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Extraction optimization for antioxidant phenolic compounds in red grape jam using ultrasound with a response surface methodology

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ABSTRACT

Optimization of the extraction methodology for antioxidant phenolic compounds in red grape jam was performed with an ultrasound-assisted system. The antioxidant phenolic compounds were extracted and analyzed by determining the total phenolic content (Folin Ciocalteu), as well as by employing free radical DPPH· and the beta-carotene/linoleic acid system. To optimize the parameters of solvent concentration, time and extraction temperature, the experiments were carried out using the central composite rotatable design (CCRD) method. Using response surface methodology (RSM), the best combinations achieved were with 60% ethanol and water for 20 min at 50 °C. The optimized parameters for this method were compared to an extraction method that has been commonly noted in the literature, which used to be the standard method, and the results were expressed in the milligram equivalent of quercetin per gram of jam (mg E.Q/g Jam). With the new method, the antioxidant potential measured by DPPH· was 70% higher than that obtained with the standard extraction method, and the antioxidant potential measured using the beta-carotene/linoleic acid system was 65% higher. In addition, a significant decrease in the total analysis time was achieved (from 10 h to 30 min), when compared to the standard method.

1. Introduction

Among fruits, grapes are a major source of antioxidant phenolic compounds, specifically flavonoids. These compounds demonstrate a great capacity for capturing free radicals, which can cause "oxidative stress", and aid in the prevention of cardiovascular diseases and some types of cancer [1–7]. In recent years, interest in natural antioxidants and their affects on health and human nutrition has greatly increased. The most commonly found flavonoids in grapes and its derivative products are flavanols (catechin, epicatechin, epigallocatechin), flavonols (kaempferol, quercetin, myricetin) and anthocyanins, in addition to other antioxidant phenolic compounds that are non-flavonoids, such as stilbenes (resveratrol) and cinnamic acid derivatives [8,9].

In a study concerning the effect of flavonoids on human health, red wine, catechin and quercetin solutions were administrated in patients to verify their action as antioxidants. It was concluded that the red wine and quercetin solutions reduced in more than 33% the levels of induced oxidation by LDL cholesterol, under oxidative stress [10,11]. Martinez et al. [12] have carried out a study using rats with acute pulmonary injuries and concluded that quercetin decreased the pulmonary oxidative stress and anti-inflammatory response associated with these injuries.

Grape jam, among other grape derivatives, is a viable alternative to the economic exploitation of fruits, adding value to the fruit and promoting access to its beneficial constituents for the entire year [13,14]. In addition, when compared with wine, grape jams and grape juices can present an advantage due to the absence of alcohol, enabling the consumption of this grape derivative by children and people with certain types of diseases, such as hepatitis [15,16].

Fruit jams are commonly used with breads, cookies, and cake fillings, among others. The main ingredients employed to prepare jam are fruit (*in natura*, pieces, juice or pulp), sugar, pectin and citric acid [13]. Although the processing of fruit can decrease the quantity of total anthocyanins and other antioxidant phenolic compounds, some studies have shown that significant amounts of these compounds can be found after three months of storage in the presence of light and at room temperature [17]. It has also been demonstrated [18] that, during jam processing, temperatures of up to 70 °C result in the inactivation of enzymes that degrade antioxidant phenolic compounds.

The most common varieties of grapes used to fabricate jams in the state of São Paulo, Brazil, are the Niagara and Isabel varieties. However, in the present work, jams were prepared with the Máximo variety (IAC 138-22), which is a result of genetically crossing the

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Siebel and Syrah varieties [19]. As this is a relatively new cultivar in the Brazilian market, the antioxidant capacity of this variety was not found in the literature; thus, it was tested in the laboratory. An increased amount of phenolic compounds and an increased antioxidant potential was found for this variety, compared to the other grape varieties used to make grape jams for the Brazilian market.

For grapes, the synthesis of resveratrol occurs mainly in the skin, and this compound is absent or is found at very low concentrations in the pulp. When the winemaking process for red grapes begins, maceration with the skin and seeds during fermentation is the principal factor that contributes to the elevated levels of resveratrol found in red wines compared to white wines. Extraction of resveratrol from the skin can be facilitated by the ethanol produced during the fermentation process [20–25]. This information indicates that it is important to create non-alcoholic grape derivatives that include grape skin in their formulations.

Antioxidant phenolic compounds are commonly extracted from plants [26], and has been developed using new environmentally friendly methods. These techniques promote a decrease in solvent consumption with an increase in the extraction ratio [27,28]. Among these techniques is ultrasound-assisted extraction, which is a simple, efficient and inexpensive alternative [27–31]. The greater efficiency achieved with ultrasound-assisted extraction is due to the acoustic cavitations effects produced in the solvent when the ultrasonic wave passes through it. This effect permits better penetration of the solvent into the sample, increasing the release of the analyzed compound from the matrix to the solvent [30].

