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Evaluation of Antioxidant and Antimicrobial Capacity of Pomegranate Peel Extract *(Punica Granatum*l.) Under Different Drying Temperatures

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Pomegranate peels (Punica granatum L.) are considered non-edible parts or byproducts obtained during juice processing and characterized by the significant presence of polyphenols such as ellagitannins, ellagic acid, gallic acid and flavonoids, associated with biological properties such as antioxidant and antimicrobial agents. The objective of this study was to establish the antioxidant and antimicrobial capacity of aqueous and ethanolic extract of pomegranate peels, freeze-dried and oven dried to 60ºC, through DPPH and ABTS radicals capture methods and Minimum Inhibitory Concentration with *Escherichia coli* ATCC 25922, *Salmonella typhimurium* ATCC 14028 and *Staphylococcus aureus* ATCC 25923, using 5000-625 µg/ml concentrations. The values indicated were the following: antioxidant activity of aqueous extract of freeze-dried pomegranate peels 595.71 ± 0.2 µmolTrolox / g (DPPH) and 1056.34 ± 0.4 µmolTrolox / g (ABTS);the ethanolic extract showed 586.96 ± 0.1 µmolTrolox / g (DPPH) and 1035.98 ± 0.2 µmolTrolox / g (ABTS), while the aqueous extract of dried peels at 60 ° C presented 351,36±0,2 µmolTrolox/g (DPPH) and 871.73 ± 0.2 µmolTrolox / g (ABTS), and ethanolic extract with 359.21 ± 0.1 µmolTrolox / g (DPPH) and 885.04 ± 0.1 µmolTrolox / g (ABTS). Similar studies have indicated that pomegranate extracts are effective in inhibiting the growth of *Escherichia coli* and *Staphylococcus aureus*. In this study, the aqueous and ethanolic extracts revealed no inhibitory and bactericidal activity for *E. coli* and *S. tyhimurium*; in addition, despite the higher antioxidant activity present in the extracts of lyophilized peels, no better antimicrobial results were pointed. All extracts indicated bactericidal activity for *S. aureus* at a minimum concentration of 650 µg / ml of extract.

1. Introduction

Pomegranate *(Punica granatum* L.) is a fruit native to the Middle East, belonging to the Punicaceae family; it is rich in phenolic compounds with strong antioxidant activity in vitro (Saxena and Vikram, 2004). Pomegranates are popularly consumed in its in natura form, like juices, foods such as jams, jellies and extracts, (Goula and Adamapoulos, 2012) which are used as plant ingredients in natural medicine and dietary supplements (Mohagheghi et al. 2011);. Pomegranate peels are considered inedible parts or byproduct obtained through juice processing (Negi et al. 2003) it is characterized by significant presence of ellagitannins and polyphenols, gallic acid and ellagic acid (Faria and Calahau, 2010) as well as flavonoids –associated with biological properties such as antioxidant and antimicrobial agents (Glazer et al. 2012).
According to Garau et al. (2007), recent decades researchers have given special attention to the use of industry-derived residues, especially the food industry, both for the reduction of the environmental damage caused by them and consequent reduction of costs to the treatment process, and for their potential use in the development of high value-added products. Recent studies have revealed characterization and extraction techniques as well as the biological potential of agro-industrial residues such as antioxidant biomolecules obtained through simple extraction from chestnut and hazelnut shells (Nazzaro et al. 2012), the drying temperature influence on the physico-chemical characteristics of dietary fibers and antioxidant capacity of orange byproducts (Garau et al. 2007), tannin extraction from nuts residues (Capparucci et al. 2011) and antioxidant activity of extracts of pomegranate peels compared with pomegranate juice extracts (Li et al. 2006). Pomegranate juice manufacturing industries generate large amounts of byproducts leading to serious environmental pollution in addition to acting as a substrate for the proliferation of insects and microorganisms.

Processed pomegranate peels usually have a high amount of moisture (approximately 66.3%) and moisture must be removed prior to production of high added-value products such as flavonoids and tannins. Drying has always been of great importance for the preservation of agricultural products and byproducts (Sánchez-Zapata et al. 2009).
The development of optimization systems for the recovery of valuable compounds may contribute to the reduction of residue; however, as a preliminary step, these byproducts require characterization in order to optimize the recovery processes.
Our study aimed at evaluating the implementation of two drying methods: conventional oven drying and freeze-drying, or lyophilization, on the antioxidant and antimicrobial potential of aqueous and ethanolic extracts of the pomegranate peel.

