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Determination of antioxidant capacity in blackberry (*Rubus glaucus*) jam processed by hydrotermodynamic cavitation compared with traditional technology

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Abstract

The objective of this study was to determine the antioxidant capacity of blackberry (*Rubus glaucus*) jam processed by hydrothermodynamic cavitation (HTD) at different temperatures (25, 45, 60, and 75 °C) compared with traditional technology (TDE). HTD is a food processing technology based on the cavitation phenomenon for the simultaneous crushing, homogenisation, and pasteurisation of whole foods. Blackberries without any treatment were used as a reference. The results indicated that the content of phenolic compounds and their antioxidant capacity were directly correlated to the type of process and temperature. However, compounds with antioxidant capacity should be further assessed in order to determine the actual effect of this technology. In addition to this, during the development of this study, savings were achieved in production processes (factory and labor expenses) due to the use of this technology, such as a 50% cost reduction

Keywords: traditional technology (evaporation); hydrothermodynamic cavitation; Blackberry jam.

Determinación de capacidad antioxidante en un dulce de mora (Rubus glaucus) procesado por cavitación hidrotermodinámica en comparación con tecnología tradicional

Resumen

Este estudio tuvo como objetivo determinar la capacidad antioxidante de un dulce de mora (Rubus glaucus) procesado por cavitación hidrotermodinámica (HTD) a diferentes temperaturas (25, 45, 60 y 75 °C) comparada con la tecnología tradicional (TDE). HTD, es la tecnología de procesamiento de alimentos basada en el fenómeno de la cavitación para la trituración, homogenización y pasteurización simultánea de alimentos integrales. La mora sin ningún tipo de tratamiento se utilizó como referente. Los resultados muestran que la cantidad de compuestos fenólicos y su capacidad antioxidante está directamente correlacionada al tipo de proceso y temperatura. Sin embargo, se debe continuar profundizando en la identificación individual de los compuestos con capacidad antioxidante para determinar efectos adicionales por el uso de la tecnología. Adicional a esto, durante el desarrollo de este trabajo, se lograron ahorros en los procesos productivos debido al uso de esta tecnología, como es una disminución en costo del 50% en gastos de fábrica y mano de obra.

Palabras clave: tecnología tradicional (evaporación); cavitación hidrotermodinámica; dulce de mora.

1. Introduction

The global demand for food that promotes health with high nutritional and nutraceutical values has been increasing. It is estimated that, by 2020, the global market for integral and functional food will reach 800 billion dollars [1]. The consumption of fruits and vegetables has been linked to health benefits, such as protection against diseases associated with oxidative stress, for example, chronic diseases. These effects have generally been

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attributed to the wide range of polyphenols, such as anthocyanins, flavonoids, proanthocyanidins, and flavonols present in fruits and vegetables [2,3]. Phenolic compounds are secondary metabolites of plants that constitute one of the most numerous and widely distributed groups of natural products in the plant kingdom. In general, these compounds can be classified into two main groups, namely: flavonoids (flavonols and anthocyanins) and non-flavonoids (stilbenes and phenolic acids). These compounds are important determinants in the sensory and nutritional properties of fruits and vegetables and have been attracting attention due to their antioxidant capacity [4,5].

Berries are highly prized for their intense colour, delicate texture, and unique flavour. Despite having several common attributes, the group is quite diverse and includes, for example, fruits such as blueberries, blackberries, raspberries, strawberries, elderberries, black and red currants, which have been postulated as a good source of phenolic compounds [6,5]. The blackberry (*Rubus* spp.) is an excellent source of natural antioxidants, particularly anthocyanins and phenolic acids [7], which have been associated with health benefits, such as antioxidant, anticonvulsant, and anti-inflammatory properties [8,3]. Antioxidants are substances that, in small quantities, have the ability to donate an electron or a hydrogen atom to a free radical. They can chelate the metal cations and thus form a stable molecule [9].