This study was carried out to optimize the parameters of solvent concentration, temperature and extraction time using a surface response methodology (RSM) and employing central composite rotatable design (CCDR), in conjunction with ultrasound bath equipment, to obtain the best extraction conditions for antioxidant phenolic compounds in grape jam produced with skin in the formulation.

2. Experimental

2.1. Materials

Grapes of the Máximo variety (IAC 138-22), employed in the preparation of jams, were obtained from a producer in the Campinas region, in the state of São Paulo, Brazil. The formulation ingredients were 60 parts grape, 40 parts refined sugar and inverted sugar syrup, 1% pectin (m/m) and 6% dry skin (m/m). The jams were produced in triplicate.

The dry skin was obtained by an air-forced drier with 3 m/s of air for 3 h. The skins were then milled and added to the jam formulation.

Ethanol and methanol solvents were of chromatography grade and were obtained from J.T. Baker. The chloroform was of analytical grade and was obtained from Alkimia. Water was purified using a Milli-Q system. Folin Ciocalteu was from Fluka. The reagents DPPH· (2,2-diphenyl-1-picrylhydrazyl), beta-carotene, linoleic acid and Tween 20 were purchased from Sigma–Aldrich (Brazil). The quercetin standard was also purchased from Sigma–Aldrich (Brazil).

2.2. Extraction of antioxidant phenolic compounds

Extractions were carried out in an ultrasonic bath (Ultra Cleaning 1400, 40 hz, 80 W, Unique, Ind. e Com. Ltd., Brazil). Samples (2 g) were placed into Erlenmeyer flasks (125 mL) with 100 mL of the extraction solvent and sonicated at various times and temperatures, as the experiment required. The temperature was controlled using a thermostat that was added to the ultrasonic bath (Fulgauge). After extraction, samples were cooled with tap water and filtered under vacuum through Whatman No. 1 paper. Subsequently, the samples were placed in a 100 mL volumetric flask, which was then filled to the mark, and used in antioxidant determination tests.

2.3. Determination of total phenolic compounds

The method used to determine the total phenolic compound (TPC) levels employed the Folin–Ciocalteu (FC) reagent, according to a procedure described in the literature [32]. An aliquot of 500 μL of sample was added to 6 mL of FC reagent and diluted to 10% (v/v) in distilled water. After 5 min, 2.5 mL of 7.5% (m/v) sodium carbonate solution in distilled water was added to the tube and the solution was shaken. The tubes were kept still for 2 h at room temperature. Subsequently, the solutions were transferred to cuvettes to measure their absorbance at 740 nm. All of the analyses were carried out in triplicate. Using quercetin as a standard, the calibration curve was prepared with 67, 100, 150, 300, 450, 600 and 750 μmols of quercetin, and the results were expressed in quercetin equivalents (mg E.Q/g Jam).

2.4. Determination of the antioxidant potential through free radical DPPH·

The antioxidant potential of the extracts was assessed using the DPPH· method. This parameter was determined to elucidate the ability of the sample to reduce free radicals [32]. The organic radical DPPH·, which is very stable, has an intense purple color that fades when it is transformed into the reduced form.

A solution of 0.039 mg/mL DPPH· was prepared [26] to present an absorbance value of 1.000 when measured at 517 nm. Volumes of 3.9 mL of DPPH· solution and 100 μL of the extract were added to each tube for the analyses. For the control solution, 100 μL of the extraction solvent was added to the tube. The absorbance readings were taken after 80 min of reaction time at room temperature and in the absence of light. All of the analyses were carried out in triplicate.

The decrease in absorbance of the sample (S) tubes was correlated to the decrease in absorbance of the control (C), resulting in an inhibition percentage of the free radical DPPH· (I DPPH), which can be expressed through the following Eq. (1):

$$\text{I DPPH}(\%) = \frac{(C - S) * 100}{C} \quad (1)$$

The calibration curve for quercetin was prepared with 67, 100, 150, 300, 450, 600 and 750 μmols of quercetin, and the results were expressed in quercetin equivalents (mg E.Q/g Jam).

2.5. Determination of the antioxidant potential through the beta-carotene/linoleic acid system

The method used for the determination of the total antioxidant potential through the beta-carotene/linoleic acid system followed the procedure described in the literature [34]. For the reactive mixture, 20 μL of linoleic acid, 200 mg of Tween 20, and 1 mg of beta-carotene were added to 5 mL of chloroform and placed in a round roto-evaporator flask. This solution was kept at 40 °C until all the chloroform had evaporated. Then, 50 mL of distilled water was added to the mixture and it was vigorously shaken.

In each tube, 6 mL of the mixture was added to 50 μL of the jam extract. In the control tube, the mixture was added to 50 μL of the extraction solvent. After these additions, the absorbance was measured (Abs 0) at 470 nm, and the tubes were placed in a water bath at 50 °C for 2 h to catalyze the oxidation reaction and