Besides controlling spoilage microorganisms, high pressure processing has demonstrated high inactivation levels on pathogenic bacteria. Guacamole was reported as a possible vehicle in 136 outbreaks along United States from 1973 to 2008 (Kendall et al., 2013).

High hydrostatic pressure (600 MPa / 87,000 psi, 3 min) inactivated efficiently *L. monocytogenes* (> 7 log cycles), *Salmonella* spp. (> 5 log cycles) and *E. coli* (> 6 log cycles) in avocado pulp and guacamole (**Figure 2**).

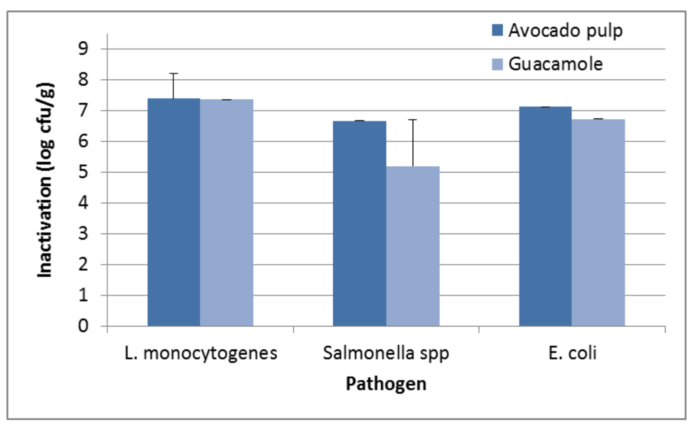

Figure 2. *HPP Inactivation of* Listeria monocytogenes, Salmonella spp., and Escherichia coli *in guacamole (600 MPa, 3 min) (Hiperbaric, not published)*

Influence of processing parameters and product characteristics

Microbial inactivation levels depend on the pressure and holding time as well factors related to food product such as water activity (a_w) or pH, and environmental factors such as temperature.

The lower water activity (a_w) (or higher Brix degrees) the lower effectiveness high pressure is (Goh *et al.*, 2007); therefore, the technology is very effective on guacamole and avocado products, since a_w is higher than 0.90.

The pH of a product is also a key factor to consider, working in synergy with HPP: the lower pH a product has, the more effective microbial inactivation by HPP is reached.

Neetoo and Chen (2012) investigated the inactivation of *Salmonella* spp. in guacamole. Peppers, an ingredient of guacamole, were inoculated by five strains cocktail of this pathogen. The storage time (0 or 24h, room temperature) of the condiments prior to HPP (500 MPa / 72,520 psi, 2 min) as well as pH (4.3 - 5.3) and the type of acidulant (vinegar and lemon juice) affected the inactivation levels of *Salmonella.*

Total inactivation (i.e. no detection (<0.7 log cfu/g)) was only reached at pH 4.3 (using vinegar or lemon juice), or when vinegar was used as acidulant, and after 24h of storage time at room temperature prior to HPP (**Figure 3**).

HPP does not inactivate bacterial spores (but mold spores are inactivated). Regarding HACCP, HPP cannot be used to control *Clostridium botulinum* or any other bacterial spore. Products with a pH higher than 4.6 are at risk for spore germination and therefore they must be kept refrigerated for the entire life of the product. We recommend acidifying HPP products below pH 4.6 whenever possible to prevent spore germination.

SENSORIAL QUALITY AND ENZYMATIC INACTIVATION

HPP is a suitable technology for controlling microbial spoilage during long periods; however, when food products are microbiologically stable, the end of their shelf life is generally limited by changes in their sensory and nutritional properties, which are mainly caused by deteriorative biochemical/enzymatic reactions.

