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Incorporation of defatted apple seeds in chewing gum system and phloridzin dissolution kinetics

Recep Gunes[a], Ibrahim Palabiyik[b,*], Omer Said Toker[c], Nevzat Konar[d], Sefik Kurultay[b]

[a] Department of Food Engineering, Faculty of Engineering, Kirklareli University, 39000, Kirklareli, Turkey
[b] Department of Food Engineering, Faculty of Agriculture, Namik Kemal University, 59000, Tekirdag, Turkey
[c] Department of Food Engineering, Faculty of Chemical and Metallurgical Engineering, Yildiz Technical University, 34000, Istanbul, Turkey
[d] Department of Food Engineering, Faculty of Architecture and Engineering, Siirt University, 56000, Siirt, Turkey

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ABSTRACT

Apple seeds are among the major natural sources of antioxidants and can be used in various industries. In this regard, antioxidant activity, total phenolic content, and individual phenolic compounds analyzes of defatted apple seed flours were firstly done in our study. According to these analyzes, total phenolic content, DPPH and ABTS radical scavenging activity of defatted seed flours were determined between 2861 and 5141 mg GAE/kg defatted seed, 21.45–43.56 μmol, and 291.50–391.79 μmol Trolox/g defatted seed, respectively. It was observed that the content of phloridzin represented 52–67% and 75–83% of the total phenolics that measured by the Folin-Ciocalteu assay and HPLC method, respectively. In the second part, chewing gums including defatted seeds were prepared and characterized in terms of phloridzin dissolution. The novel model described dissolution kinetics of phloridzin from chewing gum better than Higuchi and Korsmeyer-Peppas models. The results demonstrated that 5 min was enough for the dissolution of almost all phloridzin (88.43–96%) determined by centrifugation method and according to the model parameters, the chewing gum formulation can be optimized for providing controlled dissolution. In conclusion, chewing gum could be a suitable delivering material for phloridzin uptake, and apple seeds, a valuable agricultural by-product, could be evaluated in this way.

1. Introduction

The increase in the production of apples in parallel with the world population and the widespread consumption of apples and its derivative products have increased the amount of by-products that are released after processing. In this respect, two branches of the industry that produce large amounts of apple by-products are the juice and fresh-cut fruit salad production (Górnaś, 2015). For instance, the apple pomace (press cake) obtained during juice pressing represents approximately 20–35% of the initial amount of fruits, and the composition of pomace consists of 94.5% flesh and skin, 4.1% seeds and 1.1% fruit stems (Candrawinata et al., 2015). Therefore, the scientific studies were not only confined to fruit juices, flesh, and peels but also were carried out on apple seeds.

Recently, it has been determined that the apple seeds are a rich source of polyphenols and show significant antioxidant activities. These polyphenols are mainly composed of dihydrochalcones; hydroxycinnamic acids; flavan-3-ols and flavonols (Fromm et al., 2012; Fromm et al., 2013; Xu et al., 2016). From these compounds, phloridzin, a chalcone derivative, is known as the characteristic polyphenol of apple and it protects the plant against some pathogens (Mikulic Petkovšek et al., 2008). It has antidiabetic effect by interfering glucose adsorption by inhibition of the sodium-linked glucose transporters (Manzano and Williamson, 2010). In addition, this important compound has antioxidant activity; therefore, it can inhibit lipid peroxidation and a series of new bioactive functions of this compound have been reported recently, such as prevention of bone loss, enhancement of memory and life extension as well as inhibition of cancer cell growth (Baldisserotto et al., 2012). Nair et al. (2014) reported that fatty esters of phloridzin have potential chemotherapeutic effects against human liver cancer cells through different mechanisms. Therefore, since the discovery of these properties of apple seeds and its constituents, it has

Abbreviations: ABTS, 2,2′-azino-bis-(3-ethylbenzothiazoline-6-sulphonic acid) diammonium salt; DPPH, 2,2-Diphenyl-1-picrylhydrazyl; FC, Folin-Ciocalteu; FRAP, ferric reducing antioxidant power; GAE, gallic acid equivalent; HPLC, high performance liquid chromatography; UV–Vis, ultraviolet–visible; TEAC, Trolox equivalent antioxidant capacity; TPC, total phenolic content; Trolox, 6-hydroxy-2,5,7,8-tetramethylchroman-2-carboxylic acid

* Corresponding author. Department of Food Engineering, Faculty of Agriculture, Namik Kemal University, 59000, Tekirdag, Turkey.
E-mail address: ipalabiyik@nku.edu.tr (I. Palabiyik).

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been stated that they can be evaluated in different sectors such as food, pharmacy and cosmetic (Arain et al., 2012; Fromm et al., 2013). For this aim, different efforts are needed to increase the economic value of these seeds and to benefit from their potential health effects.

