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Effects of thermal and high pressure treatments in color and chemical attributes of an oil-based spinach sauce

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ABSTRACT

We studied the effects of high pressure processing (HPP) on physico-chemical characteristics of an oil-based spinach sauce model system. Color, chlorophylls, ascorbic acid, polyphenoloxidase activity and lipid oxidation (measured as % oleic acid, peroxide value, and *p*-anisidine content) were evaluated. Multivariate analysis of variance (MANOVA) and principal component analysis (PCA) were used to elucidate the contribution of pressure and time on the observed changes. Both pressure and time had a significant effect, with pressure be responsible for most of lipid oxidation. Color attributes were preserved or even improved after HPP, with 500 MPa for 10 min the treatment with the best results. Higher recovery of ascorbic acid content, as well as chlorophylls *a* and *b* was achieved; HPP was not so effective in triggering lipid oxidation. A storage study for 21 days at 4 °C was performed in order to assess the long-term effects of thermal and HPP. During storage, lipid oxidation was drastically inhibited in HPP-treated samples. The degradation of PPO via high pressure was found to be reversible, since partial activity was recovered after 7 days. These preliminary results demonstrate the reliability of HPP as an alternative to thermal treatment to preserve physico-chemical characteristics of oil-based vegetable sauces.

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1. Introduction

Vegetable sauces are characterized by pH and activity water values which allow their marketability for a short period of time, in refrigerated conditions, or for a longer time if pasteurization or sterilization technologies are employed. Conventional thermal processes have been used to inactivate bacteria and enzyme activity in highly perishable vegetables, showing high efficiency in the inactivation of spoilage and pathogenic microorganisms. At the same time, thermal treatments can affect the concentration of antioxidants, vitamins, carotenoids and flavonoids, as well as the organoleptic properties of vegetables (Da Cruz, Fonseca Faria, Isay Saad, André Bolini, & Cristianini, 2010). Many semi-preserved foods, including soft cheeses, sliced sausages, pasteurized milk, and vegetable sauces that are subjected to thermal treatment, are unable to guarantee commercial sterility, and consequently must be stored under refrigerated conditions for a limited time (Baiano, Tamagnone, Marchitelli, & Nobile, 2005).

On the other hand, nonthermal processing technologies for food preservation and safety are gaining widespread acceptance throughout the food industry. An example is high hydrostatic pressure processing (HPP), a technology which can be applied alone or together with other preservation methods as additional hurdles (Barrera, Blenkinsop, & Warriner, 2012; Jacobo-Velázquez & Hernández-Brenes, 2012). Food treated in this way has been shown to retain its original freshness, flavor and taste, allowing most foods to be preserved with minimal effects on appearance and/or nutritional value (Balasubramaniam, Farkas, & Turek, 2008).

Recently, attention has been paid to the effects of HPP on the color quality of green vegetables (Clariana, Valverde, Wijngaard, Mullen, & Marcos, 2011; Oey, Lille, Van Loey, & Hendrickx, 2008). HPP could increase the intensity of green characteristics, as a consequence of cell disruption and subsequent leakage of

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pigments. At the same time, high pressure produces reversible and/or irreversible changes in the protein structure of plant enzymes. Peroxidase (POD) and polyphenoloxidase (PPO), both responsible for color loss in fruits and vegetables (Yamauchi & Watada, 1991), are resistant to pressures of 600–700 MPa and 25 °C. PPO activity after high pressure treatments depends on the studied fruit or vegetable (Lopez-Malo, Palou, Barbosa-Canovas, Welti-Chanes, & Swanson, 1998; Terefe, Buckow, & Versteeg, 2014), but is generally lower than POD (Terefe et al., 2014).

All of these results indicate that HPP can be potentially useful for retaining quality characteristics of vegetables. However, few studies have been performed to investigate whether the post-effects of HPP on chlorophyll degradation and lipid oxidation. Meanwhile, it has been reported that HPP helps to retain antioxidant activity of individual fruits juices (Cao et al., 2012), but there are contradictory results regarding retention of ascorbic acid, antioxidant and polyphenols contents in foods processed by high hydrostatic pressure against thermally processed samples (Keenan et al., 2010).