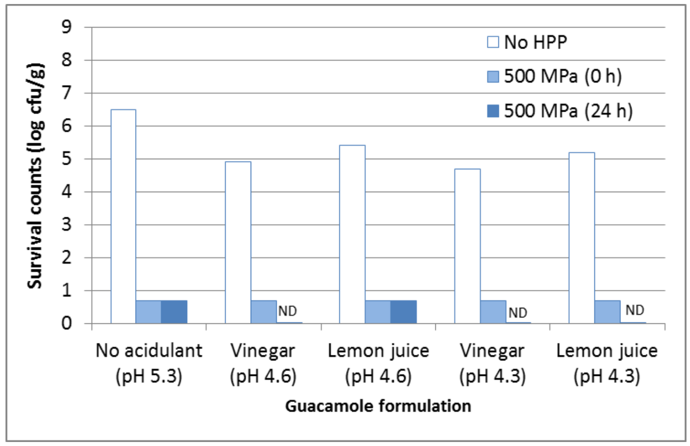

Figure 3. *Survival of Salmonella spp.in different acidulated HPP guacamole and different storage times (0h and 24h) prior pressurization (500 MPa, 2 min).*ND: not detectable. (Neetoo and Chen, 2012).*

These reactions are catalyzed by endogenous enzymes such as polyphenol oxidase (PPO) and lipoxygenase (LOX). These enzymes affect directly the components responsible of color, flavor and texture of avocado products. When pulp is removed from the intact fruit for processing, the tissues undergo partial disruption of cellular organelles releasing enzymes and their substrates causing deterioration reactions.

Depending on type of enzyme, physicochemical characteristics of the food product (pH, a_w) and the conditions of HPP process (pressure and holding time), enzymes could be partly or moderately inactivated. Storage under refrigeration (4 ºC / 39.2 ºF) minimizes detrimental quality modifications induced by residual activities (Jacobo-Velázquez and Hernández-Brenes, 2011).

Color

Enzymatic browning is the main change in appearance of avocado products and it is catalyzed by PPO. Enzymatic browning also depends on presence of O_2 in contact of food product. Vacuum and modified atmosphere packaging allow controlling O_2, slowing down browning. Both packaging technologies can be used jointly to high pressure technology.

HPP at 600 MPa (87,000 psi) for 3 min reduced immediately around 50% PPO activity on avocado puree (pH 6.35) (Jacobo-Velázquez and Hernández-Brenes, 2010). Refrigeration is essential for controlling PPO activity in guacamole and avocado products before and after HPP processing in order to slow down the enzymatic reaction. Although PPO activity reached a maximum peak at day 10, the rest of storage time, residual PPO activity remained at initial values, around 50% (Jacobo-Velázquez and Hernández-Brenes, 2010; **Figure 4**).

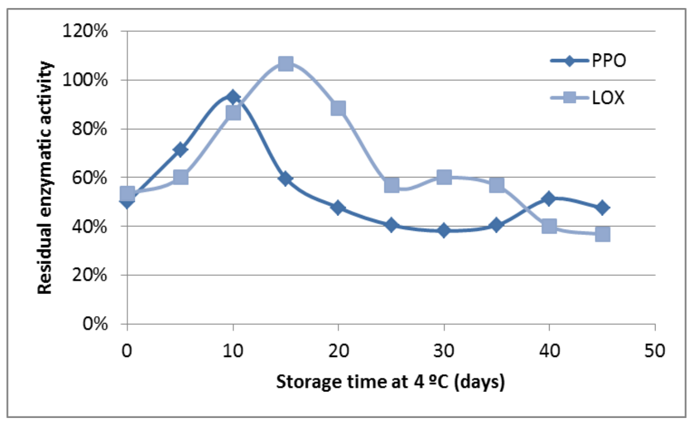

Figure 4. *Residual enzymatic activity of PPO and LOX on pressurized (600 MPa, 3 min) avocado puree at refrigerated storage (Jacobo-Velázquez and Hernández-Brenes, 2010).*

Despite of PPO is partly deactivate, several studies have shown that pressurized avocado paste does not present significant color modification compared to freshly prepared puree by using instrumental color measurements and trained sensory panelists (López-Malo, et al., 1999; Palou et al., 2000; Jacobo-Velázquez and Hernández-Brenes, 2010).

Factors affecting PPO inactivation

PPO inactivation does not depend only on HPP conditions, but pH of the product plays a relevant role on enzyme stability. López-Malo, *et al*. (1999) studied the effect of pH on inactivation of PPO in avocado puree. At low pH values and higher pressures for long holding time, the efficacy of

HPP on inactivating PPO is higher; residual activity of PPO was below 30% (**Figure 5**).

