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Assessment of Total Phenolic Compounds, Antioxidant Capacity, β-Carotene Bioaccessibility, HMF Formation, and Color Degradation Kinetics in Pumpkin Pestils

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Abstract: Pestil, often known as fruit leather, is one of the most significant traditional foods manufactured and consumed throughout Türkiye. Due to its practical consumption, the availability of numerous nutrients, and the ability to meet energy requirements, pestil is recognized as a snack food. The aim of this study was to evaluate the bioaccessibility of total phenolic compounds (TPC), antioxidant capacity (AOC), and β-carotene in pumpkin pestils dried by hot air drying (HAD), vacuum drying (VCD), and microwave drying (MD) methods using an *in vitro* digestion model. Additionally, 5-hydroxymethylfurfural (HMF) formation and color degradation of pestils were evaluated. Changes in TPC and AOC were determined using spectrophotometric methods, whereas the detections of β-carotene and HMF were carried out with high performance liquid chromatography–photodiode array detector (HPLC–PDA). Significantly higher TPC (10.99–105.70%) and AOC (15.30–118.58%, 21.88–401.04% and 89.28–482.14%, in CUPRAC, FRAP, and DPPH assays, respectively) values were observed after drying ($p<0.05$). Moreover, it was observed that there were statistically significant increases in TPC and AOC values after digestion for all pumpkin pestils compared to undigested samples ($p<0.05$). Drying process resulted in lower β-carotene content (between 32.15–61.11%) in pumpkin pestils; however, it increased the percentage of bioaccessible β-carotene (max 62.16%) in the pestil samples. Compared to HD and VCD techniques, pumpkin pestils dried with MD exhibited significantly higher TPC, AOC and β-carotene content ($p<0.05$). All of the pumpkin pestils except those dried by MD at 180 W contain HMF below the Turkish Standards Institute legal limit of 50 mg/kg. L^* value of pestils were described adequately to the zero- and first-order kinetic models while a^* and b^* values were only fitted to zero-order model. In conclusion, the findings obtained in this study pointed out that drying processes (especially by MD method) increased the bioaccessibility of TPC, AOC, and β-carotene.

Keywords: Pumpkin pestil, total phenolic compound, antioxidant capacity, β-carotene, 5-hydroxymethylfurfural, HPLC-PDA.

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1. INTRODUCTION

One of the most significant fruits produced worldwide is the pumpkin (*Cucurbita moschata*), which is valued for both its meaty shell and the seeds' ability to protect health (1). The latest recent official statistics show that Türkiye is the World's sixth pumpkins, squash, and gourds (771651 tonnes in 2021) producer with 100,853 ha of harvested area (2). To promote their increased consumption and usage for nutritional and technological purposes, it is important to be aware of the nutritional worth of food, especially fruits and vegetables. Carotenoids (especially β-carotene), water-soluble vitamins, and amino acids are all abundant in pumpkin. Due to its chemical structure, which is high in phenolics, vitamins, and antioxidants, pumpkin has a significant health-protective effect (3). On the other hand, it is known that the human health promoting activities of bioactive components depend on their bioaccessibility in the gastrointestinal system. The amount of a bioactive component that is made accessible for small intestinal absorption by the removal from the food matrix is referred to as bioaccessibility (4). The use of *in* vitro digestion methods, which are practical, reliable, and unrestricted due to ethical concerns, is quite common for the simulation of gastrointestinal conditions (5). There are many food processing factors affecting the bioacessibility of bioactive components, one of which is drying.

Cooked or pureed, pumpkin is highly regarded and has a wide range of culinary applications as a fruit or as a component in pies, soups, stews, breads, and several other meals (3). Since pumpkin contains a high percentage of water (96%), the product is susceptible to spoilage (1). Given that drying decreases the activity of water and significantly lengthens shelf life compared to fresh fruits and vegetables, it is possible to preserve food in a stable and safe ways (6).

One of the best dried products that appeals to individuals of all ages is pestil (7). Fruit pestils are made by applying various drying processes to fruit juice concentrate or puree. The pestils are directly marketed for human consumption without refrigeration and serve as economically and practically preserved versions of fruits in a variety of forms and sizes (8). Drying technique and drying temperature of the production process play a crucial role in determining the product's color, texture, minerals, vitamins, carotenoids, antioxidant capacity (AOC), total phenolic compound (TPC), and 5-hydroxymethylfurfural (HMF) formation (9). The quality of the final product may be improved by carefully choosing the raw material's drying procedure.

A common preservation technique for agricultural products is hot air drying due to its ease of usage and low cost. However, it results in the deterioration of sensitive compounds, causing the dried product' sensory and other crucial features to be lost (1).

In chemical and engineering processes, vacuum drying is a unit operation in which fresh material is dehydrated under sub-atmospheric pressures. Compared to the conventional hot air procedure at atmospheric pressure, lower pressure allows drying temperatures to be decreased and higher quality to be attained. Most frequently used materials for vacuum drying include those sensitive to temperature, low sensitivity to oxidation, and biotechnology products (10).

As a quick and efficient substitute drying method for convectional drying, microwave drying, a relatively new technology, has been suggested (11). When microwaves interact with the polar water molecules in fruits and vegetables, heat is produced, and a considerably higher drying rate than with air drying was attained (1). However this technique has adverse effects including the potential for textural damage, non-uniform heating caused by the geometry of the materials, reduced conversion of microwave energy to heat at decreased moisture content, and limited microwave penetration into the sample (6).

In the current study, pumpkin fruit was converted into pestil as a substitute product. This study is a continuation of our previous one (12). In our previous study, drying characteristics, mineral content, texture and sensory properties of pumpkin pestil produced by using microwave, hot air, and vacuum methods were investigated. The literature contains many studies on the bioaccessibility of fresh, cooked, and dried pumpkins' polyphenols and carotenoids (13-18). However, there is a very limited research on pumpkin pestil in the literature (19, 20). To the best of our knowledge, the bioaccessibility of TPC, AOC and β-carotene in pumpkin pestil has not been studied in previous researches. In this study, the TPC, AOC, β-carotene biaccessibility, color degradation kinetics, and HMF formation of the pumpkin pestil were firstly revealed. To the best of our knowledge, this is a first-ever attempt to explore the nutritional and quality factors of pumpkin pestils dried by hot air, vacuum, and microwave methods.

2. MATERIALS AND METHODS

2.1. Materials

Pumpkins (*Cucurbita moschata*) were purchased from a greengrocer in Bursa, Turkey, and kept in the refrigerator at 4±0.5°C until needed. Wheat starch, sucrose, and cinnamon, which were all from Turkey: Selva, Torku, and Bagdat brands, respectively were obtained from market.