2. Experimental

2.1Collection of samples

Pomegranates were collected from local markets in Maringa, in the Brazilian state of Paraná, in September 2013. Immediately after collection, the fruits were taken to the Laboratory for Physico-chemical Analyses of Foods, of the Department of Food Engineering of the State University of Maringa, where a cleaning process with running water was performed as well as the sanitization of the fruits with a sodium hypochlorite solution in 20 ppm for 15 minutes. The pomegranate peels were separated by hand into approximately 2 centimeter pieces and then washed in deionized water, dried at room temperature and kept away from light, followed by storage in vacuum laminated packaging and frozen at -20 ° C until the moment of processing and analysis. The figure 1 shows a chart of preparation process.

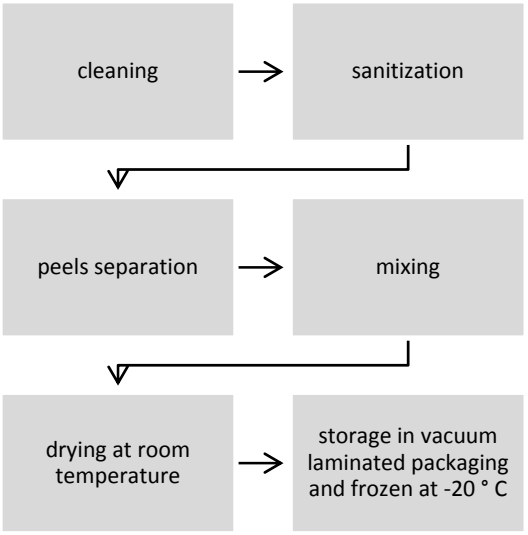

Figure 1: Preparation process from fruit to dried frizzed peels

2.2Oven drying and freeze-drying

A hundred grams of pomegranate peel fractions were used in triplicate for the drying processes; the oven drying process involved the peel samples arranged in disposable aluminium trays, with approximately 1 cm spacing between the pieces and dried in an air circulation dehydration equipment (Pardal TechnologyforAgroindustry) at a temperature of 60 ° C (± 1˚) until the peels obtained 0.3 water activity and moisture below 3% (12 h drying). For the lyophilization, or freeze-drying process, the pomegranate peels were processed in a Freeze Dryer Alpha 1-LD 4 plus Christ at -50˚C and 0.040 mbar over a period of 24 h, considering the same parameters for the activity and moisture in the oven drying process. After processed, the dried peels were ground in a Wiley mill (Marconi), standardized at a 60 mesh particle size and stored in vacuum laminated packaging to freeze at -20˚C until the moment of processing and analysis. Figures 2 and 3 show respectively the oven dry process and lyophilisation's dry process.