Storage temperature plays a relevant role on maintaining initial product color, decreasing rate of enzymatic browning and other degradation reactions over natural pigments. According to Palou et al. (2000), only HPP guacamole stored at 5 ºC (41 ºF) retained green hues for more than 10 days; higher storage temperatures (15 and 25 ºC / 59 and 77 ºF) led to changes in color.

Flavor

Activities of endogenous enzymes catalyze biochemical reactions which lead to undesirable sensory quality traits. Besides catalyzing product browning, PPO activity has also associated with the formation of undesirable flavors (Weemaes *et al*,. 1998). HPP is able to reduce partly PPO activity, slowing down enzymatic browning and the collateral undesirable effects, as it has been previously shown.

In addition to PPO, LOX enzyme is contained in the pulp of avocado. This enzyme degrades lipids and related compounds such as carotenoids, generating low molecular substances responsible of off-flavors and rancid volatile compounds (Jacobo-Velázquez and Hernández-Brenes, 2010, 2011).

LOX is less stable to pressure than PPO. At commercial HPP conditions (600 MPa / 87,000 psi, 3 min), residual LOX activity in avocado puree (pH 6.35) is reduced to 45% immediately after HPP compared to the control (Jacobo-Velázquez and Hernández-Brenes, 2010; Jacobo-Velázquez *et al.* 2012).

In HPP avocado puree, residual LOX activity during refrigeration storage (4 ºC, 39.2 ºF) shows a similar behavior than PPO (**Figure 4)**. Residual activity reaches a maximum peak at day 10, after that, LOX activity decreases below 60% at the end of storage at day 45 (Jacobo-Velázquez and Hernández-Brenes, 2010). Residual LOX activity did not affect consumers' acceptability of HPP guacamole and rancid flavor was not developed (Jacobo-Velázquez and Hernández-Brenes, 2011).

Overall sensory evaluation

Sensory evaluation of HPP guacamole using trained panelists showed no significant differences in the different sensory attributes compared to freshly-made products. The results from Palou et al. (2000) showed HPP technology preserve sensory quality of avocado products, a quite sensitive food product.

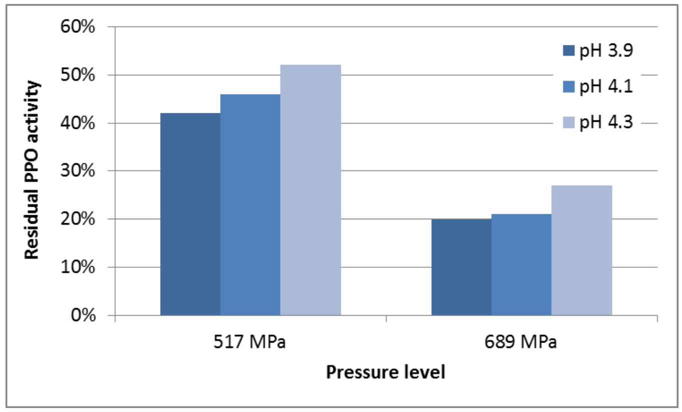

Figure 5. *Residual PPO activity in HPP avocado puree at different pressure levels (for 20 min) and different puree pH values (López-Malo, et al., 1999).*

Enzymatic browning is a shelf-life limiting factor of guacamole. Despite of HPP technology can not completely deactivate PPO enzyme. Refrigeration and packaging were a significant factor when a trained panel did not consider the changes in color of HPP guacamole as a critical descriptor (Jacobo-Velázquez and Hernández-Brenes, 2011).

NUTRIENTS RETENTION

It is well known that HPP technology has a minimal effect on low molecular-weight compounds after pressurization, contributing the conservation of sensitive molecules such as vitamins, pigments, antioxidants and other substances with relevant biological activity, sensitive to thermal treatments (Jacobo-Velázquez and Hernández-Brenes, 2012).

Vegetables are one of the important sources of nutrients and bioactive molecules in human diet. Lutein, vitamins C and E, and persenone A and B are biological active substances present in avocado pulp, protecting cells and tissues from free radicals, inflammation and carcinogenesis processes (Kim et al., 2000).