2.2. The Procedure for Producing Pumpkin Pestil and Drying

The production procedure of pumpkin pestils were given in our previous research (12). By using a mold with a length of 8 cm, a width of 8 cm and a thickness of 4 mm, all pestil samples were dried uniformly to a moisture content of 0.12 g water/g dm.

Drying treatments were performed by using hot air drying (HD) at 50 °C, 60 °C, 70 °C, vacuum drying (VCD) at 50 °C, 60 °C and 70 °C with the absolute pressures of 300 mbar and microwave drying (MD) at 90 W and 180 W power settings. All drying methods were described previously (12).

2.3. *In vitro* Digestion Procedure

The INFOGEST *in vitro* digestion model simulating gastric and intestinal digestion was applied for the determination of TPC, AOC and β-carotene bioaccessibility in pumpkin pestils with small modifications (21). As previously mentioned, simulated saliva fluid (SSF), simulated gastric fluid (SGF), and simulated small intestinal fluid (SIF) were prepared (21). Oral, gastric, and intestinal electrolyte solutions contain potassium chloride (0.5 mol/L), potassium dihydrogen phosphate (0.5 mol/L), sodium bicarbonate (1 mol/L), sodium chloride (2 mol/L), magnesium chloride hexahydrate (0.15 mol/L), ammonium carbonate (0.5 mol/L) and hydrochloric acid (6 mol/L) were prepared as specified in the protocol. To simulate the digestion in the mouth, 4 mL of SSF, 0.025 mL of 0.3 M calcium chloride, and 0.975 mL of distilled water were added to 5 g of the sample and shaken in a water bath (JB50-D, Memmert, Germany) at 37 °C for 2 minutes. Since analysis was not planned after the oral phase, the sample was not collected after this phase. For the simulation of gastric digestion, 7.5 mL of SGF, 1.6 mL of pepsin (Sigma-Aldrich, Germany) with 25000 U/mL activity, 0.005 mL of 0.3 M calcium chloride were added and the pH was adjusted to 3 with 1 M hydrochloric acid. After completing the total volume to 10 mL with distilled water, it was shaken in a water bath at 37°C for 2 hours. After collecting 10 mL of post-digestion extracts, 5.5 mL of SIF to simulate small intestinal digestion, 2.5 mL of pancreatin enzyme (Sigma-Aldrich, Germany) with 800 U/mL activity, 1.25 mL of 160 mM bile, 0.02 mL of 0.3 M calcium chloride and the pH was adjusted to 7 with 1 M sodium hydroxide (Merck, Germany). After completing the total volume to 20 mL with distilled water, it was shaken in a water bath at 37 °C for 2 hours, and then samples were collected after small intestinal digestion. After the gastric and intestinal digestion simulation, the collected liquids were centrifuged (Sigma 3K 30, Germany) at 4 °C, 3500 rpm for 10 minutes, and the supernatants were stored at -20 °C until analysis.

2.4. Extraction for Total Phenolic Compound (TPC) and Antioxidant Capacity (AOC)

Pestil samples were extracted using the method specified by Kamiloglu and Capanoglu (22). 5 mL of extraction solution (75% methanol, 0.1% formic acid-containing aqueous solution) was added to 2 g of the sample and kept in a cooled ultrasonic water bath (Bandelin Sonorex RK 510 H, Germany) for 15 minutes. Then, the samples were centrifuged at 2700 rpm at 4 °C for 10 minutes (Sigma 3K 30, Germany) and the supernatant was collected at the end of extraction. Again, 5 mL of extraction solution was added to the residue and this process was repeated 3 more times. All collected supernatants were finally combined in a tube and stored at -20 °C until analysis.

2.5. TPC Determination

TPC analysis was performed according to the spectrophotometric Folin-Ciocalteu method. For this analysis, a calibration curve (R^2=0.9992) was obtained with different concentrations in the range of 5 – 50 ppm of standard gallic acid (Sigma-Aldrich, Germany) solution. The results were calculated using the regression equation of the obtained curve and expressed as mg gallic acid equivalent (GAE)/100 g dry matter (dm). In this method, 0.5 mL of extract is mixed with 0.5 mL of Folin-Ciocalteu (diluted 3 times with distilled water) reagent. After 5 minutes, 1 mL of saturated sodium carbonate solution (35%) is added to this mixture and vortexed and diluted to 3 mL with 1 mL of distilled water. After the incubation for 30 minutes, the absorbance was read in a spectrophotometer (UV-1800, Shimadzu) at 700 nm (23).

2.6. AOC Determination with DPPH, CUPRAC, and FRAP Assays

For DPPH (1,1-diphenyl-2- picrylhydrazyl) assay, 2 mL 6×10^{-3} M DPPH reagent was added to 100 µL of sample extract. After mixing by vortex and incubation at room temperature for 30 minutes, absorbances were read at 515 nm with a UV-Vis spectrophotometer (UV-1800, Shimadzu) (24). The Trolox® standard calibration curve showed linearity in the range of 5-150 ppm (R^2= 0.9897) and the results were expressed as µmol Trolox® equivalent (TE)/ g.

Determination of AOC with the CUPRAC (Copper ion reducing antioxidant capacity) assay was performed as previously stated in the literature (25). In 100 µL of extract, 1 mL of each reagents (10 mM copper(II) chloride, 7.5 mM neocuproin, 1 M ammonium acetate, and distilled water) were added. After the mixtures were kept at room temperature for 30 minutes, absorbance was measured at 450 nm with a UV-Vis spectrophotometer (UV-1800, Shimadzu). The Trolox® standard calibration curve showed linearity in the range of 5-800 ppm (R^2=0.972) and the results were expressed as µmol TE/g.

AOC determination by FRAP assay was performed as previously stated in the literature (26). Extracted 100 µL samples were mixed with 300 µL of distilled water and 3 mL of FRAP reagent and incubated at 37 °C for 30 minutes. Their absorbance was measured at 595 nm immediately at the end of the incubation period. The Trolox® standard calibration curve showed linearity in the range of 5-200 ppm (R^2=0.9971) and the results were expressed as µmol TE/g.

2.7. β-carotene Extraction

β-carotene extraction was carried out according to Barba et al. method (27). After adding 10 mL of the 50: 25: 25 (v/v/v) mixture of n-hexane, acetone, and ethanol, respectively to the 5.00±0.01 g sample, it was vortexed for 5 minutes. After 10 minutes of centrifugation at 10,000 g and 4 °C, the supernatant was transferred to a clean tube and evaporated with nitrogen gas. Following evaporation, the residue was diluted in 1 mL of 50/50 tetrahydrofuran (THF) and methanol.