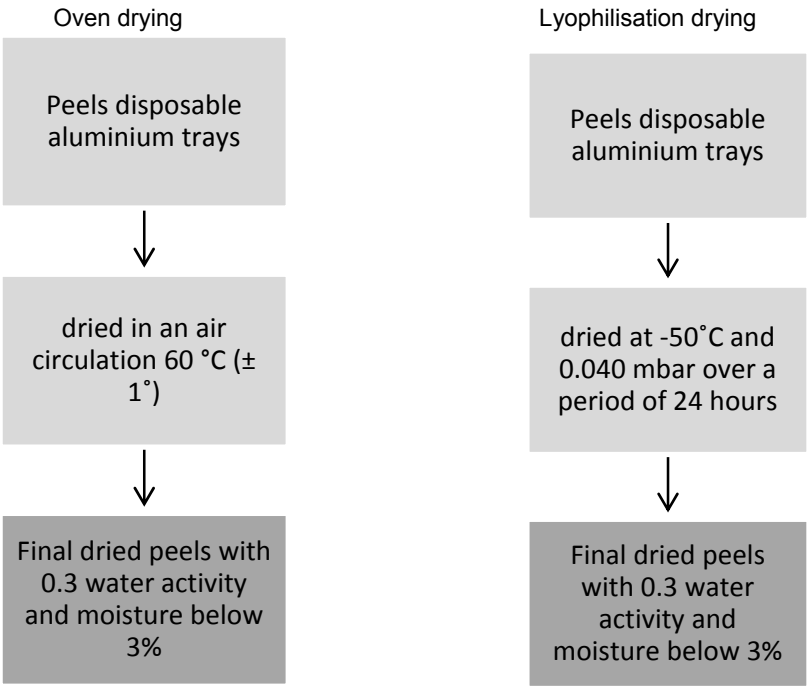

Figure 2: The oven drying process and the lyophilisation drying process

2.3Preparation of extracts

For the preparation of the extracts, the ground peels were homogenized with the solvents of deionized water and ethanolic solution at 80% at the 1:20 ratio, allowing the mixture to stand for a period of 48 hours, away from light, at room temperature. The yellow colored supernatant liquid was centrifuged (Centribio) at 3500 rpm for 15 minutes; the supernatant was vacuum filtered in a Büchner funnel with a 0.5µm filter (Zeta Plus).The filtrate was rota-vaporized (Büchi RE 120) at 50 ° C, lyophilized and stored in an amber bottle, freezing at -20 °C, identified as follows: aqueous lyophilized peel extract (LA), lyophilized peel ethanolic extract (LE) aqueous extract (60A) and ethanolic extract (60E) of peels dried at 60˚C. Figure 3 shows the process to extract bioactivities.

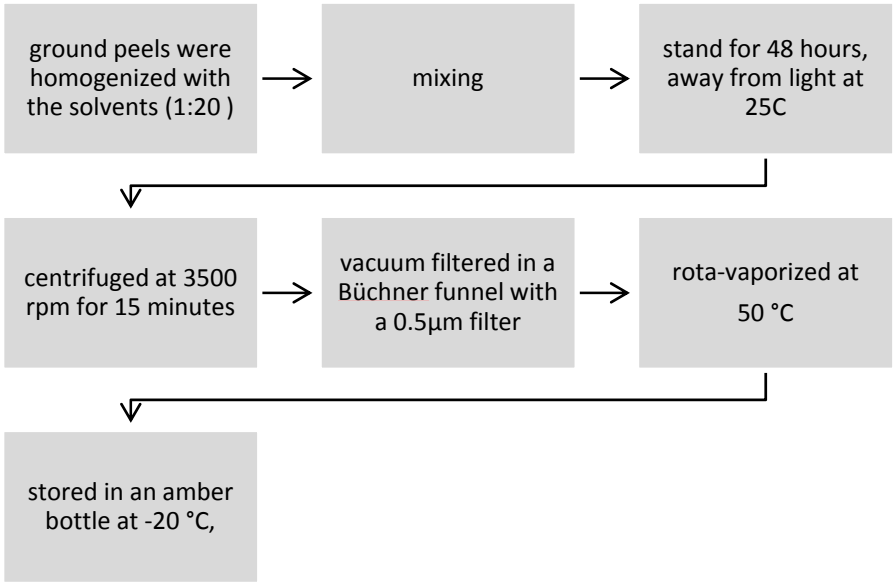

Figure 3: Process to obtain extracts

2.4Antioxidant activity: DPPH and ABTS

Determination of antioxidant activity using the capture of the free radical 2,2-diphenyl-1-picrylhydrazyl (DPPH) (Brand-Williams et al. 1995) was performed in test tubes with a 2.5ml (100µg / ml) extract, added with a 1 mL DPPH 0.3 mmol / L, homogenized through vortexing and kept away from light for 30 minutes. The reading was performed in a spectrophotometer at 518 nm; a blank sample was employed to eliminate the influence of the sample coloring, homogenizing 2.5 mL of the extract and 1 mL of the solvent used in the extraction and a negative control with 2.5ml of the solvent used in the extraction, and 1 mL of the DPPH solution. The antioxidant activity is expressed in Trolox equivalent/ g extract. Trolox. TroloxCalibration curve 0- 75µmol / L, $R^{2:}$ 0.997.

Determination of antioxidant activity using the capture of the radical 2,2-azino-bis (3-ethyl-benzthiazoline-6-sulfonic acid (ABTS) (Rufino et al. 2007) with30µl extract (100µg / mL) added with 3 ml of the ABTS radical solution (obtained by mixing 5 ml of 7 mM ABTS stock solution with a 88µl potassium persulfate solution kept in the dark at room temperature for 16 h and diluted in ethyl alcohol to obtain an absorbance of 0.70 ± 0.05 nm to 734 nm). After homogenization in vortex, the reading was performed in a spectrophotometer at 734 nanometers. The antioxidant activity is calculated in Trolox equivalent / g extract. Trolox Calibration curve (500 to 2000mMol / L). $R^{2:}$ 0.994.