Blackberries are very perishable fruits and should be consumed in very short times (days). Over-maturation, excessive softening, and attack of pathogens are the main causes of post-harvest losses [6]. Therefore, the processing of fruits into products such as juices, purees, jams, canned fruits, and marmalades can be an alternative to prolong the accessibility and the consumption of fruits such as blackberry [3]. On the other hand, processing and storage can have notable effects on the phenolic content of fruits, and can reduce or even increase their health-benefit properties [10]. Gil *et al.*, [11] demonstrated that the processing of pomegranate into juices increased their antioxidant capacity and their total phenolic content (TPC). On the contrary, the processing of strawberries into marmalades affected the flavonoid content with a decrease of 20% [12]. Similarly, the flavonoid content of red raspberries decreased slightly with processing, and more markedly during six months of storage [13,3]. Based on these findings, it is of great importance to review and determine the best treatment, given that, as mentioned above, the type of process might affect the composition and sensory characteristics of blackberries.

The concept of food preservation has changed gradually over the years. Initially, its purpose was to obtain safe products with a long lifetime. Today, fresh characteristics with a high content of nutrients and antioxidants are some of the attributes most requested by consumers, without neglecting food safety [14]. In traditional processes, the berries are crushed and pressed. The solid parts are discarded after extracting the juice, which generates an intensive accumulation of by-products, consisting of seeds, peels, and pulp remains. Through common processing, only part of the phenolic compounds are transferred to the juice, and the by-products generated are rich in anthocyanins and other compounds [15,5]. Thermal sterilisation/pasteurisation treatments ensure an effective reduction of microorganisms; however, they cause a significant loss of thermolabile compounds, and negatively affect the sensory, physicochemical, and nutritional properties of food [16,14].

For the food industry, pasteurisation is one of the most used methods for fruit drinks, such as blueberry products. Some authors have reported that this process leads to a substantial loss of bioactive compounds in berries due to oxidation and thermal degradation [17-19]. Therefore, it is necessary to adjust the intensity of the thermal treatment to a necessary minimum level that can achieve enzymatic and microbiological inactivation [20]. That is the reason why—in accordance with the aforementioned—the growing demand of consumers of minimally processed nutritious foods, together with the recent regulatory requirements, has led to a greater application of non-thermal technologies for the processing of puree, jams, marmalades, juices, and/or nectars. Such technologies have been investigated due to their potential to minimise changes in nutritional and organoleptic properties [21].

Hydrodynamic cavitation (HTD) is a new food processing technology that uses cavitation phenomena in liquids to crush and homogenise solid food particles [1]. It has a lower cost and a relatively simple configuration easily adaptable in food processing schemes, compared with other alternative technologies [22,23,42]. Cavitation is defined as the generation, formation, growth, and collapse of microbubbles due to variations in pressure in a fluid [24,1]. This process is generally produced by the mechanical rotation of a liquid at a specific speed. It can be achieved using a specialised rotor with holes known as hydrodynamic cavitators. When the rotor rotates, the action produces hydrodynamic cavitation away from the metal surface inside the holes. The cavitation effect is completely controlled within the system that facilitates the protection of the surface against damage. Subsequently, microscopic cavitation bubbles generate collapse with the discharge of shock waves that can mix together and inhibit incrustation. As a result, heat is produced with a uniform temperature distribution throughout the liquid, even without heat transfer surfaces [25]. Regarding the process equipment, the reactors used in the food industry generally consist of high pressure- and homogenisation-type reactors. They have a feed tank and two throttle valves that represent a first and second stage. The reactor is a high pressure positive displacement pump with a throttling device that follows the principle of the high pressure relief technique. By using a pump, the liquid from the feed tank is directed to the valve of the first stage. A pressure of up to 1,000 psi can be obtained in the first stage. Then, using second stage valves, the pressure can be increased to 10,000 psi with the use of different pressure relief valves. The liquid of the second stage is released into the low pressure region, and then recirculates back into the storage tank. As the throttling pressure increases, the temperature also increases. This way, cold water is recirculated in the feed tank in order to control this temperature increase. Cavitation begins at certain critical discharge pressure, which depends on the geometry of the regulation and the type of application [23].

Because of the internal heat generation, cavitation allows a single-stage processing, in which unitary operations—such as crushing, homogenisation, and heating—are performed in a closed system with limited oxygen exposure [26,23]. These process characteristics generate competitive advantages in the final product, mainly associated with a minimal degradation of food quality [27]. For example, the combination of uniform temperature with high pressure gradients was found to create favourable conditions for a single-stage processing of berries into puree-type products [28]. Similarly, this technology has been successfully used for the inactivation of enzymes (peroxidases and polyphenol oxidases) and microorganisms in cranberry puree [29,30], or to study the stability of bioactive compounds in tomato juice [23]. At other industries, HTD has been used successfully, in combination with for degradation of benzene present in wastewater [40], or to optimize the extraction of algae-oil from microalgae with different temperature and time [41]. At the scientific level, there are not many records about the advantages of using this technology; however, it is important to highlight that, by performing several unitary operations in a single stage, multiple operational and economic advantages can be generated.

