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EFFECT OF DIFFERENT DRYING METHODS ON THE PHENOLIC EXTRACTION FROM BUTTERNUT SQUASH POMACE

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ABSTRACT

The objective of this study was to observe the effect of different drying methods (freeze drying, hot air, vacuum and microwave drying) on the color parameters, total phenolic content (TPC), total flavonoid content (TFC) and antioxidant activity (AA) of butternut squash pomace (BSP). In addition, the extraction conditions of phenolic compounds were optimized using the extract of freeze-dried BSP by Response Surface Methodology. The drying conditions were 30 and 60 °C for hot air and vacuum drying and 120 and 600 W for the microwave drying. TPC, TFC and AA of dried BSP were changed between 1.88 ± 0.01–4.86 ± 0.27 mg gallic acid equivalent/g dry matter, 1.32 ± 0.05–3.09 ± 0.29 mg catechin equivalent/g dry matter and 1.45 ± 0.15–4.27 ± 0.27 mg ascorbic acid equivalent/g dry matter, respectively. This study showed that dried BSP can be used as a functional ingredient due to its bioactive potential.

Keywords: Butternut squash pomace, drying, phenolic extraction, response surface methodology

FARKLI KURUTMA YÖNTEMLERİNİN BALKABAĞI POSASINDAN FENOLİK EKSTRAKSİYONU ÜZERİNE ETKİSİ

ÖZ

Bu çalışmanın amacı, farklı kurutma metotlarının (dondurarak kurutma, sıcak hava, vakum ve mikrodalga kurutma) balkabağı posasının (BP) renk parametreleri, toplam fenolik (TF) içeriği, toplam flavonoid (TFl) içeriği ve antioksidan aktivite (AA) üzerindeki etkisini gözlemlemektir. Ek olarak, fenolik madde ekstraksiyon koşulları Yanıt Yüzey Yöntemi ile dondurularak kurutulmuş BP ekstraktı kullanılarak optimize edilmiştir. BP kurutma koşulları; sıcak hava ve vakum kurutma için 30 ve 60 °C ve mikrodalga kurutma için 120 ve 600 W olarak belirlenmiştir. Kurutulmuş BP için; TF içeriği, TFl içeriği ve AA sırasıyla 1.88 ± 0.01- 4.86 ± 0.27 mg gallik asit eşdeğeri/g kuru madde, 1.32 ± 0.05-3.09 ± 0.29 mg kateşin eşdeğeri/g kuru madde ve 1.45 ± 0.15-4.27 ± 0.27 mg askorbik asit eş değeri/g kuru madde arasında değişmiştir. Bu çalışma kurutulmuş BP'nin biyoaktif potansiyeline bağlı olarak fonksiyonel bir bileşen olarak kullanılabileceğini göstermiştir.

Anahtar kelimeler: Balkabağı posası, fenolik ekstraksiyonu, kurutma, yüzey yanıt yöntemi

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INTRODUCTION

Butternut squash or pumpkin (*Cucurbita moschata*) is in the *Cucurbitaceae* family which consist vegetables in various shape, size, and color. Nowadays, pumpkin is cultivated all over the world but intensively in Europe, America and in Asia especially in India and China (Kulczynski and Gramza-Michałowska, 2019). Pumpkin is rich in carotenoids, phenols and flavonoids and moderate in fibers, vitamins, and carbohydrates (Ping et al., 2002; Seremet et al., 2016). Pumpkin can be added to daily diet due to its potential health benefits such as anti-diabetic, anti-inflammatory, anti-carcinogenic and antioxidant (Yadav et al., 2010).

The fresh-cut pumpkin is very prone to deterioration during storage; therefore, it is processed into puree, juice and as well dried products (Jamali et al., 2018). Butternut squash pomace (BSP) is a by-product mostly obtained from juice processing which consists of the skin, seeds and pulp. After processing of the butternut squash, the large quantity of pomace remains in the environment or utilized as an animal feed. However, it can be converted into a functional ingredient that can be added into different foods due to its bioactive content. Several studies reported that various pomaces (grape pomace, apple pomace and bayberry pomace) obtained from the processing of fruits and vegetables were rich in phenolics (Bao et al., 2020; Wang et al., 2018; Zhou et al., 2009).

BSP has high moisture content; therefore, it needs a preservation method to prevent the possible microbial growths and chemical degradations (Cano-Lamadrid et al., 2018). Freezing can be considered as one of the ways to store the pomace directly but a huge freezing area would be needed. Instead of freezing, a drying method can be used due to the advantages of less storage space, reduced weight and so easier packaging and transportation. In addition, dried pomace would be convenient to add into functional food formulations. The pumpkin flour has already been used in breakfast cereals, desserts, biscuits, soups and dairy products (Seremet et al., 2016). Hot air drying is the most common technique in which the operating cost is comparatively low; however, color, texture and flavor of dried pomace can be affected negatively particularly at high temperatures and/or long time (Vashisth et al., 2011). Furthermore, the exposure to high temperatures may degrade color pigments, phenolics of the products; therefore, in order to protect these bioactive compounds, freeze-drying occurring under the low temperature and high vacuum can be an alternative method, but it has relatively high operating cost and longer drying time. Vacuum drying is another method which operates at reduced pressures and enables faster drying compared to hot air drying. Microwave drying is extremely faster to dehydrate food materials compared to previously mentioned methods and being recently used by the drying industry.

