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EVALUATION OF POLYPHENOL EXTRACTION FROM APPLE POMACE

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ABSTRACT

Food industry is searching for natural additives because consumers demand natural, safe and environmentally sound food additives. The use of different plant extracts such as grape pomace and cranberry extracts which have antimicrobial effect in food preservation has gained an increasing interest. In the latest years many researches deal with investigation of the antimicrobial effect of apple pomace extracts. Apple pomace, a residue from apple juice production, contains high amount of polyphenols which are known to have antioxidant effect. Apple pomace is a by-product of the apple juice and cider processing industry and represents about 20–35% of the original fruits. It can be used for value-added products, because it is rich in pectin, fibre, macro and micro elements and antioxidants mainly, polyphenols. Previous studies indicate that the apple pomace has high phenolic content and antioxidant activity and thus can be regarded as a valuable source of antioxidants and bioactive compounds. The contents of phenolic compounds vary greatly among different varieties of apples. Moreover, apple peels contain higher concentration of phenolic compounds compared to flesh. Conventional production of apple juice results in a juice with poor phenolic content and with only 3–10% of the antioxidant activity of the fruits that they are produced from. Thus, leaving huge amount of the phenolic compounds in the pomace. Because of this, studying extraction method for extracting high amount of polyphenol from the pomace is important. In this paper apple pomace was dried with different drying method (atmospheric and vacuum ovens, 80° C and 60°C). Extraction was performed using distilled water as solvent, and the extracts were evaluated by the colour, water soluble sugars, total polyphenol content (TPC) and antioxidant capacity.

Keywords: *Food waste, Apple pomace, Drying, Extraction, Antioxidant activity.*

INTRODUCTION

Apples (*Malus domestica* Borkh) can be processed into numerous commercial products, such as apple juice, apple compote, jams and jellies. Apple juice is loved by many people all over the word. Apples contains a lot of polyphenols and they are retained in the pomace during juice processing (Van Der Sluis *et al*., 2002; Van Der Sluis *et al.*, 2004).

Apple pomace is the solid residue that remains after milling and pressing apples for juice (Givens *et al*., 1987; Kafilzadeh *et al.*, 2008). It contains flesh and skin (94.5%), seeds (4.1%) and stems (1.1%) (Linskens & Jackson, 1999, Sudha *et al.*, 2007) and represents 20–35% of the fresh weight of the apples (Rabetafika *et al*., 2014). According to the amount of raw apple processed, 3.3 million tons of waste are produced per year.

Apple pomace has a variety of utilization, such as production of ethanol, methane, pectin, citric acid, fibres, direct burning, food additives (pomace in jams, sauces, confectionery products, toffees and bakery products), and livestock feed (Shalini *et al*., 2010; Wang & Thomas, 2008).

Fresh apple pomace spoils quickly, since they contain large amount of water and because of this, drying is necessary. Drying of the pomace is an important technical step required during the extraction process where most of the bioactive compounds are extracted better on dried pomace rather than fresh (Jung *et al*., 2015).

In recent years, interests in extracting phenolic compounds from apple pomace has increased. The pomace can be used as cheap alternative source of polyphenols (Bhushan *et al*., 2008; Cetkovic *et al*., 2008; McCann *et al.*, 2007; Soler *et al*., 2009). Several studies have shown that the phenolic compounds from the pomace are extractable using organic solvents, such as methanol, acetone and ethanol (Ajila *et al.*, 2011; Hayat *et al*., 2010 Reis *et al.*, 2012; Suárez *et al*., 2010; Van Der Sluis *et al*., 2004). However, water can also be a suitable solvent in extracting phenolic compounds from the apple pomace (Çam & Aaby, 2010; Reis *et al*., 2012, Vincent *et al.*, 2015). It has a lot of advantages: cheap, non-toxic, easily obtained and there is no aversion from the consumers.

The objective of the present paper is to evaluate the effect of drying method on polyphenol extraction from the apple pomace.

MATERIAL AND METHODS

Apple pomace from industrial juice production were obtained from Agrana Juice Ltd (Hungary).

Drying was done using the conventional oven (LP 232/1, Hungary), 200g of the pomace were spread in a drying tray; 0.5 cm pomace depth layer. Trays were then taken to the oven and dried at 60°C and 80°C and the moisture content was being monitored every hour. For a vacuum oven drying, 200g of the samples were first dried using a conventional oven to almost 10 percent moisture content at 80°C and 60°C. Thereafter, samples were dried using vacuum dryer at 60°C and a pressure of 65 mbar, moisture content was monitored every hour until reached 3 – 4 %. Dried samples were ground into fine powder using a “PRINCESS” multi chopper and

grinder and thereafter they were vacuum packaged until the day of extraction. Determination of moisture content was performed by using a MAC-50 rapid moisture analyzer (Radwag Waagen GMBH, Hilden, Germany). To determine the water activity, Novasina, LabMaster-aw equipment was used.