The potential of this technology has not been fully explored for general fruit processing. There are records of the processing of blueberry puree and tomato juice with studies addressing physicochemical, functional, and microbiological properties; however, there are no records about the processing of blackberry jam. That is why, taking into account the changes registered in some fruits according to the type of process, the goal of the present study was to determine the antioxidant capacity of blackberry (Rubus glaucus) processed by hydrothermodynamic cavitation, compared with traditional technology (evaporation).

2. Materials and Methods

2.1. Materials

Blackberries (Rubus glaucus) were obtained from Alpina Productos Alimenticios S.A. in Chinchiná, Department of Caldas, Colombia (average altitude of 1,378 mamsl, with an average temperature of 18-28 °C). Samples were collected and frozen at -18 °C until processing after approximately three days.

2.2. Processing of jams

Initially, an amount of blackberries was determined for each process, with a percentage (%) of fruit equivalent to 50% for each preparation. After this step, we prepared the jams using the traditional process (evaporation) (TDE) and hydrothermodynamic cavitation (HTD). All preparations were made in duplicate.

2.2.1 Traditional process (evaporation) (TDE)

Initially, in this process, it is necessary to obtain concentrated pulp. Then, it is mixed with the ingredients of the jam. The final step is the concentration process by evaporation. We used a fruit squeezer to obtain the pulp. After this procedure, the pulp was placed in a pan to evaporate until reaching the desired concentration (28 °Bx). It was then mixed with the ingredients of the jam (sugar, dyes, flavouring, and citric acid) and boiled until obtaining the desired concentration.

2.2.2. Hydrothermodynamic cavitation (HTD)

In this process, defrosted blackberries were mixed with the ingredients of the jam (sugar, dyes, flavouring, and citric acid) in the mixing hopper of the cavitation equipment. The cavitation process reached up to 75 °C. Samples were taken at 25 (initial temperature), 45, 60, and 75 °C, for analysis.

2.3. Determination of colorimetric properties

The colour parameters L*, a*, and b* were determined using a Hunter Lab Colour Flex EZ colourimeter. The colour difference (ΔELab*) was calculated according to equation (1). The colorimeter was standardised using the white and black calibration plate. Illuminator light was D65/10. All analyses were performed in triplicate.

$$\Delta E_{Lab} = \sqrt{L^2 + a^2 + b^2} \tag{1}$$

2.4. Quantification of phenolic compounds

The TPC was measured from the dispersion of blackberry jams in water. We used 50 µL of a freshwater solution, and added 1.5 mL of Folin extract (diluted 10 times with water). They were allowed to react for 5 minutes and subsequently we added 1.5 mL of 7.5% sodium carbonate. This preparation was allowed to react for 60 minutes in the dark at room temperature. The absorbance at 725 nm was measured in a spectrophotometer (Thermo Scientific™ spectrophotometer for Multiskan™ GO microplates). Gallic acid was used as standard to prepare the calibration curve and express the results as milligrams of gallic acid equivalents (GAE) per gram of sample (mg of GAE/g).

2.5. Antioxidant capacity

The samples were assessed to determine the antioxidant activity on the free radical 2.2-diphenyl-2-picrilhydrazil (DPPH). A calibration curve was performed with ethanolic solutions of the DPPH radical at different concentrations to determine the initial concentration of the DPPH radical at an absorbance of 0.7 ± 0.02 (765 nm). We mixed 150 ml of the sample with 2,850 ml of the DPPH solution (g/mL ethanol).