In this study, the bioactive capacity of BSP was the key parameter which would define the effectiveness of drying method. There are several studies about the influence of different drying methods on the bioactive content of phenolic extract of pomace obtained from pomegranate, citrus mandarin, grape and muscadine (Cano-Lamadrid et al., 2018; Hayat et al., 2010; Tseng and Zhao, 2012; Vashisth et al., 2011) but in regards to that, no studies were found for BSP. In order to evaluate the bioactive content of phenolic extracts, the proper extraction method and optimum extraction conditions need to be applied. Nowadays, Response Surface Methodology has been favored particularly to optimize phenolic compound extraction experiments due to time saving and low cost by researchers (Hernández-Carranza et al., 2016; Yadav et al., 2010). On the other hand, the solvent extraction method is a conventional technique used in this study among other modern extraction methods such as ultrasound, microwave-assisted and supercritical fluid extraction (Zainal-Abidin et al., 2017).

The aim of this study was to show the effect of different drying methods (freeze drying, hot air, vacuum and microwave drying) on the color parameters, total phenolic content, total flavonoid content, and antioxidant activity of the extracts

from BSP. In addition, this study analyzed the optimization of phenolic extraction from freeze-dried BSP using conventional solvent method by the aid of RSM to determine the optimum extraction conditions for dried BSP with hot air, vacuum and microwave drying.

MATERIALS AND METHODS

Materials

The butternut squash (*Cucurbita moschata*) was obtained from wholesale market hall in Osmaniye in October 2018. Folin & Ciocalteu's phenol reagent, sodium bicarbonate, gallic acid, 2,2-diphenyl-1-picrylhydrazyl, ascorbic acid, catechin, aluminum chloride, sodium nitrate, ethanol and methanol were purchased from Merck (Darmstadt, Germany).

Production of butternut squash pomace

The butternut squashes were washed and peeled off. The seeds inside of butternut squash were removed and cut into pieces. These pieces were placed to the home-use juice maker (J700, Braun, Germany, 1000 W). Afterwards, BSP were collected from the juice maker and these pomaces were stored at -20 °C until drying process.

Drying of butternut squash pomace

The obtained BSP were dried using four different methods (freeze drying, hot air, vacuum and microwave drying). Therefore, the lowest (30 °C and 120 W) and highest (60 °C and 600 W) temperature and microwave power were selected for drying experiments. The drying conditions were determined with the preliminary experiments which showed the temperature (40 and 50 °C) and microwave power (360 and 450 W) were not significantly affecting total phenolic content. BSP was dried by using freeze dryer (Alpha 2-4 LDplus, Christ, Germany) at -72 °C at 0.001 bar pressure for 48 h, hot air dryer (Memmert UN55, Germany) at 30 °C for 24 h and at 60 °C for 4 h, the vacuum dryer (Vacucell 111, Munich, Germany) at 30 °C for 4 h and 60 °C for 1 h and the microwave oven at 120 W for 16 min and at 600 W for 3 min. Dried BSP were ground into small particles by a grinder (PRG 277, Premier) and then stored at +4 °C for further analyses. The moisture content of dried BSP samples was between 5 and 10%.

Moisture content

The moisture content of fresh and dried BSP samples was determined using AOAC method with some modifications. The fresh BSP of 10 g (W_1) or dried BSP of 2 g (W_1) were weighed into cups and hold at 80 °C for 24 h in the oven. The final weight (W_2) of the samples was recorded and the moisture content (%) was determined using Eq. (1);

$$\text{Moisture content (\%)} = \frac{W1-W2}{W1} * 100$$

Color analysis

Color values (L^* (lightness), a^* (redness-greenness), b^* (yellowness-blueness)) of fresh and dried BSP samples were measured using a digital portable chroma meter (CR-400, Konica Minolta, Japan). ΔE, chroma (C) and hue (H°) values were calculated using Eqs. (1), (2) and (3). L_{ref}, a_{ref} and b_{ref} belong to fresh BSP sample.

$$\Delta E = \sqrt{(L* - L_{ref})^2 + (a* - a_{ref})^2 + (b* - b_{ref})^2} \quad \text{Eq. (1)}$$

$$C = \sqrt{a*^2 + b*^2} \quad \text{Eq. (2)}$$

$$H^\circ = \tan^{-1}\left(\frac{b*}{a*}\right) \quad \text{Eq. (3)}$$

Experimental design

Factors and their levels affecting on total phenolic content of BSP extracts were investigated using experimental design and statistical analysis performed by Design-Expert (7.0.0, Stat-Ease Inc., Minneapolis, USA) software and summarized in Table 1 and 2. The screening step (2-replicate, 2^3 full factorial design) were carried out before performing the optimization experiments. In screening experiments, the solid/solvent ratio (0.01-0.04 g/mL), temperature (25-45 °C) and time (1-3 h) were selected as independent parameters. A central composite design (CCD) with 19 runs with 5 center points were used for optimization of total phenolic content of BSP (Table 3). The independent variables were the solid/solvent ratio (0.04-0.08

g/mL), temperature (45-60 °C) and ethanol concentration (30-80%) (Table 1). Extraction time was kept at 1 h in the optimization step. Total phenolic content (mg gallic acid eqv./g dry matter) value of each run is the average of three experiments. Significance level was 0.05 for the *P*-value.

Table 1. Coded and actual levels of independent variables investigated at optimization.

Step	Factor	Sign	Actual levels	
			(-1)	(+1)
Optimization	Solid/solvent ratio (g/mL)	A	0.04	0.08
	Extraction temperature (°C)	B	45	65
	Ethanol concentration (%)	C	30	80

Phenolic extraction

The conventional solvent method was used for the extraction of phenolic compounds from BSP samples (Irakli et al., 2018). Screening and optimization experiments were carried out using freeze-dried BSP and the optimum extraction conditions for phenolic extraction were determined. In the optimization of phenolic extraction, required amount of dried BSP was placed into a centrifuge tube and mixed with 30 mL of ethanol-water mixture. Then, this mixture was homogenized (T18 digital Ultra-turrax, IKA) at 3600 rpm for 2 min and placed to the water bath (Selecta, Spain) at defined temperature for the phenolic extraction. During the extraction time, the samples were vortexed for 15 s in 5 min intervals. After the extraction, the obtained solutions were centrifuged at 3500 rpm for 15 min using the centrifuge (Universal 320 R, Hettich, Tuttlingen, Germany) and then filtered with a filter paper. The obtained extracts were stored at -20 °C for further analyses. Furthermore, the phenolic extraction from hot air, vacuum and microwave dried BSP was performed at defined optimum extraction conditions (0.08 g/mL of solid/solvent ratio, 65 °C and 56.9% ethanol concentration).