2.5Antimicrobial Activity

To assess the antimicrobial activity of the aqueous and alcoholic extracts of the pomegranate peel, we used microorganisms *Escherichia*coli ATCC 25922, *Salmonella typhimurium* ATCC 14028 and *Staphylococcus aureus* ATCC 25923 according to methodology proposed by the Clinical and Laboratory Standards Institute (2012) using ampicillin as positive control and extracts at concentrations 5000-625 µg/ml.

2.6Statistics Evaluation

The statistical analysis was performed using analysis of variance (ANOVA) and Tukey's test ($p > 0.05$) using STATISTIC 6.0 (2001) software.

3. Results and discussion

3.1Drying yield and extraction yield

The drying process yield corresponds to the dry weight and wet weight ratio. The drying processes in this study presented a 38.3% (± 0.81) yield, and a 32.6% (± 0.93) yield for oven drying at 60 ° C and freeze drying, respectively. The yield of the aqueous and ethanolic extraction in both treatments presented values above 40%;an approximate value had been found by Li et al. (2006) through extraction with a mixture of solvents methanol, ethanol and water, with31.5% of extraction yield and Ventura et al., (2013) with 50% of aqueous extract yeld. However, Singh et al. (2002) used a extraction process with agitation at 30ºCfour one hour, and found values of1.4% yield for ethanol extract; 7.53% for aqueous extract, and 9.38% for methanol extract.

3.2Antioxidant activity

The antioxidant activity of pomegranate peel extracts are presented in Table 1. According to Moure et al. (2001), the quality of natural extracts and their antioxidant performance is dependent upon not only the original plant quality, geographical origin, climate, date of harvest and storage, but also on environmental and technological factors of equal effect on the antioxidant activities of the residual sources. The solvent extraction technique is the most commonly employed for the isolation of antioxidant plant compounds; however, the extraction yield and the antioxidant activity of plant material are strongly dependent upon the nature of the extraction solvent due to the presence of different antioxidant compounds of varying chemical characteristics and polarities, which may or may not be soluble in a given solvent (Sultana et al, 2009). Thus, the results indicate a higher antioxidant activity for the ABTS method, and a significant drop for the drying treatment at 60°C relating to the same freeze-dried material. Unlike results presented in this study, Ventura et al. (2013), by adding aqueous extract of pomegranate peel in pomegranate juice jelly, was able to increase the antioxidant capacity of its products with very similar values between DPPH and ABTS methods, while the antioxidant activity of its aqueous extract is higher using ABTS than DPPH method. Borgo et al. (2010) dried leaves of Baccharis *articulata (Lam.) Pers., Asteraceae* using drying process at room temperature (25 °C ± 3 °C), air circulating oven equipment (40 °C) and micowaves oven (power of 900watts (W), operating at a frequency of 2450 MHz, and found the highest concentration of flavonoid detected in oven-dried samples with no significant difference in the pharmacological activity of all analyzed samples.

Table 1: Antioxidant activity of pomegranate peel extracts.

Sample	DPPH (µmolTrolox/g)	ABTS (µmolTrolox/g)
LA	595,71±0,2^{b}	1056,34±0,4^{a}
LE	586,96±0,1^{b}	1035,98±0,2^{a}
60A	351,36±0,2^{a}	871,73±0,2^{b}
60E	359,21±0,1^{a}	885,04±0,1^{a}

Table 2 shows the data for antimicrobial activity of pomegranate extracts. None of the extracts showed inhibitory and bactericidal activity for *E. coli* and *S. tyhimurium*. Activity was present only for *S. aureus* in the minimum concentration of 650 µg/mL of the extract. Similar studies have indicated that pomegranate extracts are effective in inhibiting the growth of gram-positive and gram-negative bacteria, especially *Escherichia coli* and *Staphylococcus aureus*.

Table 2: Minimum Inhibitory Concentration of pomegranate peel extracts (µg/ml).

Sample	*Escherichia coli*	*Salmonella typhimurium*	*Staphylococcus aureus*
LA	nd	nd	625
LE	nd	nd	625
60A	nd	nd	625
60E	nd	nd	625

nd= Not detected.

Previous research points to bactericidal activity against *S. aureus* with methanolic extracts of pomegranate peels at a concentration of 1% and inhibition of the production of the staphylococcal toxin A at 0.01% (Braga et al., 2005).There is also inhibition of S. aureus in the application of 0.01% of pomegranate peel aqueous extract, but failure to inhibit E. coli at the highest concentration used, 0.1% (Oliveira et al., 2009).

4. Conclusions

Our study indicated that conventional drying process causes a significant loss of the antioxidant capacity of extracts compared with freeze-drying process without having the data interfering with the antimicrobial test performance.

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