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Effect of microwave treatment on enzyme inactivation and quality change of defatted avocado puree during storage

Linyan Zhou [a,b], Chia Ying Tey [b], Gokhan Bingol [b,*], Jinfeng Bi [a]

[a] Institute of Food Science and Technology, Chinese Academy of Agricultural Sciences, Ministry of Agriculture, Beijing 100193, China
[b] Chemical and Materials Engineering Department, University of Auckland, 20 Symonds St, Auckland 1142, New Zealand

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ABSTRACT

We evaluated the effect of different power densities, 11.0, 7.7 and 4.4 W/g, of microwave (MW) treatment on inactivation of polyphenol oxidase (PPO) and pectin methylesterase (PME), and on changes in chlorophyll and total phenolic content of defatted avocado puree (DAP). Changes in sensory quality, such as color and rheology, during 4 weeks storage at 4 °C were also investigated. The temperature of DAP rose to 70 °C in 37 and 122 s at 11 and 4.4 W/g MW, respectively. It was observed that over 80% PPO could be inactivated in 80 s at 11.0 W/g. During storage, the residual PPO activity of untreated samples continuously increased to 250% whereas for MW treated samples the residual PPO activity remained constant at around 20%; however, no PME activity was detected. At the end of storage, for samples treated at 11 W/g for 80 s total phenolic content increased by 29.41% and negligible losses in chlorophyll *a* and *b* content occurred whereas for control samples the decrease in chlorophyll *a* and *b* was 38.2% and 37.1%, respectively. Although not significant ($p > 0.05$), the a* value of MW treated samples increased whereas the L* value decreased. MW treatment did not alter the pseudoplastic flow behavior and particle size distribution of DAP, while the apparent viscosity was slightly higher than that of untreated DAP at lower shear rates (1–10 s^{-1}).

Industrial relevance: The defatted avocado puree (DAP) can be used in cosmetics industry as an ingredient and can also be used in human consumption as it helps to reduce body weight and total hepatic fat. However, due to polyphenol oxidase (PPO) present in DAP, browning can take place and therefore PPO must be inactivated. We have found that microwave treatment could considerably lower the activity of PPO and aids in preserving the sensory and nutritional quality during storage at 4 °C.

1. Introduction

Avocado (*Persea americana*) is an important commercial tropical fruit. Worldwide consumer demand has been increasing for avocado and its related products, since it is recognized as a good source of unsaturated fatty acids, fiber, vitamins B and E, and other nutrients (Gomez Lopez, 1998). The oil content of avocado could reach as high as 15–30% depending on the variety (Werman & Neeman, 1987), which contains substantial amount of health-beneficial compounds, such as antioxidants, vitamins and phystosterols (Bae & Lee, 2008). Aqueous extraction and expeller processing are the most common methods used for production of avocado oil; however, avocado oil obtained with an alternative technique, microwave drying and subsequent cold pressing, employed by Santana, dos Reis, Torres, Cabral, and Freitas (2015) exhibited six-fold higher oxidative stability compared with ethanol-extracted oil. After extraction, the remaining defatted avocado puree (DAP) could be a good source of protein and carbohydrates (Bi, Hemar, Balaban, & Liao, 2015). However, when avocado is processed into paste and/or puree, the tissue undergoes partial disruption and releases cellular content, including polyphenol oxidase (PPO) and its substrates (phenolic compounds) and therefore causes the formation of brown pigments (Jacobo-Velázquez & Hernández-Brenes, 2010). Thus, the quality of avocado paste/puree is deteriorated and therefore inactivation of enzyme becomes essential for maintaining quality.

Thermal processing of food products with microwave (MW) technology has gained significant momentum due to its capability of providing rapid and volumetric heating. Several studies showed that in comparison with conventional heating greater or equal degree of preservation of color, bioactive compounds and antioxidant activity of fruits could be achieved by using MW. For example, Matsui, Gut, Oliveira, and Tadini (2008) found that heating green coconut water with MW to ~92 °C and holding for 47 s provided 1 log reduction of PPO whereas Campos, Souza, Coelho, and Gloria (1996) found that in a water bath at 90 °C approximately 100 s was required for the same magnitude of inactivation. Benlloch-Tinoco et al. (2015a) showed that pigment composition, including carotenoids and chlorophylls, of MW treated (1000 W for 340 s) kiwifruit puree was more similar to that of the fresh sample.

* Corresponding author.
E-mail address: bingolgo@gmail.com (G. Bingol).

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Similar findings were reported by Soysal and Söylemez (2005) such that inactivating the enzyme (peroxidase) in carrot using MW heating provided 20% more retention of Vitamin C compared with heating in a water bath.

In addition to the health benefits, sensory properties such as color and rheological properties have significant effect on consumers' purchasing decision. Therefore, at the end of processing the color should be as close as possible to the fresh product whereas the rheological properties should satisfy the consumers' expectations. Palma-Orozco, Sampedro, Ortiz-Moreno, and Nájera (2012) reported that heating mamey fruit to 70 °C by MW preserved the color without causing any significant damage to tissue and shape. At an equivalent degree of pasteurization level, Benlloch-Tinoco, Igual, Rodrigo, and Martínez-Navarrete (2015b) reported that compared with conventional thermal treatment kiwifruit puree had superior color, higher bioactive compound content (Vitamin C and total phenols) and a longer shelf-life when treated with MW. It was found that viscosity of kiwifruit puree increased when MW treatment at a power higher than 600 W was applied (Benlloch-Tinoco, Varela, Salvador, & Martínez-Navarrete, 2012). Similarly, when applied for 1 min, ultrasound treatment (375 W/cm^2) could significantly change the apparent viscosity and also the particle size of DAP (Bi et al., 2015).