Total phenolic content assay

Total phenolic content (TPC) of BSP samples were determined using Folin-Ciocalteu method (Li et al., 2015). In the analysis, 0.5 mL extract and 0.5 mL Folin-Cicocalteu reagent were mixed and then, 3 mL $NaCO_3$ solution was added. After holding the samples at dark for 30 min, the absorbance values were read at 760 nm using the spectrophotometer (Sp-3000 nano, Optima, Japan). TPC of samples was calculated as mg gallic acid equivalent/g dry matter of dried pomace (mg GAE/g DM). The experiments were carried out in three parallels.

Total flavonoid content assay

Total flavonoid content (TFC) of BSP samples were carried out according to the method of M'hiri et al. (2015). The extract of 0.3 mL was mixed with 0.3 mL of 5% $NaNO_3$ solution. Afterwards, 0.3 mL of 10% $AlCl_3$ solution was added, and the mixture was incubated for 10 min. After adding 4 mL of 10% NaOH solution to that mixture, the absorbance values were determined using the spectrophotometer at 510 nm. The experiments were performed in triplicates. Total flavonoid content was determined as mg catechin equivalent/g dry matter (mg CE/g DM).

Antioxidant activity assay

Antioxidant activity of BSP samples were performed using the method of Aruwa et al. (2019). Firstly, 0.025 g/L DPPH was prepared in 100% methanol for 2-3 h mixing. Then, 1 mL extract was mixed with 2 mL DPPH and the mixture was held at dark for 30 min. The absorbance values were read at 517 nm using the spectrophotometer. The experiments were carried out in three parallels. Antioxidant activity was expressed as mg ascorbic acid equivalent/g dry matter (mg AAE/g DM).

Statistical analysis

The effect of drying methods on the color values and bioactive content of BSP was determined using the one-way variance analysis. In order to compare the differences between means of drying methods, Duncan test was used. All data analysis was performed using SPSS trial version (SPSS Inc., Chicago, IL)).

RESULTS AND DISCUSSION

The optimization of phenolic compound extraction from butternut squash pomace

Central composite design (19 runs with 5 center points) was employed to investigate the effect of solid/solvent ratio, extraction temperature and ethanol (solvent) concentration on the TPC of the extracts of BSP (Table 3). Levels of the investigated factors were determined considering the results obtained at the screening step (data not given). The screening step (2-replicate, 2^3 full factorial design) showed that the temperature has no significant (*P*-value >0.05) effect on the TPC in the extraction process between 25-45 °C. Then, the levels of extraction temperature were increased to 45-65 °C at the optimization step. Similarly, between 1 – 3 h, extraction time has no significant (*P*-value > 0.05) effect on TPC of BPE, then in order to design a more economic process, extraction time was hold at 1 h at the optimization step. On the other hand, a dramatic increment was observed on TPC of the extracts of BSP as the solid/solvent ratio increased from 0.01 to 0.04 g/mL (figure not given). This result led to investigate a higher level of solid/solvent ratio to maximize TPC of the extracts of BSP at the optimization step. It was stated that the physical properties of the solvent changes with the varying concentration of ethanol which play role in the solubility of compounds (Cacace and Mazza, 2003). Then, the concentration of ethanol was investigated as a factor with the levels between 30 - 80 % (v/v).

The ANOVA of the TPC results obtained from the CCD was given in Table 2. According to the ANOVA (Table 2), the significant (*P*-value < 0.05) factors affecting on the TPC of the extracts of BSP were solid/solvent ratio and its interaction with the ethanol concentration.

Table 2. ANOVA table for the optimization of extraction factors effecting on total phenolic content (mg GAE/g DM) of butternut squash pomace extract.

Source of variation	Sum of squares	Degrees of freedom	*P*-value
Model	7.481	6	<0.001
A- Solid/solvent ratio	5.788	1	< 0.001
B- Extraction temperature	< 0.001	1	0.994
C- Ethanol concentration	0.409	1	0.109
AB	0.394	1	0.116
AC	0.753	1	0.037
BC	0.137	1	0.337
Residual	1.644	12	
Lack of fit	1.490	8	0.072
Pure error	0.153	4	
Total	9.125	18	

The model equation describing the TPC of the extract was depicted in Eq. 1. All main and interaction terms were included in the model equation (Eq. 1) that can be used to make predictions about the response for given levels of each factor. R-squared value of the constructed model indicates that the model is able to explain 90 % of the variation.

$$TPC = 2.91 + 0.651\,A - 0.0008\,B + 0.173\,C + 0.221\,AB - 0.307\,AC + 0.131\,BC \quad \text{Eq. (4)}$$

The model graph given in Figure 1 clearly shows that TPC of the extracts of BSP increased as the solid/solvent ratio increased from 0.04 to 0.08 g/mL. At 0.08 g/mL solid/solvent ratio, from 45 to 65 °C, extraction temperature increased the TPC slightly (Fig. 1). Ethanol concentration between 50 – 60 % has maximized the TPC (Fig. 2). Overall, the optimum parameters of the conventional extraction of total phenolics from butternut squash pomace is 0.08 g/mL solid/solvent ratio at 60-65 °C using 55 % ethanol (v/v) for 1 h (Fig. 1).