Subsequently, we stirred this material and stored it in the dark for 20 minutes (this was the standardised time at which the colour developed and there were no variations in the absorbance measurements). After this time, the absorbance was measured at 515 nm, i.e., the wavelength at which the greatest absorbance of the DPPH solution was obtained in the spectrophotometer. The percentage of DPPH inhibition was determined according to equation (2).

$$\% SE = (1 - \frac{Abs_{Sample} 517nm}{Abs_{Control} 517nm}) \times 100 \tag{2}$$

3. Analysis of the Results

3.1. Colorimetric properties

Colour is an important quality factor for food, mainly for fruit-based products, such as marmalades, jams, and other preparations. Therefore, the colour of the products should not change during storage. However, processing may alter the colour characteristics of fruit-based products due to the effects of reactions during heating, dissolution, or concentration [3]. Table 1 shows the results of the colorimetric properties of blackberry (reference value) and blackberry jam processed by traditional technology (TDE) and hydrothermodynamic cavitation (HTD) at different temperatures. The colour of the products after some type of treatment can directly influence consumer acceptance. The stability of the colour and the content of antioxidant compounds in red fruits and their processed products can be influenced by many factors, such as temperature, processing time, pH, oxygen, water activity, and storage conditions [31,3]. This way, measuring the colour of blackberries without any treatment is very useful for establishing the chromatic characteristics that define the colour of the processed jams to be obtained and what their differences will be. The component a*, associated with the red component in colour analysis, is positively correlated with anthocyanins and their stability [32]. In the present study, we found significant differences (p <0.05) between treatments. The highest values of component a* were found in blackberries without any treatment, then in jam processed by HTD, and, finally, in jam processed by TDE. This fact may be an initial indication that the process maintains a value of phenolic compounds and antioxidants that may be related to the effect of the HTD technology on the type of processing.

The changes in the three colour coordinates (L*, a*, b*) of each jam with treatment (TED or HTD) in comparison to the blackberries without any type of treatment were calculated as the total colour difference (ΔELab*). The highest ΔELab* was observed in the jam processed by TDE, in comparison to jam processed by HTD, with significant differences (p <0.05). Similarly, we observed significant changes (p <0.05) between the temperatures assessed in the jam processed by HTD; however, these changes were not significant in comparison to the jam processed by TDE. The thermal impact during evaporation and the concentration of the jam processed by TDE favoured the degradation and loss of colour [33]. A first conclusion from these results is that the ΔELab* of the jams obtained by HTD were lower compared with the jams obtained by TDE. This way, it is worth reaffirming that colour stability can depend on several factors, including the genuine matrix of food and the type of processing. Finally, the differences in perceptible colour could be classified analytically as very different (ΔELab* >3), different (1.5 < ΔELab* > 3), and little different (ΔELab* <1.5) [21,34]. Therefore, very different colour changes were observed in the two treatments in comparison to the unprocessed blackberries, including the different temperatures in HTD. On the other hand, the difference found in TDE was three times greater than in HTD, which indicates that the colour affectation was even greater when compared with unprocessed fruit, which can be attributed to the influence of the type of process mentioned above.

Table 1. Colorimetric parameters of blackberries and blackberry jam processed by traditional technology (TDE) and hydrothermodynamic cavitation (HTD) at different temperatures.

	L*	**a***	**b***	**ΔE_{Lab}^{*}**
Blackberry	15.90 ± 1.549a	32.98 ± 4.029a	13.95 ± 3.829a	-
D. TDE	11.78 ± 1.504b	19.83 ± 1.547b	7.72 ± 0.823b	15.21 ± 1.335a
D. HTD 25 °C	11.60 ±0.110c	31.57 ± 0.062c	16.35 ± 0.079c	5.12 ± 0.104b
D. HTD 45 °C	12.95 ± 0.138d	31.17 ± 0.233d	16.35 ± 0.152d	4.22 ± 0.089c
D. HTD 60 °C	12.10 ± 0.220e	31.16 ± 0.348e	16.07 ± 0.226e	4.74 ± 0.132d
D. HTD 75 °C	11.37 ± 0.050f	31.25 ± 0.078f	16.05 ± 0.040f	5.29 ± 0.010e

The means with different small letters in the same column are significantly different in each treatment (p <0.05). The results are expressed as mean ± standard deviation.
Source: The Authors.

3.2. Quantification of antioxidant components

Fig. 1 illustrates the results obtained for TPC in blackberry jam processed by TDE and HTD, beginning at 25 °C and ending at 75 °C for HTD and 90 °C for TDE in a conservation process. For TDE, the results of TPC values remained the same at the beginning and at the end of the process (decrease of 1.83%). On the other hand, in the HTD process, there was a decrease between the beginning and the end of the process (39%). The comparison between TDE and HTD indicated that the first exhibited a decrease of 44% at the beginning, and 10% at the end of the process.