2.8. Determination of β-Carotene with High Performance Liquid Chromatography - Photodiode Array Detector (HPLC-PDA)

After passing through 0.45 m membrane filters, all collected samples were injected to HPLC-PDA (Shimadzu LC-2030, Kyoto, Japan) with 10 µL volume. C18 column (250 mm × 4 mm, 5 µm, Nucleosil® 100-5) at 30 °C was used as stationary phase and methanol : acetonitrile (90:10 v/v) was used as mobile phase. Isocratic elution with a flow rate of 1 mL/ min was applied. In the identification of β-carotene, retention time in the column and characteristic UV spectra were taken into account, and spectral measurements were performed at 475 nm. The calibration curve of the β-carotene standard (Sigma-Aldrich, Germany) showed linearity in the range of 0.5-50 ppm (R^2≥0.9997), and the results were expressed as mg/100 g dm.

2.9. 5-Hydroxymethylfurfural (HMF) Determination

A modified version of Rufian-Henares and Delgado-Andrade's method (28) was used to perform the HMF analysis. 1.00±0.01 g of the sample was combined with 7 mL of distilled water and vortexed. The supernatant was then transferred to another tube after it had been centrifuged at 4500 g for 10 minutes at 4 °C. The residue was subjected to two more centrifugations after being mixed with 2 mL of distilled water. 0.250 mL of Carrez I (potassium ferrocyanide, 15% w/v) and 0.250 mL of Carrez II (zinc acetate 30% w/v) solutions were added on the collected supernatants. The volume was made up to 10 mL with distilled water following a final centrifugation. Samples taken into vials by passing through membrane filters (0.45 µm) were injected into the HPLC-PDA in a volume of 20 µL. C18 column (250 mm × 4 mm, 5 µm, Nucleosil® 100-5) at 32 °C was used as stationary phase and acetonitrile:distilled water (5 : 95 v/v) was used as mobile phase. Isocratic elution with a flow rate of 1 mL/min was applied. In the identification of HMF, retention time in the column and characteristic UV spectra were taken into account, and spectral measurements were performed at 280 nm. The calibration curve of the HMF standard (Sigma-Aldrich, Germany) showed linearity in the range of 0.5-50 ppm (R^2≥0.9999), and the results were expressed as mg/100 g dm.

2.10. Color Analyses and Calculation of Kinetic Parameters

Color measurements of the pestil samples were carried out with a chroma meter (CR-5, Konica Minolta, Osaka-Japan). Obtained *L*, a*, b** values represented lightness or darkness, redness, or greenness, yellowness or blueness, respectively. Colors of the samples were measured before and throughout drying at specified time intervals in triplicate, and the average was used for further calculations.

Degradation kinetics of color in pestil samples were investigated with the following zero-order (Equation 1) and first-order (Equation 2) kinetic models (29-31).

$$C = C_0 \pm k_0 t \quad (1)$$

$$C = C_0 \exp(\pm k_1 t) \quad (2)$$

In which; C and C_0 are measured color values (*L*, a*, b**) at time t and time 0, respectively; t is drying time; k_0 and k_1 are zero-order and first-order reaction rate constants, respectively; (+) and (-) represents formation and degradation of color values, respectively.

2.11. Statistical Analysis

For the evaluation of statistical analyses, SPSS 15.0 (SPSS Inc., USA) was employed. The Duncan's multiple range test was applied when there were significant variations between means ($p<0.05$).

3. RESULTS AND DISCUSSION

3.1. *In vitro* Bioaccessibility of Total Phenolic Content (TPC) and Antioxidant Capacity (AOC)

The effects of *in vitro* digestion on TPC of fresh and dried pumpkin pestils are given in Table 1. For undigested samples, dried fruit pestils were found to contain higher amounts of TPC (10.99–105.70%) compared to the ND mix. This could be due to the increment in the level of free flavonols by heat treatment and the concentration of phenolics as a result of drying procedures (32, 33). Additionally, heating methods may also weaken the fruit's cell walls, which encourages the release of phenolic compounds into the extraction solution and, as a result, increases the phenolic and AOC of the

extracts (33, 34). The highest TPC was recorded in pestils dried by MD at 180 W whereas the lowest content was found in the case of pestils dried by HD at 50 °C. Pumpkin pestils dried by MD had significantly higher TPC compared to HD and VCD methods ($p<0.05$). Similarly, Arslan and Ozcan (35) found that drying onion slices in both conventional and microwave ovens increased the total phenolic content of the samples, with the MD method obtaining the highest values. Furthermore, Ghanem et al. (36) found that compared to fresh peels, MD increases the TPC of dried Thompson navel orange peels. Additionally, according to Hamrouni-Sellami et al. (37), TPC increased 4.2-fold when drying sage plants by MD at 800 W as compared to fresh plants. It can be observed in Table 1 that an increase in drying temperature (especially VCD) has an important effect on TPC ($p<0.05$). The availability of precursors of phenolic molecules through non-enzymatic interactions between phenolic molecules may be the source of TPC increment at high temperatures (38).

ND mix showed an increase of 1.67 and 2.88 fold after gastric and small intestine digestion, respectively, compared to undigested one. After gastric digestion simulation, the *in vitro* bioaccessibility of pumpkin pestils increased between 1.92 and 4.05 fold. This finding is consistent with the data obtained in previous studies with vegetable juices (39), dried fruits, and nuts (40). These increases observed in TPC indicate that the extraction of phenolic compounds continues during gastric digestion and the released phenolic compounds remain stable in the acidic environment of the stomach (41). Statistically significant increases were observed in the TPC of pumpkin pestils after intestinal digestion compared to the data obtained after gastric digestion (2.84–97.14%) ($p<0.05$). In some previous studies, increases in the TPC were observed after intestinal digestion, and this was explained by the increase in the contact time of foods with intestinal fluids and the facilitation of the release of phenolic compounds by intestinal enzymes (41-43). Moreover, the increase in TPC bioaccessibility can be explained by the increased release of phenolics due to heat treatment during the drying process. Heat treatment can cause degradation of the food matrix and increase phenolic release (44, 45). In a study investigating the effect of drying on melon polyphenols, it was observed that the drying process caused an increase in the amount of bioaccessible TPC (46). In agreement with our findings, Kamiloglu et al. (47) also reported that cakes improved with black carrot pomace led to a rise in TPC following intestinal digestion.

Table 1: Changes in TPC of pumpkin pestils during *in vitro* digestion.