Table 3. Total phenolic content (TPC) in optimization experiments.

Experiment number	Solid/solvent ratio (g/mL)	Temperature (°C)	Ethanol concentration (%)	TPC (mg GAE/g DM)
1	0.06	55	55	3.12±0.16
2	0.08	45	80	2.92±0.03
3	0.06	55	55	3.62±0.08
4	0.06	55	55	2.04±0.09
5	0.06	55	97.04	2.68±0.08
6	0.06	55	12.96	1.82±0.06
7	0.04	65	30	1.87±0.14
8	0.09	55	55	2.60±0.03
9	0.06	55	55	3.28±0.09
10	0.06	71.82	55	2.97±0.07
11	0.04	45	80	3.04±0.05
12	0.04	45	30	2.57±0.03
13	0.03	55	55	2.70±0.07
14	0.04	65	80	4.29±0.09
15	0.06	38.18	55	4.08±0.23
16	0.08	65	30	3.04±0.02
17	0.08	65	80	3.96±0.09
18	0.06	55	55	2.67±0.08
19	0.08	45	30	2.12±0.03

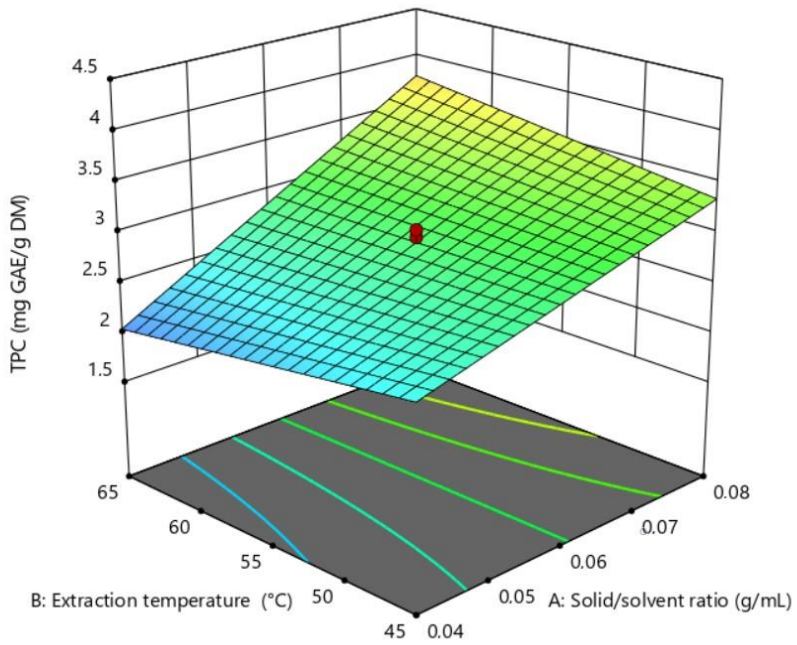

Figure 1. Effect of solid/solvent ratio and extraction temperature interaction on the TPC (ethanol concentration hold at 55%)

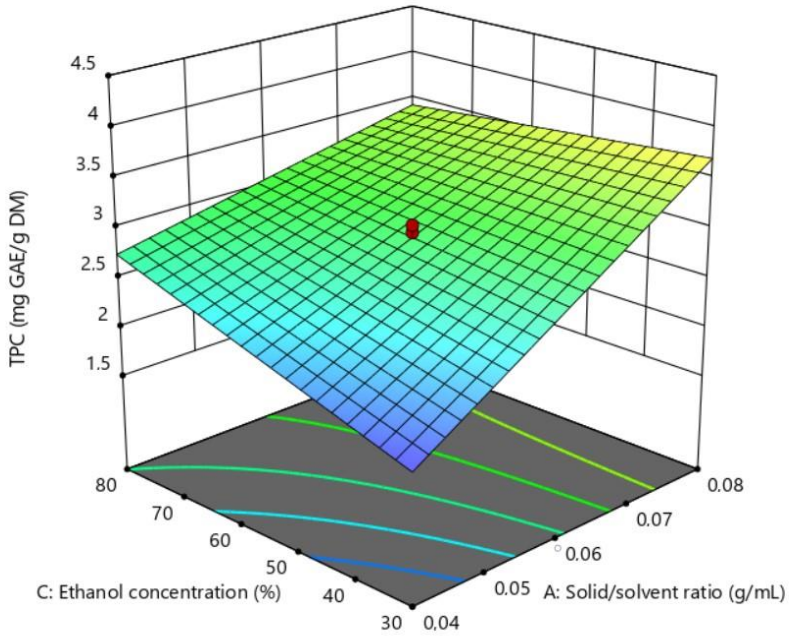

Figure 2. Effect of solid/solvent ratio and ethanol concentration interaction on the TPC (extraction temperature hold at 55 °C)

Wang et al. (2018) has reported that increase of concentration of ethanol from 0 to 50 % resulted in a noticeable increase in TPC extracted from apple pomace. Romero-Díez et al. (2019) also reported as the concentration of ethanol increased from 25 to 75 %, the concentration of anthocyanin conventionally extracted from Port wine lees increased significantly. Cacace and Mazza (2003) explained this phenomenon that the higher ethanol concentration lowers the dielectric constant resulting in a lower energy needed to break the water arrangement. Then, a higher yield of TPC can be obtained by higher ethanol concentration. The extraction temperature was found to be an insignificant factor at the optimization step however, in accordance with the increase in the solid/liquid ratio, a slight increase in TPC was observed as the extraction temperature raised from 45 °C to 65 °C (Fig. 1 and Table 3). This might be due to the enhanced release of phenolics from plant tissues with the help of softened tissue.