The main objectives of the current study were to investigate the inactivation of PPO and pectin methyleasterase (PME) by MW treatment at different power levels, and changes in product quality after MW treatment including color, chlorophyll, total phenolic, rheological characteristics and particle size distribution during 4 weeks of storage (4 ± 1 °C).

2. Materials and methods

2.1. Chemicals

Polyvinylpolypyrrolidone (PVPP), catechol, pyrocatechol, gallic acid, Folin–Ciocalteu were purchased from Sigma Aldrich, New Zealand. All the other chemicals were obtained from ECP Ltd. (Auckland, New Zealand).

2.2. Avocado

DAP was provided by a local producer in New Zealand and was kept frozen at −29 °C until used for experiments. Frozen sample was thawed at 4 °C for 12 h prior to experiments.

2.3. Microwave treatment

The microwave treatment of DAP was carried out in a modified domestic microwave oven (Microwave Work Station-240, FISO Technologies, Canada). 100 g sample was tempered to an initial temperature of 10 ± 0.5 °C and then was treated in the microwave oven in a 150 mL glass beaker at different power densities (4.4, 7.7, and 11.0 W/g) for up to 90 s. The temperature during treatment was measured using fiber optic probes (FOD-NS-967 A; FISO Technologies Inc., Canada) with an accuracy of ±0.5 °C. The tip of the fiber optic probe was placed at the radial-center of the beaker and was fixed with a plastic holder and the temperature data was collected approximately every 0.5 s by a personal computer. The treated samples were immediately submerged in ice-water.

2.4. Dielectric properties measurement

Dielectric properties (dielectric constant, ε′ and dielectric loss factor, ε″) of DAP were measured using open-ended coaxial probe (85070E, Agilent Technologies, Malaysia) connected to a network analyzer (E5062A, Agilent Technologies, Malaysia). The dielectric properties of sample were measured after it reached temperature in the range of 20–90 °C (about 5 °C intervals) in a water bath.

2.5. Shelf-life study

The control (untreated) and microwave treated samples were packed into 50 mL sterile airtight plastic tubes and stored at 4 °C for 4 weeks. The color, rheology and particle size distribution were measured at 0, 3, 6, 10, 17, 24, 31 days.

2.6. Polyphenol oxidase assay

The extraction of polyphenol oxidase (PPO) from DAP and analysis of PPO activity were performed according to the method described by Woolf et al. (2013) with small modifications. Briefly, 3 g of DAP was homogenized with 10 mL phosphate buffer (0.1 M, pH 6.5, containing 5% poly(vinylpolypyrrolidone) (PVPP)), and the mixture was centrifuged at 5525*g* at 4 °C for 15 min (4 K15, Sigma, Germany). The supernatant was collected and the crude extract was kept at 4 °C before analysis.

The assay mixture consisted of 3 mL 0.1 M pyrocatechol dissolved in 0.1 M phosphate buffer and 0.5 mL crude extract. The absorbance change at 420 nm was recorded for 1 min using a UV-VIS spectrometer (Lambda 35 UV/VIS, PerkinElmer, USA). An enzyme activity unit was defined as an increase of 0.1 in absorbance at 420 nm per min. The residual activity (RA) of PPO was defined as below:

$$\text{Residual activity}\ (\%) = \frac{\text{specific activity after treatments}}{\text{specific activity before treatments}} \times 100 \quad (1)$$

2.7. Pectin methylesterase assay

Pectin methylesterase (PME) activity was determined by autotitrator (800 Dosino, Metrohm, Switzerland) using the method previously described by Benlloch-Tinoco, Igual, Rodrigo, and Martínez-Navarrete (2013) and Duong and Balaban (2014) with some modifications. Briefly, the reaction mixture consisted of 5 mL of DAP and 20 mL of 1% apple pectin (70–75% esterification, Fluka) containing 0.1 M NaCl, which was previously adjusted to pH 7. PME activity was measured by recording the amount of 0.05 M NaOH required for static titration at pH 7 for 5 min. One unit of PME activity (U/mL) was calculated by using Eq. (2).

$$\text{PME}(\text{U/mL}) = \frac{V \times N \times 1000}{V_s \times t_r} \quad (2)$$

where V and N are the volume (mL) and normality of NaOH, respectively, V_s is volume of sample (mL) and t_r is the reaction time (min).

2.8. Chlorophyll assay

The chlorophyll *a* and *b* concentrations were measured by slightly modifying the method described by Van Loey et al. (1998). 20 mL acetone (80% *v*/v) was added to 2 g of DAP in a 50 mL centrifuge tube, and mixed by using a vortex mixer and then centrifuged at 5525*g* at 4 °C for 15 min (4 K15, Sigma, Germany). The supernatant was collected and adjusted to 20 mL with acetone (80% v/v). The absorbance values at 645 nm and 663 nm (A_{645} and A_{663}) were measured at 25 ± 2 °C using a plate reader (EnSpire 2300, Perkin Elmer, USA). The chlorophyll *a* and *b* concentrations were calculated using the following conversion formulas:

$$\text{Chlorophyll } a = 12.71 \times A_{663} - 2.59 \times A_{645} \quad (3)$$

$$\text{Chlorophyll } b = 22.88 \times A_{645} - 4.67 \times A_{663} \quad (4)$$