	Undigested	**Simulated gastric digestion**	**Simulated small intestinal digestion**
TPC (mg GAE /100 g dm)			
Non-dried mixture (ND mix)	40.65±0.00 ^{cC}	67.98±11.79 ^{eB}	117.24±15.17 ^{fA}
HD 50 °C	45.12±4.06 ^{cB}	172.32±2.47 ^{cA}	177.22±3.67 ^{eA}
HD 60 °C	74.30±12.51 ^{abC}	142.42±7.70 ^{dB}	225.33±12.34 ^{cdA}
HD 70 °C	67.95±2.31 ^{bC}	145.35±4.43 ^{dB}	286.55±3.66 ^{bA}
VCD 50 °C & 300 mbar	52.95±2.91 ^{cC}	181.69±4.99 ^{bcB}	199.78±5.08 ^{deA}
VCD 60 °C & 300 mbar	53.89±12.27^{cB}	187.65±15.64 ^{bcA}	207.53±10.79 ^{deA}
VCD 70 °C & 300 mbar	69.17±0.40 ^{bC}	176.05±11.08 ^{cB}	290.48±12.00 ^{bA}
MD 90 W	69.10±9.09 ^{bC}	197.87±8.80 ^{bB}	259.84±45.90 ^{bcA}
MD 180 W	83.62±7.76 ^{aB}	338.65±19.24 ^{aA}	377.61±54.21 ^{aA}

Values followed by different lowercase letters within the same column are significantly different ($p < 0.05$)
Values followed by different capital letters within the same row are significantly different ($p < 0.05$)

Changes in AOC as a result of *in vitro* digestion of pumpkin pestils and ND mix were determined and the results are given in Table 2. The highest AOC values were obtained with the CUPRAC method, followed by the FRAP and DPPH methods, respectively: CUPRAC > FRAP > DPPH. This may be due to the fact that DPPH assay only measures hydrophobic antioxidants, whereas FRAP assay only measures hydrophilic antioxidants and CUPRAC assay measures both of them (48). In addition, the formation of biologically unrelated nitrogen radicals in the DPPH method causes the antioxidant capacity to be underestimated from its true value (49). As for the TPC of the undigested samples, there were increases in the range of 15.30–118.58%, 21.88–401.04% and 89.28–482.14%, respectively, in the CUPRAC, FRAP and DPPH assays after drying. This increment was explained by Nicoli et al. (50) as the result of the formation of Maillard reaction products with high AOC. According to some studies, dried

fruits have superior antioxidant qualities because of their high polyphenol content and because their monomer derivatives are produced as a result of the complex polymeric molecules' hydrolysis or breakdown during dehydration (51, 52). Consistent to this findings, Karabacak (53) reported that drying cause an increment in CUPRAC values of blackthorn pestils compared to paste mixture. In contrast, dried fruits like pepino (54) and chokeberry (55) showed a decrease in AOC after dehydration. The fruit variety, drying techniques, drying applications and extraction and analysis methodologies of AOC could be the cause of the differences between these researches (33). Additionally, thermal treatments have the ability to significantly increase the overall antioxidant capacity of processed fruits and vegetables, even while drying destroys heat-, light-, and oxygen-sensitivephytochemicals (50, 56). Due to diversity of fruits and dehydration treatments, various results have been reported.

Compared to HD and VCD techniques, pumpkin pestils dried with MD exhibited significantly higher AOC ($p<0.05$). Similar to this, Benlloch-Tinocoet al. (57) found that microwave heating increased the AOC of kiwi fruit more than traditional heating provided.

After gastric digestion, a statistical increase was observed in the AOC values, as well as in the TPC results ($p<0.05$). However, varying results were obtained after small intestinal digestion. It was observed that there were statistically significant increases in AOC values after digestion for all pumpkin pestils compared to undigested samples ($p<0.05$). This finding is consistent with a previous studies for plant-based milks and blackthorn leathers in the literature, and the increase in AOC was explained by the release of phenolic compounds by enzyme activities after digestion or the formation of new compounds with antioxidant properties (58). Additionally, the variations in pH values caused by the deprotonation of the hydroxyl moiety located on the aromatic ring of phenolic compounds could be the cause of the increases in AOC observed after small intestinal digestion. Based on these findings, it may be assumed that intestinal cells may be sufficiently protected from oxidative stress by superior polyphenol scavenging activity because the intestine has a lower pH than the stomach (59).

3.2. *In vitro* Bioaccessibility of β-Carotene

β-carotene is a carotenoid found in pumpkins that has been reported to exhibit beneficial health effects (13). For this reason, the concentration of β-carotene was determined in the pumpkin pestils and their ND mix before and after digestion by HPLC analysis. Chromatograms of β-carotene standard and pumpkin pestil were given in Figure 1a and Figure 1b, respectively. β-carotene content of ND mix was 59.53 mg/100 g dm. It is clear from the findings in Table 3 that drying caused a large loss of β-carotene ($p<0.05$) (32.15–61.11%). While the lowest loss was observed in MD at 180 W (32.15%), HD at 70 °C (61.11%) showed the highest degradation of β-carotene. Similar to this research, Hernández-Ortega et al. (60) found that carrot pomace powders dried by microwave method had higher β-carotene than samples dried with hot air. Since β-carotene is a component that is sensitive to oxidative thermal degradation, the short drying time of MD may decrease its degradation. Carotenoids' sensitivity to heat, oxygen, light, and enzymes may be the cause of the decrease in β-carotene after drying. Similarly, previous studies have reported that significant reductions in carotenoid content of coriander seeds (61), carrots, sweet potatoes, yellow bell peppers, broccoli (62), and pumpkin (63) were observed after drying. β-carotene was more degraded in HD method (between 56.89 – 61.11%) when compared with VCD method (between 35.14 – 52.48%). The VCD method could remove the oxygen in the oven and thus limit the carotenoid loss (17). Due to the high internal temperature and the high exposure to ambient oxygen in the HD method, the relative β-carotene losses in pumpkin pestils are significantly higher than those of the samples dried by VCD.

Table 2: Changes in AOC of pumpkin pestils during *in vitro* digestion.