Pomace extracts were obtained by adding 15 g of apple pomace into deionised water (450 ml distilled water (1:30 w/v)) at room temperature. The mixtures were placed into a sonication bath (Bandelin, RK 52), at 35 kHz for 1 hour. Obtained solution was filtered by Whatman filter paper No.1, using vacuum pump. Solvent from the obtained filtrate was removed using rotary evaporator (IKA, Model: RW 10C S99) and further removed on circulating air oven (60°C) in a petri dishes. Weight of the obtained extracts was determined and diluted accordingly with distilled water to obtain a final extract solution with a concentration of 200mg mL^{-1}.

Total phenolic contents were determined using the Folin–Ciocalteu colorimetric method as described by Singleton & Rossi (1965). Briefly, 1250µL of Folin reagent (1:10 v/v Folin; distilled water) was added in the test tube followed by 150µL of methanol (4:1 v/v methanol; distilled water). Then, 100µL of the sample was added and allowed to stand for 1 minute, followed by the addition of 1000µL of sodium carbonate. The results were expressed in gallic acid equivalents (GAE, µg/mL pomace extract).

Antioxidant capacity was determined using Ferric Reducing Ability of Plasma (FRAP) assay (Benzie & Strain, 1996). FRAP reagent was prepared by using acetate buffer (pH 3.6), 2, 4, 6-tripyridyl-s-triazine (TPTZ), and $FeCl3 \times 6\ H2O$. The reagent and samples were mixed thoroughly in the tube. The absorbance was taken at 593 nm after 5 min. Results were expressed as ascorbic acid equivalent (mg ascorbic acid/mL extract) using an ascorbic acid standard calibration curve. Colour was determined according to C.I.E.LAB system using a tristimulus colorimeter (Konica Minolta CR 410, Minolta Canada Inc.).

Statistical analysis was performed using one factor complete randomized ANOVA using IBM SPSS version 20.

RESULTS AND DISCUSSION

The data of drying apple pomace using atmospheric oven at different temperatures and atmospheric oven combined with vacuum oven is shown in Fig.1. Depending on operational conditions (atmospheric or vacuum), air velocity (1–2 m/s), temperature (60–105 °C), and amount of sample, apple pomace drying takes about 3–10 h (Wang et al., 2007). Raw apple pomace had an initial moisture content of 69 %, and drying procedure was continued until the final moisture content of the sample was reached (2.94 - 4.28%; 3 h and 6 h) (Table 1.). In case of atmospheric drying at 80°C during the first 2 h (to 11.67-17.17%) the moisture content of apple pomace decreased quickly. Thereafter, moisture content decreased slightly.

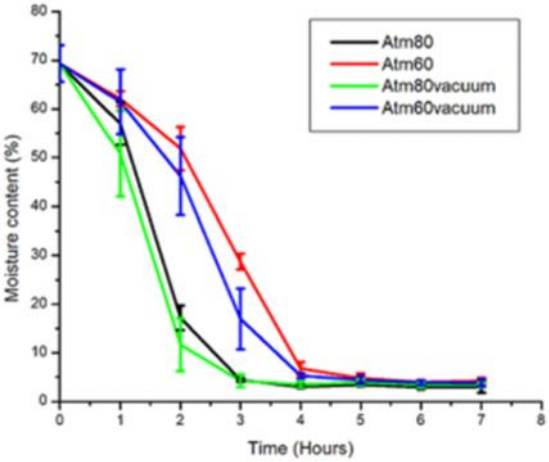

Figure 1. Decrease of moisture content during drying of the apple pomace (Gonelimali et al., 2021).

Atm80 and Atm60 stand for drying the pomace at 80 and 60 using conventional atmospheric oven respectively. Atm80vacuum and Atm60vacuum represent drying the pomace at 80°C conventional oven plus vacuum drying and 60°C conventional oven plus vacuum drying respectively.

Table 1. Shows time, final weight and percentage recovery after drying the pomace using a conventional oven and a combination of a conventional oven with vacuum drying to reach a moisture content of about 4%. The final weight was the highest in case of using 60°C atmospheric drying, and the smallest weight was obtained in case of 80°C atm+ Vacuum drying method, however, there was no significant difference. The recovery shows similar tendency. The highest recovery % was achieved using 60°C atmospheric drying, and the smallest recovery was in case of 80°C atm+ Vacuum drying method.