In the current study, three processing conditions at selected points from the optimum region were repeated (0.08 g/mL of solid/solvent ratio, 65 °C extraction temperature and ethanol concentration of 54.9, 55.1 and 56.9 %) and validated with the average convergence of 16.07%. The average TPC obtained from the pumpkin pomace under these optimum conditions was 3.35 mg GAE/g DM. Similarly, Hernández-Carranza et al., (2016) used RSM for phenolic extraction and the optimum extraction conditions and TPC of the extracts from apple pomace, orange and banana peels were reported as 51 °C - 7.9 h and 3.89 mg GAE/g DM, 60 °C-12 h and 7.29 mg GAE/g DM and 60 °C-12 h and 4.90 mg GAE/g DM, respectively.

The effect of different drying methods on the color

The color parameters (L^*, a^*, b^*, ΔE, C and H°) of fresh and dried BSP were shown in Table 4 and 5. L^* (80.05 ± 1.47), a^* (1.94 ± 0.09), b^* (44.73 ± 1.41), ΔE (67.77 ± 1.92) and C (44.77±1.41) values of freeze-dried BSP were notably higher

than the ones of other drying methods (hot air, vacuum and microwave). H° values were changed between 55.85 ± 5.51 and 89.64 ± 0.01 for all drying methods, and the highest H° was calculated for microwave drying at 600 W. Similar to our findings, Aydin and Gocmen (2015) reported significantly higher *L** (88.36 ± 0.04) , *a** (10.22 ± 0.09) and *b** (56.79 ± 0.90) values for the powder obtained from freeze-dried pumpkin slice than the ones of hot air-dried (60 °C and 24 h) pumpkin slice. Another study also showed that the hot air dried (60 °C) pumpkin slices has lower *L** (38.53 ± 0.90) and ΔE (14.56 ± 1.21) values as compared to *L** (76.03 ± 2.29) and ΔE (30.51 ± 3.69) of freeze-dried pumpkin slices (Monteiro et al., 2018). On the other hand, *L** value of microwave-dried (120 W) BSP was in agreement with the *L** (25.85 ± 0.12) of microwave-dried (160 W) pumpkin slice reported by Alibas (2007). The lower *L** values obtained in hot air dried product compared to freeze-dried ones could be explained by the browning reactions due to the higher temperatures during the hot air drying (Monteiro et al., 2018).

Table 4. *L**, *a** and *b** values of fresh and dried butternut squash (BSP) samples (FD: freeze-dried, HAD: hot air drying, VD: vacuum drying and MWD: microwave drying).

Drying treatment	L*	a*	b*
Fresh	28.90^{ab} ±1.07	0.09^{ab}±0.06	0.40^{a}±0.25
FD	80.05^{c} ±1.47	1.94^{e}±0.09	44.73^{b}±1.41
HAD-30 °C	29.47^{b} ±0.63	0.03^{ab}±0.02	0.53^{a}±0.21
HAD-60 °C	29.55^{b} ±0.70	0.14^{bcd}±0.02	0.37^{a}±0.18
VD-30 °C	27.76^{ab}±1.28	0.10^{abc}±0.08	0.51^{a}±0.19
VD-60 °C	28.12^{ab}±0.70	0.22^{cd}±0.05	0.62^{a}±0.19
MWD-120 W	27.09^{a}±0.27	0.23^{d}±0.07	0.36^{a}±0.18
MWD-600 W	29.10^{ab}±0.01	0.01^{a}±0.01	1.59^{a}±0.01

Different letters in the same column indicate a statistical difference between the means ($p < 0.05$).

Table 5. ΔE, C and H° values of fresh and dried butternut squash (BSP) samples (FD: freeze-dried, HAD: hot air drying, VD: vacuum drying and MWD: microwave drying).

Drying treatment	ΔE	C	H°
Fresh	-	0.42^{a} ±0.24	82.28^{abc} ±18.93
FD	67.77^{b} ±1.92	44.77^{b} ±1.41	87.51^{bc} ±0.10
HAD-30 °C	0.68^{a} ±0.48	0.53^{a} ±0.14	87.09^{bc} ±1.54
HAD-60 °C	0.68^{a} ±0.68	0.40^{a} ±0.16	65.20^{ab} ±16.2
VD-30 °C	1.28^{a} ±1.12	0.52^{a} ±0.18	76.14^{abc} ±12.35
VD-60 °C	0.94^{a} ±0.49	0.66^{a} ±0.18	69.89^{abc} ±7.65
MWD-120 W	1.82^{a} ±0.28	0.42^{a} ±0.19	55.85^{a} ±5.51
MWD-600 W	1.28^{a} ±0.01	1.59^{a} ±0.01	89.64^{c} ±0.01

Different letters in the same column indicate a statistical difference between the means ($p < 0.05$).

The effect of different drying methods on the total phenolic content

Total phenolic content of the extracts obtained from fresh and dried BSP was given in Figure 3. The lowest (1.88 ± 0.01 mg GAE/g DM) and highest (16.44 ± 0.39 mg GAE/g DM) TPC was found in the extracts of microwave dried BSP at 120 W and fresh BSP. Except fresh BSP, the highest TPC was obtained as 4.86 ± 0.27 mg GAE/g DM in the extracts of microwave dried BSP at 600 W. When hot air and vacuum drying were compared, TPC of the extract from hot air dried BSP at 30 °C was slightly but significantly higher than TPC of the extract of vacuum dried BSP at 30 °C. Furthermore, lower TPC values for the extracts of hot air and vacuum dried BSP at 30 °C was obtained compared to the ones dried at 60 °C. This could be related with the oxidation of polyphenols due to prolonged exposure of BSP to oxygen at lower drying temperature in the hot air

drying process. Aydin and Gocmen (2015) reported TPC of the powder of freeze-dried and hot air dried pumpkin slice as 8.99 ± 0.19 and 11.13 ± 0.04 mg GAE/g DM, respectively which were higher than our findings. On the contrary to the study of Aydin and Gocmen (2015), we found that TPC was higher in freeze-dried BSP than hot air dried one supported by the studies of Tseng and Zhao (2012) and Demirkol and Tarakci (2018). These findings could be associated with the principle of freeze drying where the formation of ice crystals in plant tissues destroys the cell wall and structure, thus releasing phenolic compounds from the tissue matrix and facilitating extraction (Demirkol and Tarakci, 2018). In addition, the bioactive compounds of dried material are mostly protected under the low temperature and vacuum of the freeze drying (Tseng and Zhao, 2012). On the other hand, the most effective method on TPC was microwave drying at 600 W; however, drying at 120 W did not provide the similar effect. Kammoun Bejar et al. (2011) reported that higher TPC (1.8 mg caffeic acid/g DM) was obtained with microwave drying at 450 W compared to that of 180 W. The wider and looser fiber matrix structure might be obtained at higher microwave power and therefore the phenolic extraction could become easier (Kammoun Bejar et al., 2011). Furthermore, the microwave energy could increase the release of free phenolic compounds from the plant matrix (Hayat et al., 2010).

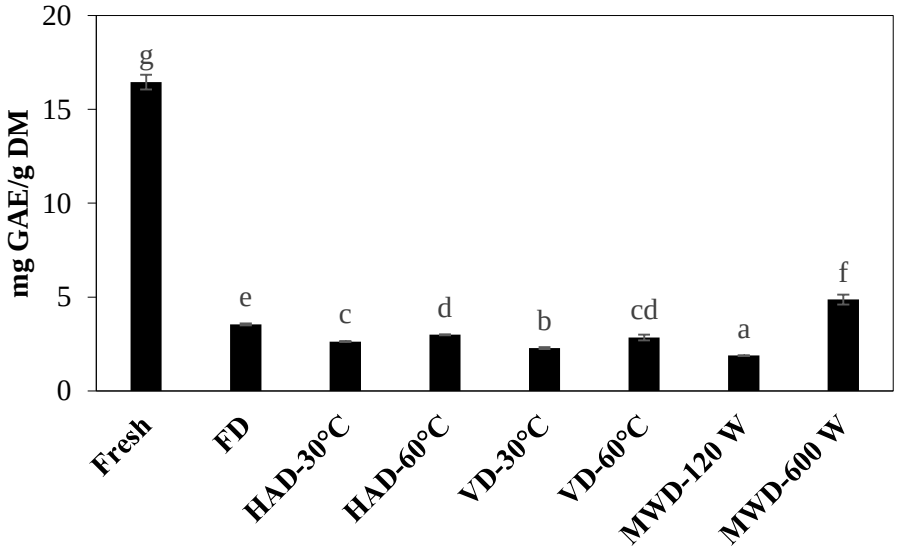

Figure 3. Total phenolic content (TPC) of fresh and dried BSP samples (FD: freeze-dried, HAD: hot air drying, VD: vacuum drying and MWD: microwave drying).

The effect of different drying methods on the total flavonoid content

Total flavonoid content of extracts of fresh and dried BSP with different methods was shown in Figure 4. The lowest (1.32 ± 0.05 mg CE/g DM) and highest TFC (18.89 ± 0.32 mg CE/g DM) were obtained in the extracts of hot air dried BSP at 30 °C and fresh BSP, respectively. In this study, similar TFC values were seen for the extracts obtained from other drying methods. There were no significant effects of different drying methods and temperature/microwave power on the TFC values. On the contrary to our findings, previous studies reported the significant influence of different drying methods on TFC. Tseng and Zhao (2012) found that freeze drying was the most effective method on TFC of grape pomace and peel among different methods (hot air and vacuum drying at 40 °C and freeze drying). Hayat et al. (2010) reported that the increase of microwave power from 125 W to 500 W in drying of mandarin pomace increased significantly TFC values and the highest TFC was determined as 5.81 ± 2.80 mg/g DM.

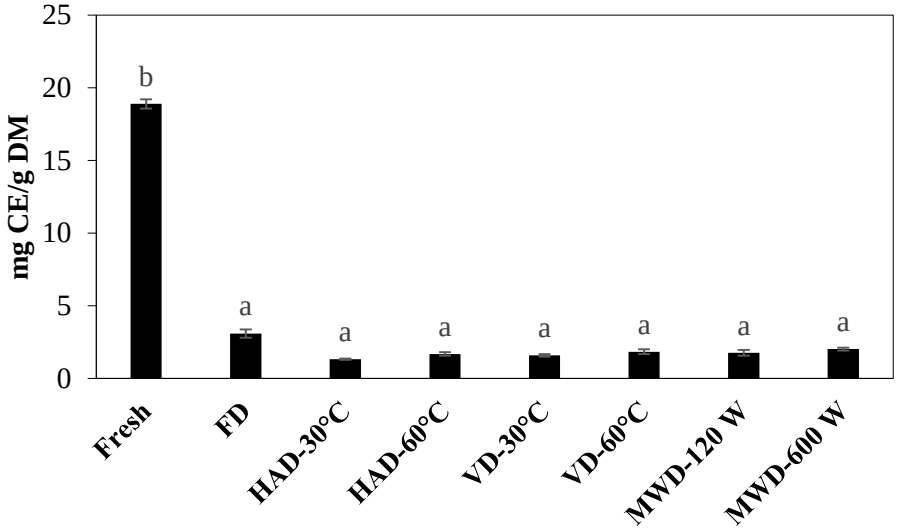

Figure 4. Total flavonoid content (TFC) of fresh and dried BSP samples (FD: freeze-dried, HAD: hot air drying, VD: vacuum drying and MWD: microwave drying).

