Isolation of phenolic substances from pomegranate residues

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INTRODUCTION

In recent years, there has been an increase in the consumption of pomegranates (*Punica granatum L.*) due to their high concentrations in ingredients of significant antioxidant activity such as tannins, phenolic acid, and flavonoids (i.e. anthocyanins etc.) [1]. Apart from their direct consumption or juice production, the interest is also focused on the isolation of bioactive substances from the fruit of pomegranates or their residues. The combination of advanced physicochemical separation processes is widely employed for the isolation of substances of high added value at high purity [2]. Apart from the worldwide need to decrease waste and increase process sustainability via isolation of significant by-products, nowadays there is a great demand in greener fuels such as bioethanol. The production of bioethanol is done either by fermentation of hydrolyzed corn starch or sucrose juices or by fermentation of a substrate such as lignocellulosic biomass which is of lower cost. More specifically, the yeast *Saccharomyces cerevisiae* produces ethanol via sugars fermentation [3]. Herein, pomegranate residues extracted at appropriate conditions and after sieve filtration it was separated to liquid extract to be submitted to fermentation (LF) and residual pulp (RP). Next, a parametric study was conducted to optimize the extraction of total phenolic content (TPC) and carbohydrates from the RP. Total Phenolic Content (TPC) and carbohydrates were measured in: i) LF and ii) in RP which was separated to liquid (LP) and humid solid (HS). HS was further submitted to extractions under various conditions.

MATERIALS AND METHODS

Frozen pomegranate residues were thawed at 4ºC for 15-20 h and they were cut in cubic pieces (~1x1 cm) and they were ground at *ca.* 3 mm. Next, 50 g of the ground residues were put in 250 mL Duran bottles and 125 mL of H_2SO_4 solution 0.05M (95-97%, Sigma Aldrich) were added in each bottle. The bottles were placed in an autoclave for extraction/hydrolysis at 121ºC, 1 atm and for 20 min. The samples were cooled at room temperature, and they were sieve-filtrated (sieve opening: 1 mm) and separated to liquid (LF, liquid to be used as raw material for the preparation of fermentation substrate) and residual pulp (RP). The RP was vacuum filtered, and it was separated into 50% of humid solid (HS) and 50% of liquid phase (LP). The HS (humidity 73.1%) was further submitted to extraction under various conditions. First, extractions were done using water as solvent, at room temperature, under stirring for 30 min and for various solid/liquid volume ratios (100, 200 and 400 g/L). Next, 200 g/L were extracted under stirring, for 30 min and using water as solvent at various temperature conditions (20, 40, 60 and 100ºC). Followingly, the effect of solvent was tested by conducting extractions of 200 g/L solid/solvent, under stirring, for 30 min at ethanol/water volume ratios 0, 40, 70, 99 % v/v. Next, extractions were done using 200 g/L solid/solvent under stirring and at room temperature, using water for solvent for various time durations (15, 30 and 60 min). Furthermore, the effect of double extraction process with fresh solvent was tested with extraction of 400 g/L of solid/solvent, using water as solvent at 25ºC for 30 min at each extraction. Total phenols were measured using the Folin-Ciocalteu method. L-tryptophan reagent was used to measure the total carbohydrates concentration. A UV-Vis spectrophotometer (Shimadzu UV-1601) was used for both measurements [2].

RESULTS AND DISCUSSION

High concentrations of TPC and carbohydrates were found in LF and LP. TPC was equal to 5073mg/L in LF and 5350mg/L in LP, while carbohydrates were 25358mg/L and 30352 mg/L correspondingly. HS was further submitted to extractions and Table 1 summarizes the measured values. First, the effect of the solid/liquid waste was tested, and it was found that the higher concentrations were obtained for the lower solid/liquid ratio (i.e. 100 g/L). Next, the effect of temperature was tested, and it was concluded that both TPC and carbohydrates were increased with increasing temperature. The temperature of 100ºC may involve experimental errors due to condensation effect. Concerning the effect of used solvent, TPC was maximized for ethanol/water ratio volume equal to 0.7, while carbohydrates were maximized at the lower ratio of ethanol/water volumes (i.e. 0.4 v/v). The duration of the extraction process did not strongly affect

the concentration of TPC and carbohydrates. Concerning the effect of a second extraction with fresh solvent, TPC was 2.5 times lower at the second extraction than in the first extraction, while carbohydrates were 17 times lower.

Table 1. TPC and carbohydrates concentrations obtained from extractions at various conditions.

Tested Parameter		TPC (g/Kg)	Carbohydrates (g/Kg)
Ratio solid/liquid (g/L):	100	5.98	21.71
	200	4.82	19.37
	400	3.86	17.17
Temperature (ºC):	20	4.82	19.37
	40	6.32	19.7
	60	8.16	22.15
	100	10.05	30.44
EtOH Percentage (%):	0	4.82	19.37
	40	8.89	22.44
	70	9.22	17.58
	99	7.51	18.3
Duration of extraction (min):	15	4.73	18.97
	30	4.82	19.37
	60	4.97	20.38
Sequence of extractions:	1	3.86	17.17
	2	1.36	4.22

CONCLUSION

Pomegranate residues were extracted, and after sieving, LF was separated by the RP which was further separated to liquid (LP) and solid (HS). High concentrations of TPC and carbohydrates were found in both liquids (LP and LF). The HS was extracted under various conditions. The extraction process was found to be optimized for 40-70 % v/v ethanol/water, at 60ºC, and for a ratio solid/waste equal to 100g/L. So far, it was found that significant quantities are included in RP and in a next step, High Performance Liquid Chromatography (HPLC) shall be employed to identify the substances. Isolation and quantification of TPC and carbohydrates in fermented liquid (after ethanol removal) will also be among the objectives of future research.

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