Other antioxidants and bioactive compounds present in avocados are phenolics, monounsaturated fatty acids, lycopene and other carotenoids such as xanthins. The presence of these compounds is desired from the creation of guacamole until before its consumption.

Carotenoids

These pigments are relevant in human physiology since they have antioxidant and vitamin A activity. The concentration of total carotenoids measured in HPP (600 MPa / 87,000 psi, 3 min) avocado puree was 2.3 times higher than in unprocessed puree (Jacobo-Velázquez *et al.,* 2012). High pressure processing increased the measurable content of the most of carotenoids in avocado puree immediately after HPP compared to fresh avocado puree (Relative concentration $C/C_0 = 1$) (**Figure 6)**

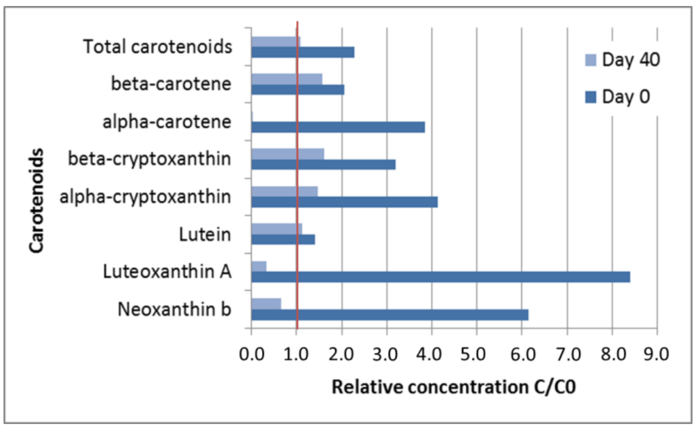

Figure 6. *Relative concentration of total and individual carotenoids in HPP (600 MPa, 3 min) avocado puree (where fresh-prepared avocado puree composition has a relative concentration of 1.0 –red line-) at day 0 and day 40 at refrigerated storage (Jacobo-Velázquez et al., 2012)*

Carotenoids are bound to proteins in the plant cell membrane surface. Since high pressure induces changes in protein structure, the complex protein-carotenoids may be disassembled, facilitating the extraction of these compounds from food matrices (Sánchez-Moreno *et al.,* 2003). Researchers stated the differences for individual carotenoid can be attributed to the different degree of association carotenoid-binding protein.

Increase in the extractability of carotenoids in HPP guacamole during refrigerated storage may lead to a positive impact on the bioavailability of these compounds; although it has not been demonstrated from a pharmacokinetic standpoint.

Although during refrigerated storage (day 40), the total content of carotenoids decreased, it was still higher than the content in unprocessed puree (**Figure 6**). Decrease in carotenoids content during storage may be related to degradation by residual LOX (Jacobo-Velázquez *et al.*, 2012).

Antioxidant activity

Additionally to determine how processing technologies modify the concentration of the antioxidant species in food products, it is also important to measure the effects of processing on the overall antioxidant activity of the foods, since it is related to the biological beneficial effects such as preventing oxidative damage to important biomolecules such as DNA, proteins and lipids.

HPP immediately decreases about 25% of the total antioxidant activity (determined by ORAC method) in avocado paste (Jacobo-Velázquez *et al.*, 2012). However, from day 5, avocado paste recovered its initial antioxidant activity (Control values), reaching a maximum peak at day 10. After 15 days at refrigerated storage (4 ºC, 39.2 ºC), antioxidant activity went back to control values (**Figure 7**).

According to Jacobo-Velázquez *et al.* (2012), the dynamics of antioxidant activity during refrigerated storage is a complex process: initial reduction due to enzymatic degradation of vitamin C and oxidation of polyphenols by residual PPO activity. After initial decrease, antioxidant activity would increase since some antioxidants such as carotenoids and phenolic compounds start to release from tissues and cells affected by pressurization, reaching a maximum value at day 15 (**Figure 7**).

Stabilization of total antioxidant activity might be due to a balance between the releasing of compounds from the interior of plant cells and degradation of antioxidants caused by residual PPO and LOX enzymes.