The effect of different drying methods on the antioxidant activity

Antioxidant activity of the extract of BSP was determined using DPPH method. Antioxidant activity of fresh and dried BSP was presented in Figure 5. The antioxidant activity of the extracts obtained from 120 W microwave dried BSP and fresh BSP were determined as the lowest 1.45 ± 0.15 mg AAE/g DM and 6.09 ± 0.38 mg AAE/g DM, respectively. Aydin and Gocmen (2015) reported antioxidant activity of the powder obtained from freeze and hot air oven (60 °C and 24 h) dried pumpkin slice as 5.57 ± 0.17 and 30.0 ± 0.39 μmol trolox/g, respectively. On the contrary, Demirkol and Tarakci (2018) indicated that significantly higher antioxidant activity was obtained in the freeze-dried grape pomace compared to the that of oven dried pomace. Similarly, the highest antioxidant activity (34.65 mg AAE/g) was reported in the freeze-dried grape pomace among other drying methods (oven and vacuum drying at 40 °C) (Tseng and Zhao, 2012). However, in this study the effect of drying methods except microwave drying at 600 W was found similar on the antioxidant activity of the extracts from BSP.

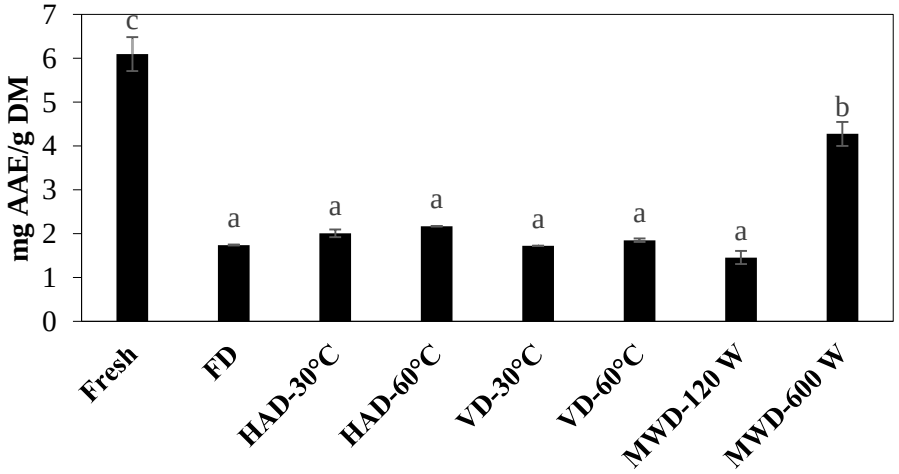

Figure 5. Antioxidant activities of fresh and dried BSP samples (FD: freeze-dried, HAD: hot air drying, VD: vacuum drying and MWD: microwave drying).

CONCLUSION

The solid/solvent ratio and its interaction with ethanol concentration significantly (p<0.05) affected TPC of extracts obtained from BSP. TPC increased with increasing the solid/solvent ratio from 0.04 to 0.08 g/mL. In addition, TPC reached to the maximum level at 50-60% of ethanol concentration. The optimum conditions for phenolic extraction were determined as 1 h extraction time, 0.08 g/mL, 65 °C and 56.9% (v/v) ethanol concentration. The highest TPC and antioxidant activity was obtained in the extracts of microwave dried BSP at 600 W among drying treatments. In addition, TPC of the extract from BSP increased with the increase in temperature from 30 °C to 60 °C in both hot air and vacuum drying. The highest TFC was seen in freeze-dried BSP. As a result, this study showed that the butternut squash pomace has a potential to incorporate into formulations to produce functional foods. Also, our results suggested that the selection of the appropriate drying method is very important in the food industry.

CONFLICT OF INTEREST

The authors declared no conflicts of interest for this work.

AUTHOR CONTRIBUTION

Büşra Gündoğdu: Investigation; Özge Süfer: Drying idea suggestion and data analysis; Meric Simsek: Conceptualization, methodology, formal and data analysis, supervision, writing-original draft

REFERENCES

Alibas, I., 2007. Microwave, air and combined microwave-air-drying parameters of pumpkin slices. *LWT - Food Sci. Technol.* 40, 1445–1451. https://doi.org/10.1016/j.lwt.2006.09.002

Aruwa, C.E., Amoo, S., Kudanga, T., 2019. Phenolic compound profile and biological activities of Southern African *Opuntia ficus-indica* fruit pulp and peels. *LWT* 111, 337–344. https://doi.org/10.1016/j.lwt.2019.05.028

Aydin, E., Gocmen, D., 2015. The influences of drying method and metabisulfite pre-treatment onthe color, functional properties and phenolic acids contents and bioaccessibility of pumpkin flour. *LWT - Food Sci. Technol.* 60, 385–392. https://doi.org/10.1016/j.lwt.2014.08.025

Bao, Y., Reddivari, L., Huang, J.Y., 2020. Enhancement of phenolic compounds extraction from grape pomace by high voltage atmospheric cold plasma. *LWT* 133, 109970. https://doi.org/10.1016/j.lwt.2020.109970

Cacace, J.E., Mazza, G., 2003. Optimization of extract,on of anthocyanins from black currants with aqueous ethanol. *J. Food Sci.* 68, 240–248.

