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Synergistic effect of thermosonication to reduce enzymatic activity in coconut water

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A R T I C L E I N F O

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A B S T R A C T

In order to minimize the depreciation of coconut water quality caused by the conventional heat treatment, non-conventional preservation methods have been studied. Ultrasound technology can be suitable as an alternative thermal treatment for several food matrices, due to its efficiency in microbiological and enzymatic inactivation. The objective of this work was to evaluate the effect of the thermosonication to reduce the enzymatic activity in coconut water and optimize the optimal operating parameters (amplitude and time) and corresponding specific acoustic energy required for the complete enzymatic inactivation. Ultrasound presented an additive effect to the heat treatment on the inactivation of the polyphenoloxidase (PPO) and peroxidase (POD). It was verified that from 500 to 550 mW/mL a great inactivation of the PPO and POD is achieved, already for a complete inactivation of these enzymes the specific acoustic energy necessary is 655.80 mW/mL. It was also verified that the both enzymes should be taken into account for a US process since PPO and POD presented very similar resistance.

Industrial relevance: This work demonstrates the great potential of the use of thermosonication for the processing of heat-sensitive products, such as coconut water. Thought this innovative processing the food industry will be able to reduce the damages caused by the heat and consequently to obtain higher quality products.

1. Introduction

Coconut water is a sweet refreshing drink contained in the green coconut (*Cocos nucifera* L.) much appreciated because of its pleasant sensory characteristics, low caloric value and because it is considered a natural isotonic due to its richness in minerals such as sodium and potassium (Prades, Dornier, Diop, & Pain, 2012a). The beverage is sterile and stable when it is inside the fruit, however, soon after extracted and exposed to the air some oxidation reactions, especially promoted by its naturally enzymes polyphenoloxidase and peroxidase begin (Matsui, Gut, Oliveira, & Tadini, 2008). These reactions have a negative effect on sensorial and nutritional qualities of the coconut water (Campos, Souza, Coelho, & Glória, 1996; Duarte, Coelho, & Leite, 2002). Besides that, microbiological contamination also compromises its stability and spoilage may occur in a few hours from extraction (Reddy, Das, & Das, 2005).

The coconut is a typical tropical regions tree, this way the coconut as well as the coconut water is not available in all locations and throughout the year at uniform prices (Reddy et al., 2005; Prades, Dornier, Diop, & Pain, 2012b). Due to the inconvenience of transporting coconut water inside the fruit itself, it is necessary that it is extracted, processed and packaged. Thermal treatment (pasteurization or sterilization) is the main conservation method applied for coconut water preservation due its efficiency for enzyme inactivation, however, product quality is depreciated, therefore non-conventional preservation methods have been studied (Campos et al., 1996; Reddy et al., 2005). Technologies as ultrafiltration (Jayanti, Rai, Dasgupta, & De, 2010), high-pressure homogenization (Dosualdo, 2007), microwave heating (Matsui et al., 2008), CO_2 in dense phase (Damar, Balaban, & Sims, 2009), γ-irradiation (Awua, Doe, & Agyare, 2011) and ultraviolet (Augusto, Ibarz, Garvín, & Ibarz, 2015) have already been studied and some have even been applied as alternative methods of coconut water conservation. However, according to Augusto et al. (2015) unconventional technologies, such as ultra-filtration, micro-filtration and high pressure technology, still result in undesirable changes and/or are ineffective at inactivating the PPO and POD enzymes.

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Ultrasonic waves are sound waves with a higher frequency (generally above 20 kHz) that cannot be sensed by the human ear which cause mechanical and thermal changes due to the cavitation phenomena and can also induce cells disruption (Thrasyvoulou, Manikis, & Tselios, 1994; Deora et al., 2013; Millan-Sango, Abela, McElhatton, & Valdramidis, 2014). This technology has been shown to be effective for inactivating micro-organisms (Bermúdez-Aguirre & Barbosa-Cánovas, 2012; Millan-Sango, McElhatton, & Valdramidis, 2015; Martínez-Flores, Garnica-Romo, Bermúdez-Aguirre, Pokhrel, & Barbosa-Cánovas, 2015) and enzymes, including PPO and POD (Ercan & Soysal, 2011; Costa et al., 2013; Dias et al., 2015; Huang et al., 2017) in a great diversity of food matrices; been suitable as an alternative to thermal and other treatments since it presents smaller losses in the quality of the product (Dias et al., 2015; Martínez-Flores et al., 2015). Besides that, the ultrasound has already been applied to coconut water combined with supercritical carbon dioxide for microbial inactivation (Cappelletti, Ferrentino, & Spilimbergo, 2014) and recently it was evaluated the effect of this technology for inactivating peroxidase (POD) in green coconut water (Rojas, Trevilin, Funcia, Gut, & Augusto, 2017).