Among food products, chewing gum is widely consumed all over the world and has potent for delivering of bioactive compounds. As well as usual consumption, its formulations have been evaluated for several drugs and plant materials so as to improve their releasing and bioavailability (Paradkar et al., 2016). Therefore, it has been used as a drug delivery system in some medical treatments and stated that the gum formulations offer several advantages over the tablet or liquid formulations (Swamy et al., 2012). The benefits of chewing gum in medical applications include local and systemic effect, consumption without water, high acceptance by children and teenagers, fewer side effects, suitable stability and effective on the dry mouth (Aslani and Rostami, 2015). Besides all these, taking into account that the mild production conditions, having the longest duration of staying in mouth compared to other foods and low dose active ingredients are being the prime candidates for the formulation (Chandran et al., 2014; Konar et al., 2016); it turns out that chewing gum may be a potential delivering material in the administration of bioactive compounds.

To the best of our knowledge, no study has been carried out on the evaluation of defatted apple seeds in food products and therefore, the first purpose of this research is to determine the phenolic profiles and antioxidant activities of the phenolic extracts obtained from defatted seed flours of 5 different commercial apple cultivars after oil extraction. Secondly, considering the advantages of chewing gum as a potential delivering system and scientific studies based on the effects of phloridzin as an antioxidant and antimicrobial agent, it was decided to formulate a new chewing gum with defatted apple seed flour and to determine the dissolution kinetics and mechanism of phloridzin from the chewing gum matrix.

2. Materials and methods

2.1. Materials

The apple seeds used in the research were obtained from the apples collected at Kirklareli and Tekirdag/Turkey during the autumn harvest period (September-October-November). Five different apple cultivars (Fuji Zhen Aztec, Granny Smith, Pink Lady, Super Chief, Jeromine), which have high economic value and widely produced in the region, were used. Fruit flesh firmness test and water-soluble dry matter analyzes were performed to determine that the apples were in the appropriate commercial harvest condition. As a result of these analyzes apples were harvested at commercial maturity: flesh firmness 6–8.5 kg/ cm^2 and sugar content 11–14 °Brix.

DPPH, ABTS, Trolox, Gallic acid and Folin Ciocalteu's phenol reagent were provided from Sigma-Aldrich (St. Louis, MO, USA). HPLC-grade methanol and *n*-hexane were purchased from Merck, Darmstadt, Germany. All other chemicals and reagents used were of analytical grade.

2.2. Preparation of apple seeds

The seeds were collected manually from the apples without any damage by cutting all sides of the whole fruit with a knife to remove the fruit flesh and reveal the seed cores. Then the seeds were removed from the seed cores and during this process; empty, rudimentary and blackened seeds were separated from normal ones and were not included in the analyzes. The seed samples were dried at 55 °C for 5 h and taken in separate cellophane packages according to the cultivar. After the cellophane packages had been wrapped in aluminum foil, they were kept in the dark at room temperature until further analyzes (Górnaś, 2015). Prior to analyzes, the apple seeds were ground with a domestic coffee grinder (Siemens MC 23200). Crude oil of the ground dry seeds (10 g) of each cultivar was removed with *n*-hexane in a Soxhlet extractor for 8 h. For all samples, after the crude oil analysis, the defatted seed flours were dried at 50 °C for 3 h in order to remove any residual solvent (*n*-hexane) left (Chan et al., 2013).

2.3. Preparation of phenolic extracts from defatted seed flours

Extraction of phenolic compounds from the defatted seed flours was performed according to the method described by Karaman et al. (2015). Defatted seed flours (2.5 g) were mixed with 25 mL 80% methanol and held in an ultrasonic bath for 30 min at room temperature. The tubes were centrifuged at 5000 rpm for 5 min and the supernatants were filtered using a 0.45 μm filter. Each extract was transferred into dark glass bottles and kept at −18 °C for further analysis.

2.4. Total phenolic content analysis of defatted seed flour extracts

The total phenolic content was measured by the Folin-Ciocalteu method of Singleton et al. (1999) with some modifications. Firstly 7.5 mL distilled water was pipetted into the test tubes. Then, 100 μL of the phenolic extracts which were prepared as described above and 500 μL Folin-Ciocalteu reagent were put into these tubes. The tubes were mixed thoroughly and left to stand for 3 min. Then, 1 mL saturated Na_2CO_3 solution was added to each test tube and 900 mL distilled water was added in order to reach a final volume of 10 mL. Finally, the tubes were left to stand for 1 h at room temperature in the dark and the absorbance was read at 720 nm in a UV–Vis spectrophotometer (Shimadzu, Tokyo, Japan). The results were expressed as mg gallic acid equivalents (GAE)/kg defatted seed flour using the calibration curve of gallic acid ($R^2 = 0.9986$) and taking into account the dilution rates applied. Each measurement was performed in triplicates and results were shown as mean values with a ± standard deviation.