Cano-Lamadrid, M., Lech, K., Calín-Sánchez, Á., Rosas-Burgos, E.C., Figiel, A., Wojdyło, A., Wasilewska, M., Carbonell-Barrachina, Á.A., 2018. Quality of pomegranate pomace as affected by drying method. *J. Food Sci. Technol.* 55, 1074–1082. https://doi.org/10.1007/s13197-017-3022-9

Demirkol, M., Tarakci, Z., 2018. Effect of grape (*Vitis labrusca* L.) pomace dried by different methods on physicochemical, microbiological and bioactive properties of yoghurt. *LWT* 97, 770–777. https://doi.org/10.1016/j.lwt.2018.07.058

Hayat, K., Zhang, X., Farooq, U., Abbas, S., Xia, S., Jia, C., Zhong, F., Zhang, J., 2010. Effect of microwave treatment on phenolic content and antioxidant activity of citrus mandarin pomace. *Food Chem.* 123, 423–429. https://doi.org/10.1016/j.foodchem.2010.04.060

Hernández-Carranza, P., Ávila-Sosa, R., Guerrero-Beltrán, J.A., Navarro-Cruz, A.R., Corona-Jiménez, E., Ochoa-Velasco, C.E., 2016. Optimization of antioxidant compounds extraction from fruit by-products: Apple pomace, orange and banana peel. *J. Food Process. Preserv.* 40, 103–115. https://doi.org/10.1111/jfpp.12588

Irakli, M., Chatzopoulou, P., Ekateriniadou, L., 2018. Optimization of ultrasound-assisted extraction of phenolic compounds: Oleuropein, phenolic acids, phenolic alcohols and flavonoids from olive leaves and evaluation of its antioxidant activities. *Ind. Crops Prod.* 124, 382–388. https://doi.org/10.1016/j.indcrop.2018.07.070

Jamali, S.N., Kashaninejad, M., Amirabadi, A.A., Aalami, M., Khomeiri, M., 2018. Kinetics of peroxidase inactivation, color and temperature changes during pumpkin (*Cucurbita moschata*) blanching using infrared heating. *LWT* 93, 456–462. https://doi.org/10.1016/j.lwt.2018.03.074

Kammoun Bejar, A., Kechaou, N., Boudhrioua Mihoubi, N., 2011. Effect of microwave treatment on physical and functional properties of. *J. Food Process. Technol.* 2, 1–7. https://doi.org/10.4172/2157-7110.1000109

Kulczynski, B., Gramza-Michałowska, A., 2019. The profile of carotenoids and other bioactive molecules in various pumpkin fruits (*Cucurbita maxima* Duchesne) cultivars. *Molecules* 24. https://doi.org/10.3390/molecules24183212

Li, X., Wasila, H., Liu, L., Yuan, T., Gao, Z., Zhao, B., Ahmad, I., 2015. Physicochemical characteristics, polyphenol compositions and antioxidant potential of pomegranate juices from 10 Chinese cultivars and the environmental factors analysis. *Food Chem.* 175, 575–584. https://doi.org/10.1016/j.foodchem.2014.12.003

M'hiri, N., Ioannou, I., Mihoubi Boudhrioua, N., Ghoul, M., 2015. Effect of different operating conditions on the extraction of phenolic compounds in orange peel. *Food Bioprod. Process.* 96, 161–170. https://doi.org/10.1016/j.fbp.2015.07.010

Monteiro, R.L., Link, J.V., Tribuzi, G., Carciofi, B.A.M., Laurindo, J.B., 2018. Effect of multi-flash drying and microwave vacuum drying on the microstructure and texture of pumpkin slices. *LWT* 96, 612–619. https://doi.org/10.1016/j.lwt.2018.06.023

Ping, W., Jiecai, L., Qingyan, Z., Lizhen, H., 2002. Studies on nutrient composition and utilization of pumpkin fruit. *J. Inn. Mong. Agric. Univ. Sci. Ed.* 23, 52–54.

Romero-Díez, R., Matos, M., Rodrigues, L., Bronze, M.R., Rodríguez-Rojo, S., Cocero, M.J., Matias, A.A., 2019. Microwave and ultrasound pre-treatments to enhance anthocyanins extraction from different wine lees. *Food Chem.* 272, 258–266. https://doi.org/10.1016/j.foodchem.2018.08.016

Seremet, L., Botez, E., Nistor, O.V., Andronoiu, D.G., Mocanu, G.D., 2016. Effect of different drying methods on moisture ratio and rehydration of pumpkin slices. *Food Chem.* 195, 104–109. https://doi.org/10.1016/j.foodchem.2015.03.125

Tseng, A., Zhao, Y., 2012. Effect of different drying methods and storage time on the retention of bioactive compounds and antibacterial activity of wine grape pomace (Pinot Noir and Merlot). *J. Food Sci.* 77. https://doi.org/10.1111/j.1750-3841.2012.02840.x

Vashisth, T., Singh, R.K., Pegg, R.B., 2011. Effects of drying on the phenolics content and antioxidant activity of muscadine pomace. *LWT - Food Sci. Technol.* 44, 1649–1657. https://doi.org/10.1016/j.lwt.2011.02.011

Wang, L., Boussetta, N., Lebovka, N., Vorobiev, E., 2018. Effects of ultrasound treatment and concentration of ethanol on selectivity of phenolic extraction from apple pomace. *Int. J. Food Sci. Technol.* 53, 2104–2109. https://doi.org/10.1111/ijfs.13835

Yadav, M., Jain, S., Tomar, R., Prasad, G.B.K.S., Yadav, H., 2010. Medicinal and biological potential of pumpkin: An updated review. *Nutr. Res. Rev.* 23, 184–190. https://doi.org/10.1017/S0954422410000107

Zainal-Abidin, M.H., Hayyan, M., Hayyan, A., Jayakumar, N.S., 2017. New horizons in the extraction of bioactive compounds using deep eutectic solvents: A review. *Anal. Chim. Acta.* 979, 1–23. https://doi.org/10.1016/j.aca.2017.05.012

Zhou, S.-h., Fang, Z.-x., Lü, Y., Chen, J.-c., Liu, D.-h., Ye, X.- q., 2009. Phenolics and antioxidant properties of bayberry (*Myrica rubra* Sieb. et Zucc.) pomace. *Food Chem.* 112, 394–399. https://doi.org/10.1016/j.foodchem.2008.05.104