It is already well known that the ultrasound used alone is not very effective, so it should be combined with other technologies (Martínez-Flores et al., 2015). For example, in the study by Rojas et al. (2017) a reduction of only 27% of the enzymatic activity of the peroxidase in coconut water was verified during US processing, at control temperature (25 °C). When ultrasound is combined with heat (thermosonication), it can promote a further reduction of the enzymatic activity compared to the conventional heat treatment (Zhang et al., 2017) or can accelerate pasteurization/sterilization process, thus lessening both the duration and intensity of thermal treatment and consequently resulting in less adverse effect on nutritional and sensory quality (Chemat, Huma, & Khan, 2011). Current studies have not elucidated the possible synergistic, additive or antagonistic effect of temperature and ultrasound on the activity levels of both POD and PPO. Additionally, there is a need to further optimize such processes in relation to the operation parameters for achieving complete enzymatic inactivation.

Therefore, the objective of this work was to evaluate the effect of the thermosonication to reduce the enzymatic activity in coconut water. The optimal operating parameters (amplitude and time) and corresponding specific acoustic energy required for the complete enzymatic inactivation thought response surface methodology (RSM) in conjunction with central composite rotatable design (CCRD), are identified.

2. Materials and methods

2.1. Materials

Green coconuts of 4–5 weeks maturity and without any visible damage on outside were purchased in Brazilian local market and, after washing in chlorinated water, the water coconut was manually extracted using a special knife to perforate the fruit mesocarp. The water extracted from several fruits was filtered in filter paper (Whatman No. 4) to remove the solid parts in order to avoid interferences in spectrophotometric readings and mixed in a cooled tank to promote the water homogenization of all fruits. Then, the coconut water was divided into 23 portions (i.e., 22 treatments and the control) of 60 mL and was frozen in glass jars until the processing.

2.2. Experimental design

For the evaluation of the effect of the thermosonication on reducing the enzymatic activity and in order to optimize the ultrasonic conditions required for the complete enzymatic inactivation of the coconut water a central compostable rotatable design was performed (Box & Wilson, 1951; Box & Hunter, 1957).

The independent variables studied were the amplitude and time. Amplitude levels were 50% (− 1) and 90% (+ 1), while for the time the levels were 6.5 min (− 1) and 13.5 min (+ 1). Considering the axial points (− 1.41 and + 1.41) and 3 central points, the design totaled 11 treatments (Table 1).

Table 1
Codified and levels of the amplitude and time variables according to the Central Compostable Rotatable Design (CCRD) and the specific acoustic energy (SAE) of each treatment.

Treatments	Amplitude (%) X_1		Time (min) X_2		SAE (mW/mL)[a]
1	(− 1)	50	(− 1)	6.5	508
2	(− 1)	50	(+ 1)	13.5	499
3	(+ 1)	90	(− 1)	6.5	653
4	(+ 1)	90	(+ 1)	13.5	665
5	(− 1.41)	40	(0)	10	448
6	(+ 1.42)	100	(0)	10	715
7	(0)	70	(− 1.41)	5	618
8	(0)	70	(+ 1.41)	15	671
9	(0)	70	(0)	10	690
10	(0)	70	(0)	10	664
11	(0)	70	(0)	10	717

[a] Calculations were performed as described in Section 2.3.

The equations describing the enzyme activity (polyphenoloxidase and peroxidase) in relation to the specific acoustic energy were obtained using a multiple linear regression analysis based on which response surfaces were generated. Analysis of Variance, normal probability and residual plots were used in order to validate the predictive capacity of the models. The calculations were carried out using the software Chemoface (Nunes, Freitas, Pinheiro, & Bastos, 2012) and Matlab (The MathWorks Inc., Natick, MA).

2.3. Ultrasound treatments

An ultrasonic processor Q500 (QSonica), 20 kHz with a ultrasonic horn of 1 cm diameter was used. This was immersed 0.5 cm in equal volumes of coconut water (70 mL) and sonicated according to the conditions described in the Table 1, through continuous mode.

The variation of the temperature over time was recorded and analysed in order to estimate the acoustic power (W) and the specific acoustic energy (mW/mL). Fig. 1 shows the temperature profile of each thermosonication treatment. At the end of the treatment, the samples were immediately cooled in an ice-water bath before the enzyme activity determination.

The acoustic power applied to each treatment was quantified by the equation based on the calorimetric method suggested by Baumann, Martin, and Feng (2005).

$$\text{Power}(\text{W}) = mC_p\left(\frac{dT}{dt}\right)_{x=0} \tag{1}$$

where, m [kg] is the mass (0,070 kg), C_p [J/kg·°C] is the coconut specific heat capacity (4056.5 J/kg·°C) and dT/dt [°C/s] is the ratio of change of temperature during sonication.

The specific acoustic energy of each treatment was then calculated in mW/mL taking into account the acoustic energy (W) and volume of coconut water sample (mL).

2.4. Thermal treatments

Aiming to have a control treatment to assess the type of effect (e.g., synergistic/additive) of the thermosonication, a conventional thermal treatment (without ultrasound) was carried out in a temperature/time controlled water bath replicating the same temperature profile obtained in each of the 11 ultrasonic treatments. After recording the temperature profile during the ultrasound process (Section 2.3) a similar temperature ramp was chosen in the thermal water bath (Q215S2 model – QUIMIS). A sample of 10 mL of coconut water was placed in a glass test