In this work we evaluate the effects of high pressure processing on physico-chemical characteristics of a vegetable sauce model system consisting in an oil-based spinach sauce. Spinach (*Spinacia oleracea*) provides not only texture and color to food preparations, but it is also a good source of vitamin C, vitamin A, dietary fiber, and minerals, especially iron (Toledo, Ueda, Imahori, & Ayaki, 2003). Among other qualities, we evaluated color, ascorbic acid content, PPO residual activity and chlorophylls content, as well as lipid degradation (free fatty acids, peroxides and *p*-anisidine) in HPP treated samples and compared those results with spinach sauce submitted to a traditional thermal treatment. Multivariate analysis of variance (MANOVA) was used to study the effect of pressure and time on chemical and color parameters. To visualize the similarities and differences in overall impact of the different processes on spinach sauce quality, all analyzed parameters were brought together in a principal component analysis (PCA). We chose the treatments which showed the best results in color and chemical attributes to perform a 21 d storage which simulated commercial storage conditions.

2. Materials and methods

2.1. Sample preparation

All ingredients were purchased in a local market (Pullman, WA, USA). Spinach sauce was prepared as follows: 72 g/100 g fresh spinach, 25 g/100 g commercial extra virgin olive oil, 2 g/100 g citric acid and 1 g/100 g salt were combined and mixed in an Osterizer™ blender, then the physicochemical properties of the fresh sauce were analyzed. Samples (100 g) were vacuum sealed inside flexible 3 mil, 75 μm thickness, polypropylene pouches 15 cm × 24 cm × 4 cm (Ultravac Solutions, Kansas City, MO, USA) for thermal and high-pressure processing, to avoid any contact between the pressurization fluid and the samples. PPO assays, pH and color characterization were performed immediately after processing; an aliquot of each sample was in an upright freezer at −45 °C, before vitamin C, lipid oxidation and chlorophylls analyses were performed.

2.2. High pressure processing

The pouches were placed inside the cylindrical chamber vessel (0.1 m internal diameter, 0.25 m internal height) and processed with a high hydrostatic pressure unit (Engineered Pressure Systems, Inc., Andover, MA, USA). The unit was operated with an electrohydraulic intensifier pump (Hochdruck- Systeme GmbH, AP 10-0670-1116, Sigless, Austria). Come up time was 0.8 min and the process conditions were 400, 500, and 650 MPa for 5 and 10 min at 20 °C (indicated as 400-5, 500-5, 650-5 and 400-10, 500-10 and 650-10 in the rest of the paper). The pressurization fluid was 5% Mobil Hydrasol 78 water solution. Depressurization time was less than 10 s, which was not included in the process time. Three samples per cycle were treated. The pressurization samples were cooled immediately in a cold water bath to avoid further reaction.

2.3. Thermal treatment

Pouch samples were subjected to thermal heating (TT in the rest of the paper) by immersing in a water bath (Memmert GmbH, WB22, Schwabach, Germany) for 30–45 s at which time they had achieved a core temperature of 70 °C. They were held at this temperature for 10 min. The temperature of the sauce during the heat process was monitored using K-type thermocouples connected to a datalogger (Jenco, Model 7000 APL) inserted through a septum glued onto the outer surface of the pouches. Then the samples were taken out and immediately immersed in ice water in order to preserve the residual activity of the enzymes.

2.4. pH and color

The pH was determined by direct immersion of the electrode with a potentiometer (Orion Research Inc., Boston, Massachusetts, USA).

Color determination was carried out using a CR-200 spectrophotometer (Minolta Camera Co., Osaka, Japan) equipped with a standard illuminant D65. The instrument was calibrated using a white color tile standard. L^* (lightness), a^* (redness), b^* (yellowness), C^* (chroma, 0 at the center of the color sphere), and H° (hue angle, red = 0°, yellow = 90°, green = 180°, blue = 270°) were quantified on each sample using a 10° position of the standard observer (CIE, Paris, France, 1978). Ten measurements were conducted on random points on at least 3 samples.

The net color difference (ΔE^*) was determined using L^*, a^* and b^* values, comparing them with the value of an unprocessed sample:

$$\Delta E^* = \sqrt{(\Delta L^*)^2 + (\Delta a^*)^2 + (\Delta b^*)^2} \quad (1)$$

Hue angle (H°) was determined using the following relationship:

$$H^\circ = \tan^{-1}\left(\frac{b^*}{a^*}\right) \quad (2)$$

and the chroma or saturation index (C^*) was evaluated using the equation:

$$C^* = \sqrt{(a^*)^2 + (b^*)^2} \quad (3)$$

2.5. Extraction of fat fraction and lipid oxidation analyses

A sample (10 g) was placed in a flask with 50 mL of n-hexane and mixed using a magnetic stir for 5 min. The organic fraction was separated from the spinach leaves by vacuum filtration with a Whatman No. 41 paper filter. Then the filtered organic fraction was recovered and filtered again on anhydrous sodium sulfate to remove water traces. After filtration, the organic solvent was evaporated using a Rotavapor (model Buchi 461). The extracted oil was subjected to the following analyses: