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COLLOIDAL GAS APHRON EXTRACTION OF BIOACTIVE COMPOUNDS FROM BRAZILIAN PINOT NOIR GRAPE POMACE

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ABSTRACT – Residues from the food industry are a problem worldwide. Alternatives have been developed in an attempted to extract compounds from fruit pomace in other to apply those as a natural component in different products. This work has the objective to produce two extracts: (1) ethanolic extract and (2) hot water extract; and characterize the chemical composition of these extracts for future application. In sequence an extraction using surfactants (Colloidal Gas Aphron) was applied in order to obtain a more concentrate and pure extract, in a foam form with better physical properties. The ethanolic extraction was more efficient, obtaining 4 times more bioactive compound (in 100g of pomace: 2670.63 mg Gallic Acid; 65.70 mg anthocyanins and 45,564.78 mmol of Trolox). However the CGA was able to concentrate more the compounds from hot water extract, 61.62% of phenolics compounds were concentrated in the Aphron phase.

KEYWORDS: Colloidal Gas Aphron, grape pomace, phenolics, antioxidante capacity, anthocyanins.

1. INTRODUCTION.

Grape is one of the largest fruit crops in the world, and about 80% of the production goes to winemaking industry (Antoniolli et al., 2016). Wine and grape juice industry has as main residue the grape pomace, which is a solid byproduct that represents approximately 30% (w/w) of the grape amount used in the process. Resulting in a management issue both ecologically and economically (Drosou et al., 2015). Brazil is the world's third highest producer, with approximately 290,000 tons of grape pomace per season (Sousa et al., 2014).

Usually grape pomace is destined to animal feed and composting (Tournour et al., 2015). However, grapes are known by their high phenolic content, and a large fraction of this is retained in the pomace after winemaking process. Based on that, the recovery of these compounds are of great

interest for food, pharmaceutical and cosmetic industries (Tournour et al., 2015; López-Miranda et al., 2016).

An alternative method to extract and concentrate biocompounds such as phenolics, is Colloidal Gas Aphron (CGA). CGA are microbubbles (10-100 µm) made of surfactant generated by intense stirring (8,000 rpm). Surfactants has the ability to interact with surfaces and form micelles, hexadecyltrimethylammonium bromide (CTAB) and polyoxyethylenesorbitan monolaurate (Tween 20) are examples of cationic and nonionic surfactants respectively (Santos-Ebinuma et al., 2016). Studies shows several applications for CGA: water purification, soil remediation, protein recovery and biological recovery (Molaei & Walters, 2015).

Based on that, this work had the objective to apply Colloidal Gas Aphron method to extract and concentrate biochemical compounds from hot water and ethanolic extract from Brazilian Pinot noir grape pomace.

2. MATERIAL AND METHODS

2.1 Extractions methods

A hot water and an ethanolic extraction were conducted in order to obtain relevant chemical compounds from the blackcurrant pomace flour. The hot water extraction used water as solvent, the solvent:solute ratio was 12:1, the temperature used was 100^{o}C during 1 hour. The ethanolic extraction used pure ethanol as solvent, with a solvent:solute ratio of 8:1, at 60^{o}C for 2 hours. Extractions were conducted in water bath.

2.2 Extracts profile

The extracts were analysed according to total phenolic content, total anthocyanins and antioxidant capacity. All analysis were performed in triplicate. The methods used are described below.

Antioxidant Activity - ABTS free radical-scavenging activity (Re *et al*., 1999). Extracts were diluted 1:10 for this analysis. The radical ABTS was generated by persulfate oxidation of ABTS. A mixture of ABTS (7.0mM) and potassium persulfate (2.45 mM) formed the radical cation ABTS. A solution of this radical and ethanol had the absorbance measured at 734nm. An aliquot of 20µL of the diluted sample reacts with the radical solution, and the decrease of absorbance was measured at 734 nm. Trolox was used as a positive control, and water as a negative control.

Total Phenolics – Folin Ciocalteau method (Singleton & Rossi, 1965; Georgé *et al*., 2005). Extracts were diluted 1:10 for this analysis. A 200 µL sample aliquot was mixed with Folin Ciocalteau reagent, sodium carbonate buffer (20%) and distilled water. After 2 hours the absorbance was measured at 760nm. A standard curve using gallic acid was prepared, and the result was expressed as mg gallic acid equivalent / grams of pomace flour.

Total anthocyanins - Determined using the pH differential method (Lee *et al*., 2005), where the absorbance was measured at 520 and 700 nm. Extracts were diluted 1:10 for this analysis. An aliquot of 2g measured using a balance, were diluted using both buffer Potassium Chloride (pH 1.0) and Sodium Acetate (pH 4.5). After 20 minutes samples were read in a spectrophotometer. The results are expressed as mg/100g of pomace.

2.3 Extracts storage stability

To determine the difference between hot water and ethanolic extracts, they were stored for 2 weeks in fridge. The total anthocyanin content stability was observed, and the difference between hot water and ethanolic extraction was investigated.

2.4 High performance liquid chromatography (HPLC) of hot water and ethanolic extract

After the extraction method previously described, the extracts were filtered using a 0.22µm. The mobile phase was 99.9% acetonitrile and 0.1% of Trifluoroacetic acid, with the following gradient: 0-10 min/ 0% B, 10-45 min/ 32% B, 45 – 50 min / 90% B, 50-60 min/ 90%, 60-65 min / 5%. The

analysis were done in an Agilent 1100 series HPLC system equipped with a diode array detector. Separation was performed on a Nova-Pak C18 column (250 X 4.6 mm).

2.5 Colloidal Gas Aphron extraction

Colloidal Gas Aphron (CGA) is a technique used for the extraction of chemical compounds. Based on the utilization of surfactants and its chemical interaction with the target compound. For this work two surfactants were used: Tween 20 and Cetyl trimethylammonium bromide (CTAB). The method consists in pumping the surfactant solution into a flotation column containing grape extracta. Ater 5 min agitation (8000 rpm), the surfactant solution is in a foam form. As the surfactant foam is pumped up throw the column, it carries chemical compounds. The conditions used for this experiment were in 60 mL of the extract into the column, and pump the surfactant foam at 27.8 rpm.

3. RESULTS AND DISCUTION

The profile of biochemical compounds for hot water and ethanolic extract of grape pomace had a significant difference. Ethanolic extraction was about 4 times more efficient than hot water (Table 1).

Table 1: Bioactive compounds profile of hot water and ethanolic extracts from brazilian Pinot noir grape pomace.

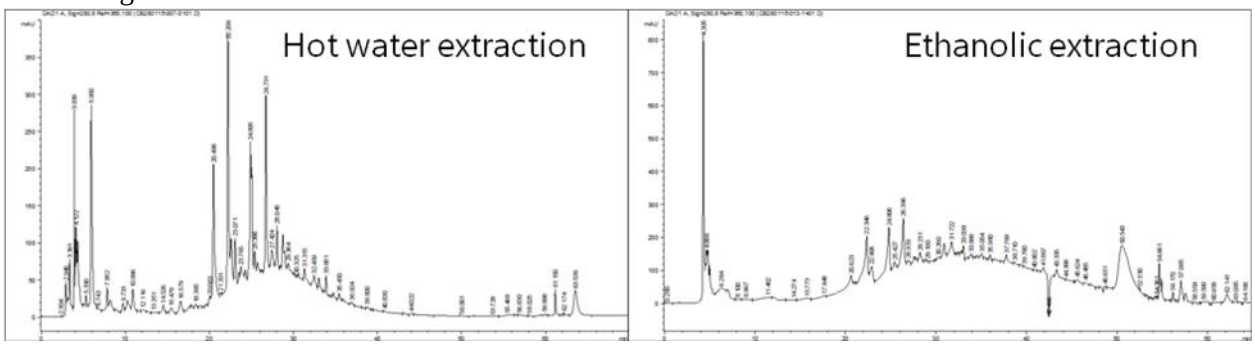
Extraction method	Total phenolics (mg/100g pomace)		Total anthocyanins (mg/100g pomace)		Monomeric anthocyanins (mg/100g pomace)		Antioxidant capacity (mmol Trolox/ 100g pomace)	
	average	SD	average	SD	average	SD	average	SD
Hot water extraction	625.33	99.61	15.30	0.77	67.59	4.31	14,558.33	9,015.49
Ethanolic extraction	2,670.63	28.41	65.70	0.72	283.70	4.72	45,564.78	5,392.66