	Undigested	**Simulated gastric digestion**	**Simulated small intestinal digestion**
DPPH (μmol TE/g dm)			
ND mix	0.28±0.06 ^{eC}	1.37±0.07 ^{eA}	0.80±0.13 ^{dB}
HD 50 °C	0.64±0.13 ^{dB}	1.47±0.04 ^{deA}	1.27±0.16 ^{cA}
HD 60 °C	0.60±0.07 ^{dB}	1.58±0.19 ^{cdA}	0.89±0.34 ^{dB}
HD 70 °C	0.53±0.02 ^{dC}	1.65±0.10 ^{bcA}	1.30±0.11 ^{bcB}
VCD 50 °C & 300 mbar	1.22±0.11 ^{cC}	1.67±0.11 ^{bcA}	1.48±0.02 ^{bcB}
VCD 60 °C & 300 mbar	1.22±0.06 ^{cA}	1.52±0.01 ^{cdeA}	1.32±0.30 ^{bcA}
VCD 70 °C & 300 mbar	1.21±0.09 ^{cB}	1.65±0.03 ^{bcA}	1.61±0.04 ^{bcA}
MD 90 W	1.44±0.05 ^{bB}	1.79±0.06 ^{bA}	1.65±0.20 ^{abAB}
MD 180 W	1.63±0.07 ^{aB}	2.01±0.04 ^{aA}	1.95±0.02 ^{aA}
FRAP (μmol TE/g dm)			
ND mix	0.96±0.06 ^{eA}	1.02±0.04 ^{eA}	0.17±0.08 ^{dB}
HD 50 °C	1.18±0.07 ^{deB}	1.46±0.09 ^{dAB}	1.96±0.42 ^{bA}
HD 60 °C	1.27±0.08 ^{cdA}	1.67±0.42 ^{cdA}	1.37±0.18 ^{bcA}
HD 70 °C	1.17±0.06 ^{deB}	2.05±0.34 ^{cA}	1.82±0.13 ^{bcA}
VCD 50 °C & 300 mbar	1.23±0.34 ^{cdeA}	2.01±0.18 ^{cA}	1.77±0.57 ^{bcA}
VCD 60 °C & 300 mbar	1.23±0.19 ^{cdeB}	1.90±0.27 ^{cA}	1.22±0.43 ^{cB}
VCD 70 °C & 300 mbar	1.47±0.03 ^{cA}	1.76±0.04 ^{cdA}	1.44±0.37 ^{bcA}
MD 90 W	2.94±0.09 ^{bA}	3.10±0.13 ^{bA}	1.44±0.13 ^{bcB}
MD 180 W	4.81±0.14 ^{aB}	6.02±0.17 ^{aA}	2.74±0.28 ^{aC}
CUPRAC (μmol TE/g dm)			
ND mix	1.83±0.08 ^{eB}	6.82±0.68 ^{fA}	0.13±0.08 ^{eC}
HD 50 °C	2.25±0.05 ^{dB}	10.27±0.29 ^{dA}	2.80±0.52 ^{dB}
HD 60 °C	2.25±0.03 ^{dB}	8.68±0.54 ^{eA}	8.36±0.23 ^{cA}
HD 70 °C	2.11±0.05 ^{dC}	13.05±0.26 ^{cA}	12.15±0.39 ^{bB}
VCD 50 °C & 300 mbar	2.14±0.06 ^{dC}	13.06±0.44 ^{cA}	10.75±0.58 ^{bB}
VCD 60 °C & 300 mbar	2.18±0.10 ^{dC}	14.94±1.32 ^{bA}	12.65±1.07 ^{bB}
VCD 70 °C & 300 mbar	2.46±0.09 ^{cC}	15.15±0.40 ^{bA}	12.21±1.93 ^{bB}
MD 90 W	3.27±0.14 ^{bB}	15.39±1.19 ^{bA}	14.58±0.91 ^{aA}
MD 180 W	4.00±0.14 ^{aC}	17.08±0.97 ^{aA}	14.65±1.70 ^{aB}

Values followed by different lowercase letters within the same column are significantly different ($p < 0.05$)
Values followed by different capital letters within the same row are significantly different ($p < 0.05$)

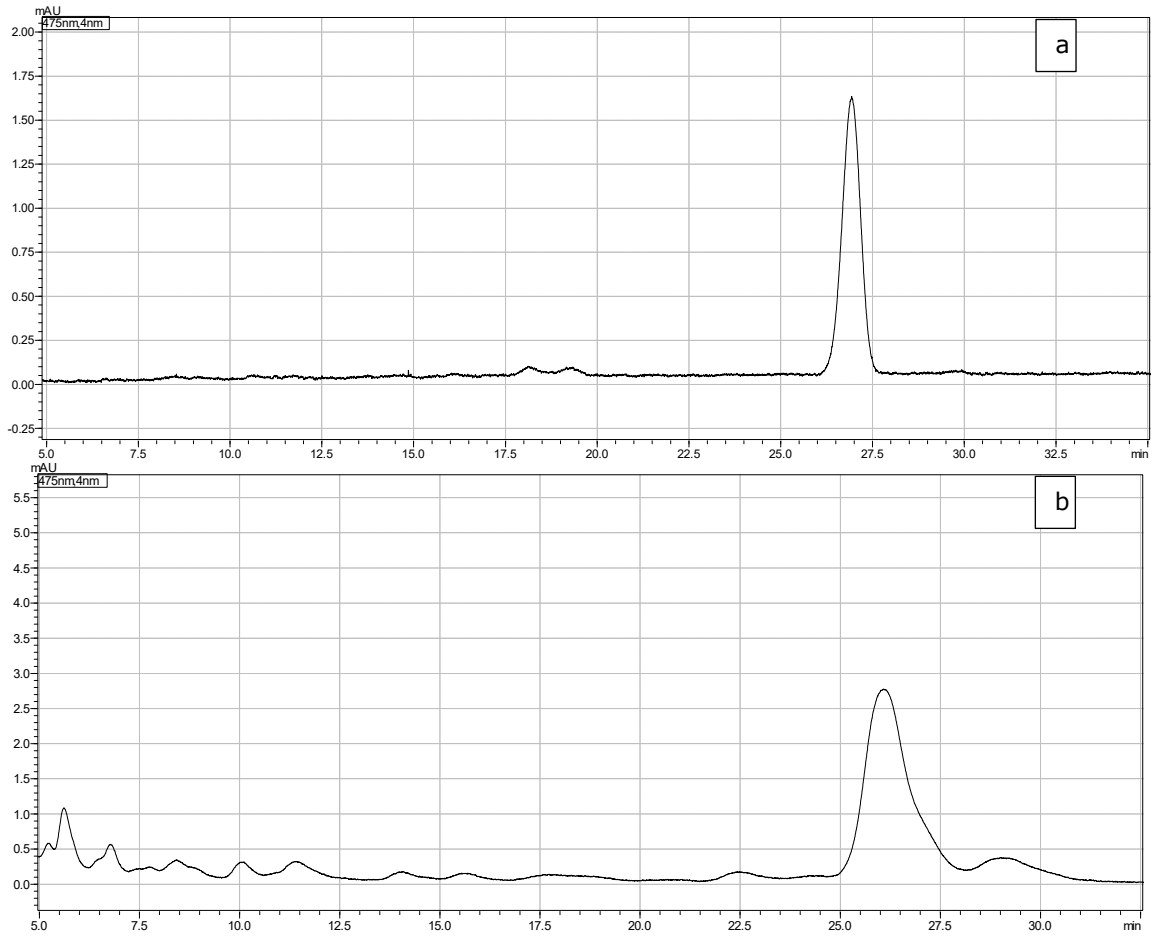

Figure 1: HPLC chromatogram of β-carotene standard (a) and pumpkin pestil sample (b).