The degradation of phenolic compounds may be related to the effect of temperature and the increase of solids content during heating [35,3]. Hager et al., [36] studied the effect of juice extraction, puree, canning, and freezing treatments on phenolic compounds of blackberries (Apache cv) and observed that canning and freezing had little effect on phenolic compounds; however, significant changes were observed during the juice extraction process. This finding is in correlation with what was found in the present study for TDE and HTD. We observed a high difference in TPC content from the beginning of the process, which could be directly related to the type of extraction used for obtaining the juice. This effect can only be affirmed for TDE throughout processing, given that there was a minimum decrease in TPC value (0.007) at the end of the procedure, in comparison to the value observed at the beginning of the procedure.

Fig. 2 illustrates the results obtained for TPC in blackberry jam processed by HTD at different temperatures (25, 45, 60, and 75 °C). In general, and according to the results, it can be affirmed that, as there was an increase in temperature (between 15 and 20 °C) in HTD processing, there was 5 to 20% decrease in TPC. Martynenko, Astatkie and Satanina [1] stated that size reduction, heat treatment, and cavitation during the HTD process facilitated the process of releasing bioactive compounds into solution, thus making them available for analytical measurements and final consumption. Similarly, Martynenko and Chen [28] stated that, although HTD processing results in the degradation of anthocyanins and polymeric colour formation, the total phenolic contents in the purees processed with HTD had been significantly higher than in commercial juices. This fact was mainly due to the exclusion of pulps and fruit residues during pulp processing, which did not favour the preservation of these types of compounds. It has been reported that approximately 12-50% of anthocyanins and other polyphenols are preserved in pulp residues during juice production [18,19], which may be in line with the TPC values found in our study. Although there was a decrease in TPC at higher temperature, there was a higher TPC value in relation to TDE from the beginning of the process. This way, these results are in line with the initial hypothesis of a positive effect of HTD on phenolic compounds.

On the other hand, we observed that temperatures between 25 and 60 °C did not cause significant differences ($p < 0.05$) in the results of TPC. However, the comparison of the four temperatures (up to 75 °C) indicated significant differences ($p > 0.1$). With these results, we can affirm that, at temperatures above 70 °C, TPC was significantly affected in blackberry jam processed by HTD. Similar results had been reported by Terán et al. [23], who found that the TPC in tomato juice had had great stability during treatment with HTD at different temperatures and times (35 - 62 °C for 10 minutes) . On the other hand, the phenolic compounds of watermelon juice had decreased at 80 °C during 15 minutes [37].

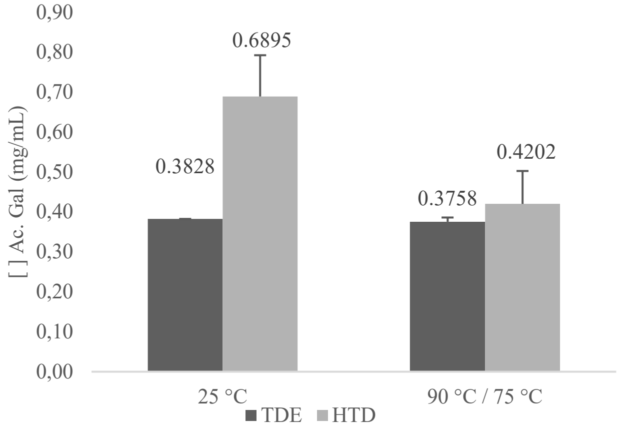

Figure 1. Total phenolic content in blackberry jam processed by traditional technology (TDE) and thermodynamic cavitation (HTD).
Source: The Authors.

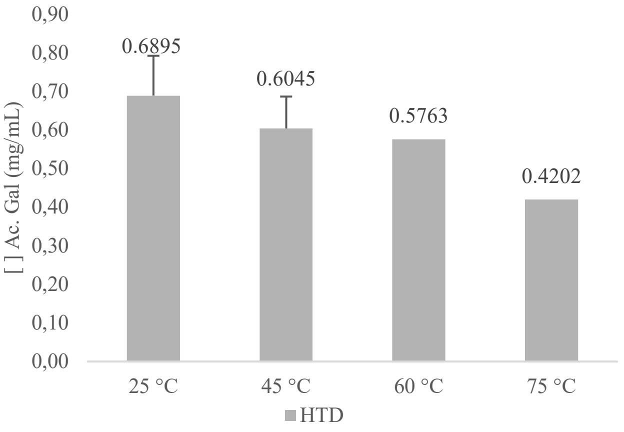

Figure 2. Total phenolic content at different temperatures (25, 45, 60, and 75 °C) in blackberry jam processed by thermodynamic cavitation (HTD).
Source: The Authors.