2.5. ABTS radical scavenging activity analysis of defatted seed flour extracts

The ABTS method was used according to Xu et al. (2016) with some modifications to determine the ABTS radical scavenging activity of phenolic extracts. Firstly, 4.9 mM potassium persulfate solution ($K_2S_2O_8$) and 14 mM ABTS solution were prepared with distilled water and were transferred to an amber bottle at a ratio of 1:1 (v/v). Then, the bottle was left in a dark place at room temperature for 12–16 h to allow the formation of ABTS radical, and at the end of time dark blue color was observed. 1 mL of this stock solution was diluted with methanol to an absorbance of 0.70 ± 0.02 at 734 nm. Various volumes of methanolic extracts (10-15-25 μL) were mixed with 2.0 mL ABTS solution into spectrophotometer cuvettes. The cuvettes were left in the dark for 6 min at room temperature. After that time, the samples were measured against the blank (methanol) with the UV–Vis spectrophotometer (Shimadzu, Tokyo, Japan) at 734 nm. A calibration curve ($R^2 = 0.9996$) was obtained by using Trolox standard solution at concentrations from 50 to 2000 μM. ABTS radical scavenging activity of the extracts was expressed as μmol Trolox/g defatted seed flour. Each measurement was performed in triplicates and the results were expressed as mean values ± standard deviation.

2.6. DPPH radical scavenging activity analysis of defatted seed flour extracts

The DPPH method was conducted according to the method of Thaipong et al. (2006) with some modifications. Various volumes of methanolic extracts (100-150-200 μL) obtained from each cultivar were pipetted into spectrophotometer cuvettes. Then, 2.850 mL 0.1 mM DPPH (prepared with methanol) solution was added. The cuvettes were left in the dark for 30 min at room temperature. At the end of the time, the absorbances were determined against the blank (methanol) using

the UV–Vis spectrophotometer (Shimadzu, Tokyo, Japan) at 517 nm. A calibration curve ($R^2 = 0.9991$) was obtained by using Trolox standard solution at concentrations ranged between 50 and 1000 µM. DPPH radical scavenging activity of the extract was expressed as µmol Trolox/g defatted seed flour. Each measurement was performed in triplicates and the results were given as mean values ± standard deviation.

2.7. Quantitative determination of phenolics in defatted seed flour extracts by HPLC

Analysis of phenolic compounds of the extracts was performed according to Belwal et al. (2017) method, using HPLC (LC-10AT, Shimadzu Liquid Chromatography, Japan) coupled to binary pump and diode array detection unit (SPD-M2OA). The extract was filtered with a 0.2 µm membrane filter (Merck-Millipore, Germany) and injected in a C18 reverse-phase column (5 µm, 250 × 4.6 mm, Purosphere, Merck, Darmstadt, Germany). The mobile phase was a mixture of methanol and 0.1% orthophosphoric acid (40:60) and flow rate and the detector was 0.8 mL/min and PDA detector, respectively. The detector screen the compounds from 254 to 330 nm of wavelength with a total run time of 40 min. To detect and measure the concentration of phenolic compounds, 8 standard phenolics (ellagic acid, (−)-epicatechin, ferulic acid, (+)-catechin, gallic acid, chlorogenic acid, phloridzin, and caffeic acid) have been used. Stock solutions (20, 40, 60, 80, 100 mg/l) of standards were prepared in methanol and the calibration and linearity of the curve were calculated with the correlation coefficient. Each measurement was performed in triplicates and the results were expressed as mg/kg dry weight of defatted apple seed flour. On the other hand, the percentage of phloridzin monomer in total phenolic contents determined by both HPLC and Folin-Ciocalteu method was assessed in the study.

2.8. Production of chewing gum enriched with defatted apple seed flours

Amount of defatted seed flour added to chewing gum was determined by sensory test. According to the sensory analysis, the addition of 1% seed flour did not negatively affect the quality of chewing gum. Addition of other amounts (5, 10%) affected the taste, odor and hardness values of the chewing gum. Therefore, in the present study, chewing gum was enriched with defatted apple seed flour at 1% concentration. In the production step, chewing gum base (Maykim, Turkey) was heated to 70 °C and defatted apple seed flour (1%) mixed with glucose syrup (20%), powdered sugar (53%), glycerin (1%), lecithin (0.25%) and sorbitol (0.25%) were added to gum base and mixed for 5 min. In order to ease mixing, the prepared blend was heated to 70 °C and mixed for 10 min again. After homogenizing, the samples were molded and stored at room temperature.