Table 1. Drying time, final moisture content, final weight and percentage recovery of the pomace using different drying method (Gonelimali et al., 2021)

Drying method	**80°C atm.**	**60°C atm.**	**80°C atm. + Vacuum**	**60°C atm. + Vacuum**
Time (hours)	3	6	3	6
Final Moisture content	2.94[a]	4.28[a]	3.42[a]	3.82[a]
Final weight (g)	41.92 ± 2.42	45.25 ± 1.26	39.97 ± 2.84	40.98 ± 2.34
Recovery (%)	20.96[a]	22.63[a]	19.99[a]	20.49[a]

Superscript letters, a: no different groups based on the statistical analysis by drying methods

The water soluble sugar content of the extracts were between 6.2-6.9%. According to the ANOVA results, the drying method had not significant effect on the water soluble sugar content (p=0.056).

Table 2 shows colour values of the extracts. L* defines the lightness of the pomace extracts. In case of Atm 80°C+vac drying method, the L* value is smaller than in the two other cases. In the case of a* values (green–red opponent colors, with negative values toward green and positive values toward red), values were in range of 2.51- 6.73.

The b* values (blue–yellow opponents, with negative numbers toward blue and positive toward yellow) were in the range of 5.75 - 14.83. For both values a* and b*, there is similar tendency as in the case of L*values. The effect of drying method on the colour values of the extracts was evaluated by one-way analysis of variance (ANOVA). The drying method had significant effect on colour values of the extracts (p=0.000).

Table 2. Color values of the extracts

	Atm_80	**Atm_60**	**Atm_80_vac**	**Atm_60_vac**
L*±SD	27.78 ± 0.94^{c}	32.00 ± 1.19^{a}	28.92 ± 0.0^{c}	30.47 ± 0.21^{b}
a*± SD	2.51 ± 0.60^{c}	6.14 ± 0.07^{a}	3.58 ± 0.11^{c}	6.73 ± 0.10^{b}
b*± SD	5.75 ± 0.72^{a}	14.55 ± 2.17^{b}	6.11 ± 0.44^{a}	14.83 ± 1.07^{b}

Superscript letters, a, b, c: indicate significance difference by drying methods

Table 3 contains the total phenolic content (TPC) of the apple pomace extracts. The TPC of the final apple pomace extract was the highest (479 μg mL^{-1}) in case of atmospheric drying at 80°C + vacuum drying, and was lowest when atmospheric 60°C was used (396 μg mL^{-1}). The effect of drying method and the TPC content of the extracts was evaluated by one-way analysis of variance (ANOVA). The drying method had no significant effect on TPC content of the extracts (p value at 95% confidence: 0.074).

Table 3. TPC content of the extract (μg mL^{-1} extract)

	Atm_80	**Atm_60**	**Atm_80_vac**	**Atm_60_vac**
TPC±SD	445 ± 107^{a}	396 ± 51^{a}	479 ± 16^{a}	410 ± 7^{a}

Superscript letters, a: no different groups based on the statistical analysis by drying methods

Table 4 shows the antioxidant capacity of the apple pomace extract expressed in ascorbic acid equivalent (μg ascorbic acid/mL extract). The highest antioxidant capacity was obtained when the sample was dried at 80°C using a combined atmospheric and vacuum oven. Lowest value was determined in samples dried at

80°C atmospheric oven. According to the statistical analysis, drying method has significant effect on the antioxidant capacity (p values at 95% confidence: 0.000). This shows the importance of optimizing the drying method since there was no significant difference on recovery of the apple pomace as well as total phenolic content when dried using different methods. However, there was a significant difference on antioxidant activity which is an important properties of the extracts.

Table 4. FRAP values of the extract (μg AS mL^{-1} extract)

	Atm_80	**Atm_60**	**Atm_80_vac**	**Atm_60_vac**
FRAP value ±SD	$496±36^{b,c}$	$426±86^{a,b}$	$521±166^{c}$	$374±79^{b}$

Superscript letters, a, b, c: different groups based on the statistical analysis by drying methods

CONCLUSIONS

This work is an important starting point to valorize apple pomace, a very cheap and common by-product that is obtained in tons during processing of the apples to produce juice. It has been demonstrated that different drying method has an effect on antioxidant activity of the extracts from the apple pomace. Drying the pomace at 80°C using conventional oven in combination with vacuum dryer or without, results in polyphenols with higher amount of antioxidant activity when water is used as an extraction solvent. Apple pomace could be considered as a valuable type of by-products, a source of polyphenols, which could be suitable for the functional foods. Comparison of water as extraction solvent with other organic solvents, further purification of the apple pomace, identification and stability examination of the phenolic fractions are necessary to be performed in further studies. Apple pomace should be regarded as a valuable product and has potential as a value-added ingredient for functional foods as natural antioxidants and functional food ingredients (jams, jellies, juices and biscuits).

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