3.3. *Antioxidant Capacity*

Fig. 3 illustrates the antioxidant capacity of blackberry jam processed by TDE and HTD, beginning at 25 °C and ending at 75/90 °C. Blackberry jam processed by TDE exhibited lower values at the initial temperature in comparison to HTD. As the temperature increased (ending at 75/90 °C), the antioxidant capacity exhibited the same behaviour using TDE or HTD. Likewise, the results were very similar to those of TPC in the two processes. There was a decrease of 10% in TDE, and 16% in HTD. The results of the antioxidant capacity had a direct correlation with the results of TPC, which indicates that the antioxidant activity depended on all the phenolic compounds, and the anthocyanins were partially responsible for this activity. This finding may be related to the fact that TPC is the main potential responsible for the antioxidant properties reported in fruit jams, for example. The antioxidant activity is not limited to this type of compounds but is related to the degradation of antiocyanins [31,3]. Koca and Karadeniz [38] observed that the antioxidant activity in blueberries was highly correlated with phenolic content, and less linearly correlated with anthocyanin content. A relationship between antioxidant capacity and TPC has been suggested; however, it should be complemented with the anthocyanin content for final differentiation to observe the actual effect between different treatments. Some studies [39] have suggested that TPC other than anthocyanins contributed positively to total antioxidant activity [5].

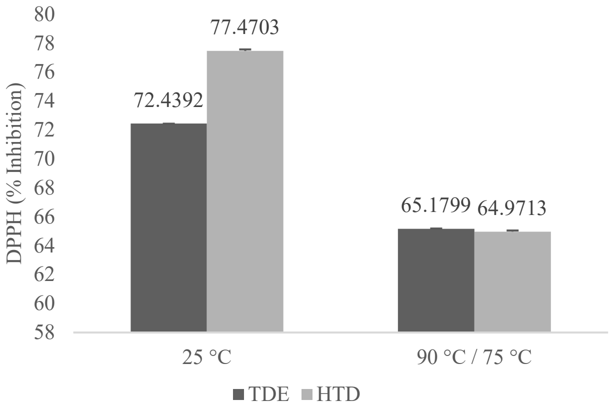

Figure 3. Antioxidant capacity in blackberry jam processed by traditional technology (TDE) and thermodynamic cavitation (HTD).
Source: The Authors.

The present study can lead to a general conclusion, according to which the reduction in size caused by HTD releases bio-compounds, thus making them more vulnerable to light and oxygen. This way, the lowest temperature did not favour these bio-compounds, and the protection of the compounds from light and oxygen might be a priority. Similarly, the type of heat transfer used in each process might have directly affected the stability of these compounds.

Multiple unitary operations and thermal conservation stages are used for preparing a fruit jam by traditional technology. On the other hand, the use of the principle of cavitation generates the integration of the process stages (extraction, grinding, homogenisation, and conservation). With this integration, operational savings in factories and operating costs (labour) can reach up to 50%, according to the equipment and process line. These savings were precisely observed during the conduction of the present study. These results confirm the industry interest for considering both processing costs and implementing greener processing technologies [42].

4. Conclusions

The present study determined the antioxidant capacity of blackberry jam processed by traditional evaporation technology (TDE) and hydrothermodynamic cavitation (HTD). The results indicated that blackberry (Rubus glaucus) is a significant source of phenolic compounds, with a high antioxidant capacity. The effect of processes of TDE and HTD on antioxidant capacity did not indicate significance differences. HTD shows a notable decrease in phenolic and antioxidant activity at temperature higher than 65 °C, in comparison to TDE. However, at a range from 20 to 60 °C phenolic compounds and antioxidant activity were more stable with HDT. Therefore, it is suggested to keep the HTD process up to a maximum temperature of 65 °C. Nevertheless, greater understanding of the antioxidant capacity directly associated with the anthocyanin content is still required, in order to determine the final effect that can be achieved by HTD processing.

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