*SD: Standard deviation

Ethanol is the most used solvent to extract phenolics and anthocyanins, among the advantages it could be highlighted that it is a GRAS organic solvent and a low cost reagent.

The higher efficiency of the ethanolic extraction was also proved by HPLC analysis (Figure 1).

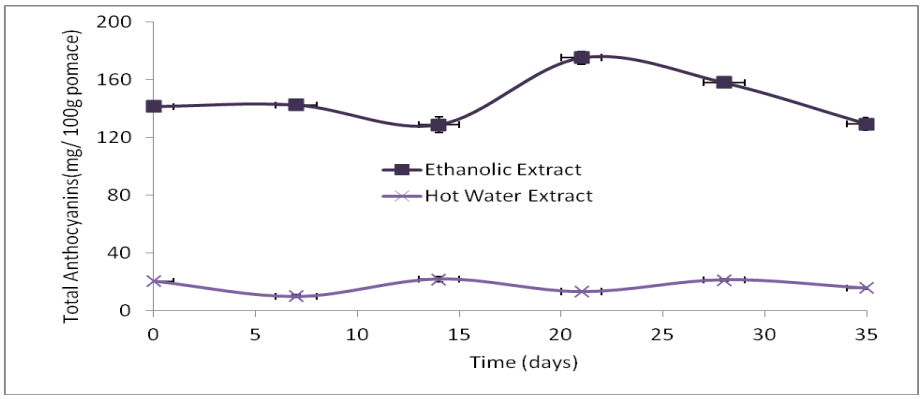
Figure 1: HPLC comparative analysis between hot water and ethanolic extract from grape pomace. Chromatograms from 280nm.



In general more compounds and in a higher amount was extracted using ethanol, however some specific compounds were more extracted using hot water. In this way hot water extraction could be more selective and specific than ethanolic extraction.

In order to analyse the stability of anthocyanins in both extracts, a 5 week storage test was conducted. The results (Figure 2) showed that both extracts were stable during 7°C storage. This information is relevant to the potential to use those extracts as a food ingredient.

Figure 2: Total anthocyanins stability from grape pomace hot water and ethanolic extract during 5 weeks at 7°C.



In order to concetrate the phenolics compounds a CGA method was applied (Figure 3).

Figure 3: CGA extraction of phenolics compounds from grape pomace ethanolic extract.

The CGA application allows to concetrate the phenolics compounds in the Aphron phase. Tween 20 had a better recovery when applied to hot water extraction, probably due to a negative interation beetween Tween and ethanol interfering in the Aphron stability. CTAB had also a higher recovery when applied to hot water, however as it is not a GRAS reagente, it could only be used for cosmetic and pharmacological industries.

Table 1: Recovery of phenolics compounds using Colloidal Gas Aphron methodology.

Extraction methods	Phenolics recovery	
	Tween 20	CTAB
Hot water extraction	61.62%	67.58%
Ethanolic extraction	33.20%	14.96%

4. CONCLUSION

The ethanolic extract was more selective than the hot water, and the compounds extracted by this method were more concentrated. An important result of the ethanolic extraction is the slightly higher colour stability of this exract during storage over 5 weeks at 7 °C.

The CGA extraction is an advantage for the final application because it could improve the solubility and the stability of chemical compounds. Further studies should focus on investigating these advantages. Based on the differences presented for each extraction and the advantages and disadvantages of each surfactant, the choice should be according to the final application.

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