Pumpkin pestils and their ND mix's bioaccessibility were decreased after gastric digestion with the ratio of 74.87–83.52% and 67.81%, respectively. The gastric phase's agitation and incubation were found to significantly decrease particle size and disrupt the food's structure, which facilitated the easier release of carotenoids from the cells and hence the β-carotene in the pumpkin pestils was found to be decreased (64).

There were statistically significant increases in β-carotene values after digestion for all pumpkin pestils compared to undigested samples ($p<0.05$). β-carotene in pumpkin pestils increased gradually with the pH value rising in the small intestine phase (pH = 7). It's probable that a high pH value will make carotenoids more stable (65). Bioaccessibility of β-carotene in pumpkin pestils dried by MD at 180 W (62.16%) was significantly higher than their ND mix (53.84%). By loosening the food matrix, heat processing is thought to increase the bioaccessibility of β-carotene and so facilitate its absorption. The kind and intensity of food processing affect the food matrix and, consequently, the bioaccessibility of carotenoids (66). The higher bioaccessibility of carotenoids by heat treatment was also reported from the other studies (13, 66, 67).

3.3. HMF Content

HMF is used as an indicator of non-enzymatic browning reactions. Since HMF is not found in fresh and unprocessed foods, its formation is directly related to the heat intensity applied to the food (28). Under the influence of heat, Maillard reactions involving reducing sugars and amino acids can take place during food preparation and preservation. The nutritional content of food is reduced, unfavourable taste and color changes occur, and product quality degrades as a result of the HMF formation, a significant Maillard reaction intermediate product, whose amount is restricted in many products due to its potential carcinogenic effect (68). The Maillard reaction is influenced by a lot of variables, including pH, temperature, metal ions, sugar type, and other factors (9). According to the Turkish Standards Institute (Grape pestil, TS12680), the legal limit for the HMF concentration of pestil is 50 mg/kg (69). In the current study, the HMF content of the pumpkin pestil was found to be between 29.73 to 115.86 mg/kg dm (Figure 2). The pumpkin pestil dried by MD at 180 W had the highest HMF concentration while the lowest HMF was obtained by VCD at 50 °C & 300 mbar method. All of the pumpkin pestils except dried by MD at 180 W contain HMF below the

Turkish Standards Institute legal limit (Grape pestil, TS12680) of 50 mg/kg. Due to the rapid increase of temperature in sample during microwave heating, it may be thought that the pestil samples were subjected to a high temperature and had higher HMF content as a result of this (70). Additionally, it was seen that the HMF content increased along with the magnetron power in MD method. Therefore, the HMF concentration in the product is affected by factors such as drying temperature, processing time, presence or absence of oxygen and magnetic waves (71). In both HD and VCD methods, pumpkin pestils dried at a medium temperature of 60 °C contained lower HMF than those dried at other temperatures (50 °C and 70 °C). This can be explained by the fact that drying at 60 °C takes a shorter time than at 50 °C and is less exposed to Maillard reactions than that at 70 °C. Similarly, Wang et al. (72) reported that HMF formation in bee pollen increased with the increase in pulsed vacuum drying temperature. Additionally, Kanar and Mazi (70) investigated the change of HMF in pollen samples dried by freeze drying, hot air, vacuum, microwave, and microwave-assisted vacuum drying methods. They reported that hot air and vacuum drying methods did not cause a significant increase in the HMF content of the pollen. On the other hand, researchers, similar to this study, reported that the amount of HMF during microwave drying changed significantly depending on the applied microwave power. They reported that the highest amount of HMF was obtained from the samples dried with microwave and microwave assisted vacuum drying at 450 and 600 W power levels.

Table 3: Changes in β-Carotene of pumpkin pestils during *in vitro* digestion.

	Undigested	**Simulated gastric digestion**	**Simulated small intestinal digestion**
β-carotene (mg/100 g dm)			
ND mix	59.53±0.52 ^{aB}	19.16±0.66 ^{aC}	91.58±5.31 ^{aA}
HD 50 °C	24.15±0.26 ^{hB}	4.31±0.20 ^{dC}	25.21±0.18 ^{gA}
HD 60 °C	25.66±0.21 ^{gB}	4.23±0.15 ^{dC}	29.19±1.60 ^{fA}
HD 70 °C	23.15±0.23 ^{iB}	3.94±0.09 ^{dC}	24.97±1.56 ^{gA}
VCD 50 °C & 300 mbar	31.95±0.03 ^{dA}	7.73±0.15 ^{cB}	31.50±0.30 ^{efA}
VCD 60 °C & 300 mbar	38.61±0.16 ^{cB}	8.75±1.68 ^{cC}	48.06±0.81 ^{cA}
VCD 70 °C & 300 mbar	28.29±0.32 ^{fB}	4.45±0.06 ^{dC}	33.52±0.79 ^{deA}
MD 90 W	31.00±0.17 ^{eB}	4.64±0.09 ^{dC}	36.19±0.70 ^{dA}
MD 180 W	40.39±0.43 ^{bB}	10.15±0.26 ^{bC}	65.50±2.14 ^{bA}

Values followed by different lowercase letters within the same column are significantly different (p < 0.05)
Values followed by different capital letters within the same row are significantly different (p < 0.05)