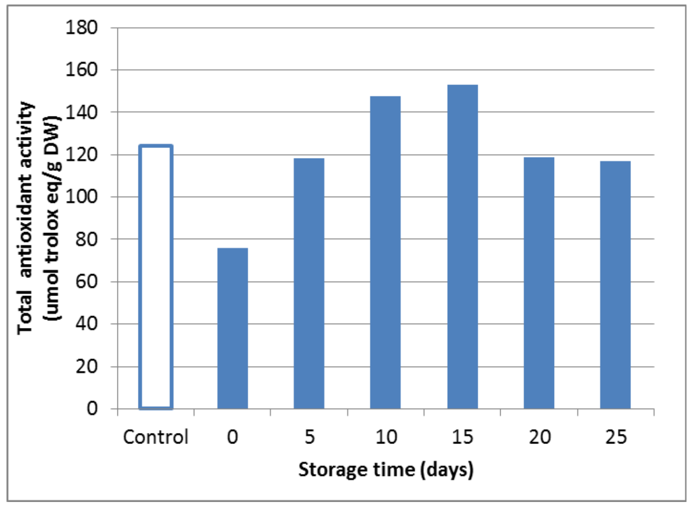

Figure 7. *Total antioxidant activity (µmol Trolox eq/ g dry weight) throughout refrigerated storage of HPP (blue bars) avocado paste (600 MPa, 3 min) compared to control (white bar) (Jacobo-Velázquez et al., 2012).*

CONCLUSIONS

Guacamole and avocado products have successfully been processed by high hydrostatic pressure around the globe since 1996. HPP technology increases the shelf-life of the avocado products, maintaining, and in some cases enhancing, their nutritional quality, while the products keep the freshness and sensory attributes of home-made product, avoiding the use of additives.

High Pressure Processing technology started its industrial evolution hand in hand with the avocado processing industry. You can see how avocado processing industry is applying HPP technology on its products:

http://www.hiperbaric.com/en/avocado-products

You can get more information about Hiperbaric and hi pressure processing of foods on the next links:

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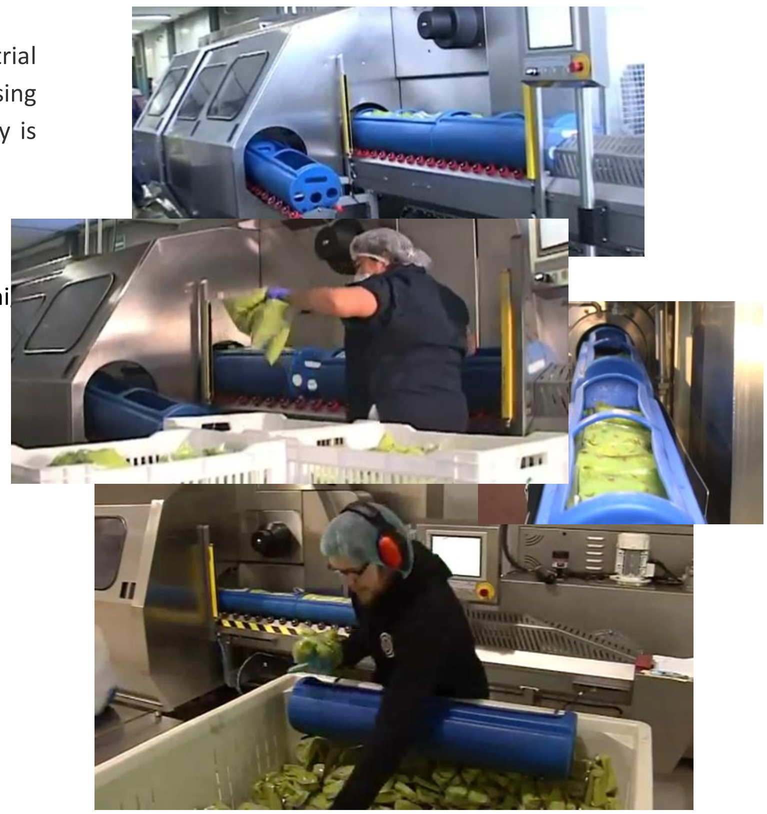

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