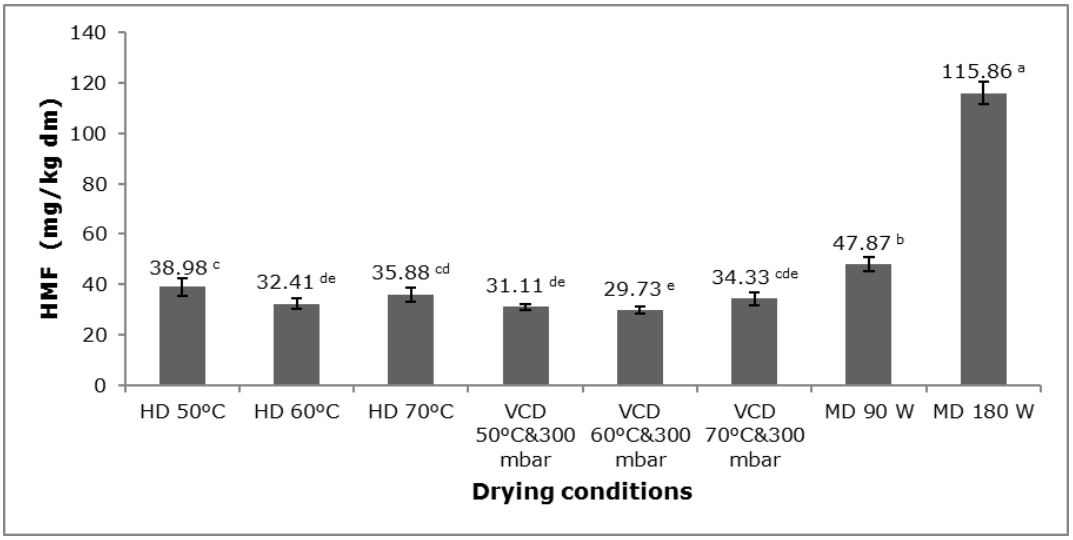

Figure 2: HMF content in pumpkin pestils.
Different lowercase letters in bars displays significant differences (p<0.05)

3.4. Kinetics of Color Change During Drying

The results of color values obtained from HAD, VD, and MWD of pumpkin pestils were presented in Figures 3, 4, and 5. As it can be observed from Figure 3a and 3b, *L** decreased through drying time. The reduction in *L** values changed from 32.81 (paste mixture) to 27.65 and 31.09 between the drying treatments representing the most, and the least damage occurred by MWD at 180 W and VD at 50 °C & 300 mbar, respectively. Besides, the decrement in *L** values indicated that pumpkin pestils became darker. A similar behaviour was reported by Maskan (29), Dadali et al. (30), Swain et al. (31) and Hou et al. (73). It has been defined that, falling values in brightness of the pestils might be resulted from the non-enzymatic Maillard reaction. The results for *a** values were given in Figure 4a and 4b. During different drying conditions an increment in *a** values were found, explaining that the pestils lost their color and became more red. This increment might be explained by decomposition of the pigments and the formation of the browning pigments (29, 74, 75). Similar results were expressed by other researchers in yellow sweet pepper (31), spinach (30), and purple carrot (76). A decrement in *b** value (Figure 5a and 5b) was also observed from the drying conditions. It was reduced from 40.50 to 15.04 during HAD at 60°C. This might be attributed to the decomposition of carotenoid pigments due to longer drying times and higher drying temperatures leading to the detriment of yellowness (77).

Zero-order and first-order kinetic models were utilized for the determination of color changes in pumpkin pestils and kinetic parameters achieved from these models are represented in Table 4. The results revealed that *L** values were fitted to both of the zero- and first-order kinetic models whereas the zero-order kinetic model was found to be appropriate for *a** and *b** values of pumpkin pestils.

*L** values of pumpkin pestils were described adequately both to the zero- and first-order kinetic models as a result of very close R^2 values. On the other hand, zero order kinetic model had higher R^2 for *a** and *b** color values when compared to the first order reaction model. As a result of this situation, zero order kinetic model was better fitted than the first order model in terms of *a** and *b** values.

Current findings were in agreement with the literature in which several researchers reported that zero and first order models were fitted to the *L** values of kiwifruit (29), and zero order kinetic model was better fitted to *a** and *b** values of cabbage (78).

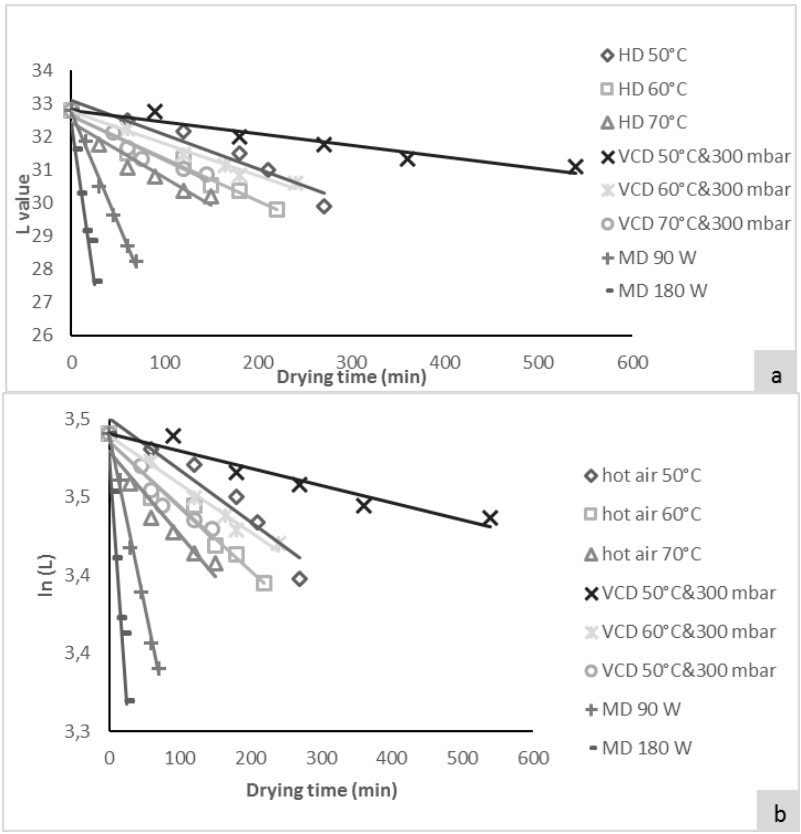

Figure 3: Kinetics of *L** color value changes in pestil samples as a function of drying time for different methods: zero-order model (a) and first-order model (b)

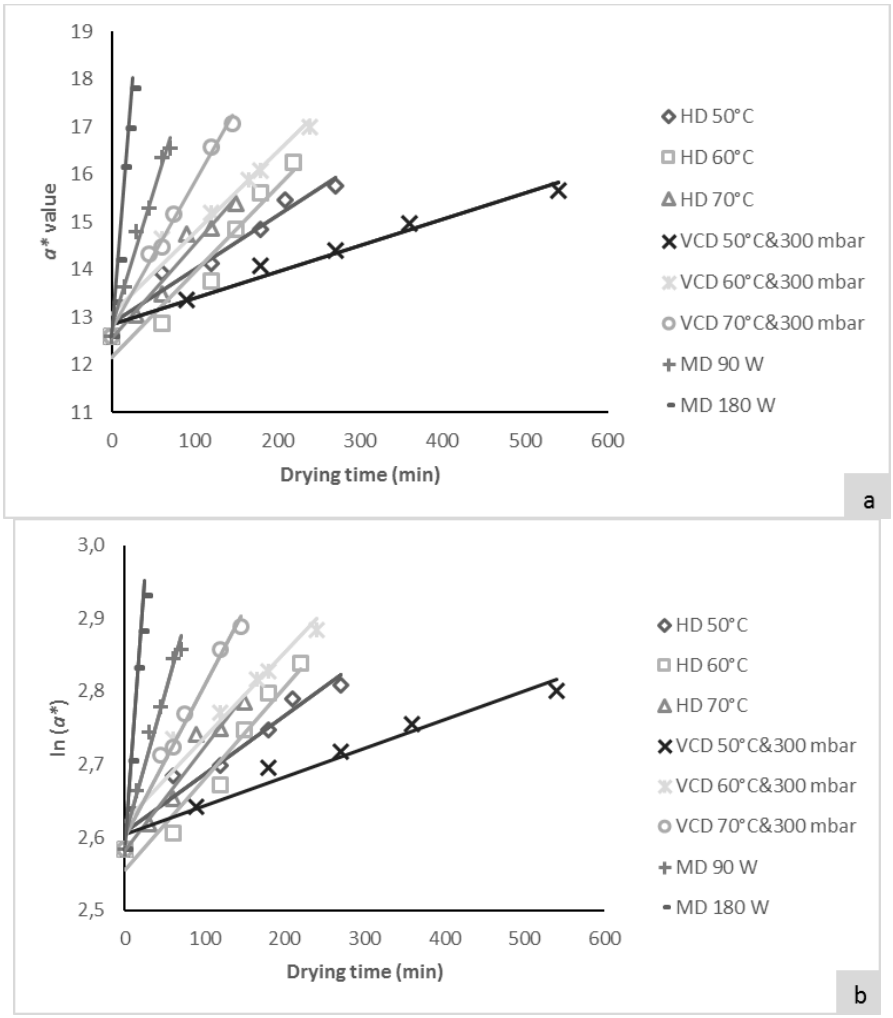

Figure 4: Kinetics of *a** color value changes in pestil samples as a function of drying time for different methods: zero-order model (a) and first-order model (b).

Table 4: The kinetic parameters of zero-order and first-order models for color values of pumpkin pestil.

Color parameter	Drying conditions	Zero-order model			First-order model		
		k_0(min^{-1})	C_0	R^2	k_1(min^{-1})	C_0	R^2
L*	HD 50 °C	0.0104	33.1030	0.9289	0.0003	3.5004	0.9221
	HD 60 °C	0.0129	32.6250	0.9542	0.0004	3.4855	0.9562
	HD 70 °C	0.0167	32.4280	0.9204	0.0005	3.4791	0.9265
	VCD 50 °C & 300 mbar	0.0035	32.8040	0.9286	0.0001	3.4907	0.9313
	VCD 60 °C & 300 mbar	0.0097	32.7550	0.9805	0.0003	3.4893	0.9819
	VCD 70 °C & 300 mbar	0.0135	32.6380	0.9305	0.0004	3.4856	0.9337
	MD 90 W	0.0666	32.7330	0.9932	0.0022	3.4897	0.9954
	MD 180 W	0.1933	32.3560	0.9520	0.0064	3.4778	0.9587
a*	HD 50 °C	-0.0114	12.8640	0.9542	-0.0008	2.5565	0.9452
	HD 60 °C	-0.0178	12.1500	0.9441	-0.0013	2.5047	0.9357
	HD 70 °C	-0.0197	12.5460	0.9599	-0.0014	2.5321	0.9592
	VCD 50°C&300 mbar	-0.0056	12.8400	0.9716	-0.0004	2.5546	0.9599
	VCD 60 °C & 300 mbar	-0.0171	13.0590	0.9510	-0.0012	2.5716	0.9317
	VCD 70 °C & 300 mbar	-0.0310	12.7350	0.9912	-0.0021	2.5507	0.9831
	MD 90 W	-0.0571	12.7800	0.9828	-0.0039	2.5517	0.9747
	MD 180 W	-0.2176	12.6030	0.9846	-0.0144	2.5413	0.9783
b*	HD 50 °C	0.1121	45.3540	0.9255	0.0040	3.8746	0.9115
	HD 60 °C	0.1271	42.0560	0.9835	0.0048	3.7962	0.9728
	HD 70 °C	0.1850	44.6770	0.9444	0.0065	3.8555	0.9241
	VCD 50 °C & 300 mbar	0.0391	40.0990	0.9346	0.0056	3.8136	0.8744
	VCD 60 °C & 300 mbar	0.0861	41.5610	0.9565	0.0028	3.7489	0.9412
	VCD 70 °C & 300 mbar	0.1694	44.6200	0.9544	0.0056	3.8486	0.9452
	MD 90 W	0.3214	45.8580	0.9036	0.0101	3.8670	0.8758
	MD 180 W	0.5726	42.6430	0.9337	0.0161	3.7596	0.9216

4. CONCLUSION

To the best of our knowledge, this is the first study to evaluate the effects of drying processes on the *in vitro* bioaccessibility of TPC, AOC, and β-carotene of pumpkin pestils. Additionally, HMF formation and color degradation kinetics after drying processes were also assessed.

The results of this study showed that the bioaccessible phenolic compounds, antioxidant capacity, and β-carotene increased as a result of drying the pumpkin pestils by HD, VCD, and MD methods. The highest TPC, AOC, and β-carotene values were observed by MD method in all drying treatments. However, pumpkin pestils dried by MD at 180 W showed HMF content above the Turkish Standards Institute's legal limit of 50 mg/kg for grape pestil. After drying *L** and *b** values decreased while *a** value increased significantly ($p<0.05$). The degradation of *L** value during drying was described well by both of the kinetic models (zero- and first- order) while *a** and *b** values were only fitted to zero-order kinetic model.

In this study, it has been seen that a functional product with high bioaccessibility of phenolic compounds and carotenoids can be developed by using pumpkin in fruit pestil production.

The MD method at 90 W can be recommended for both of the highest bioaccessible phenolic, antioxidant, and carotenoid content and reasonable HMF formation. In future studies, *in vivo* studies are also needed to fully understand the effect of drying processes on the bioaccessibility of pumpkin pestil's phenolics and carotenoids. Additionally, to reduce HMF formation, pre-cooking can be applied under vacuum at low temperatures, the amount of sugar added to the product formulations can be optimized, and new drying methods can be applied that allow the product to dry faster while preserving its nutritional value at lower temperatures.

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