2.9. Determination of dissolution kinetics of phloridzin in chewing gum matrix

Samples of chewing gums prepared with 5 different defatted apple seed flours were cut into small pieces with the aid of a sharp knife while still frozen and then 2.5 g of each sample was weighed and transferred to falcon tubes with 15 mL ultrapure water. In order to reflect the chewing process in the mouth, each tube was first centrifuged at 4500 rpm for 1 min. Then 2 mL were pipetted from these tubes and filtered through a 0.45 µm filter to amber autosampler vials. For each tube, the same volume (2 mL) of ultrapure water was added in order to make total volume again 15 mL and the tubes were then vortexed again for 1 min. Centrifugation process was repeated for 3, 5, 10 and 20 min in the same tubes, respectively and the filtrates obtained after the same procedure were transferred to amber autosampler vials. The amount of phloridzin passing through ultrapure water after centrifugation at different times was determined according to the HPLC analysis described above. Each measurement was performed in triplicates and the results were expressed as mean values ± standard deviation.

The experimental time versus dissolved phloridzin amount was modeled using the following equations to determine the best model to describe the dissolution kinetics of the phloridzin from chewing gum system;

1) Novel model

$$y = a \times (1 - b^{x}) \tag{1}$$

where y is the concentration of the dissolved phloridzin, x is centrifugation time, a and b are parameters represent the maximum concentration be dissolved during centrifugation and dissolution rate, respectively.

2) Higuchi Model

$$y = a \times x^{0.5} \tag{2}$$

where a is a model constant.

3) Korsmeyer-Peppas Model

$$y = a \times x^{b} \tag{3}$$

where a is a model constant and b is the dissolution rate.

2.10. Statistical analysis

The software SPSS 16.0 for Windows (SPSS Inc., Chicago, IL, USA) was used for analysis of variance (ANOVA) and Tukey's multiple comparison tests in order to determine significant differences between the samples. Also, two-tailed Pearson's correlations test was conducted to identify correlations among the means.

3. Results and discussion

3.1. Total phenolic contents and radical scavenging activities of defatted seed flour extracts

According to the results, as shown in Table 1, the highest amount of total phenolic content in the defatted seed flours was detected in Super Chief (5141 mg GAE/kg defatted seed flour), while the lowest was found in Fuji Zhen Aztec (2861 mg GAE/kg defatted seed flour). Amount of total phenolic in the defatted seed flours significantly differed among the cultivars in the study ($p < 0.01$). Antioxidant activity assays indicated that ABTS values of all apple cultivars were higher than that measured by DPPH. It indicated that DPPH decolorizing reaction was not supported by constituents of the extracts and thus showed a little activity. The highest $TEAC_{ABTS}$ value of the defatted seed flours was determined in Pink Lady (391.8 µmol Trolox/g defatted seed flour) and the lowest in Granny Smith (291.5 µmol Trolox/g defatted seed flour) ($p < 0.01$). Additionally, the highest $TEAC_{DPPH}$ value of

Table 1
Total phenolic contents, ABTS and DPPH radical scavenging activities of the defatted apple seed flours.

Cultivar	TPC (mg GAE/kg DS)	ABTS (µmol Trolox/g DS)	DPPH (µmol Trolox/g DS)
Fuji Zhen Aztec	2861 ± 23^{d}	363 ± 2^{b}	24.28 ± 1.01^{c}
Granny Smith	3581 ± 48^{c}	292 ± 4^{d}	33.12 ± 0.02^{b}
Jeromine	4096 ± 98^{b}	368 ± 4^{b}	32.04 ± 0.77^{b}
Pink Lady	3616 ± 31^{c}	392 ± 2^{a}	21.45 ± 0.27^{d}
Super Chief	5141 ± 46^{a}	343 ± 5^{c}	43.56 ± 0.83^{a}

Each value was expressed by mean ± SD.
There is no statistical difference between the results indicated by the same letter within the same column ($p > 0.01$).
DS: Defatted seed.

defatted seed flours was determined in Super Chief as 43.56 μmol Trolox/g defatted seed flour and interestingly the lowest in Pink Lady as 21.45 μmol Trolox/g defatted seed flour ($p < 0.01$).

In a previous study, Leahu et al. (2013) found that the total phenolic content of apple and grape seeds were 75.2 ± 5.21 mg GAE/100 g and 62.1 ± 2.15 mg GAE/100 g, respectively. It is clearly observed that the total amount of phenolic in apple seeds in this study is very low compared to the results of our study. This can be caused by the apple cultivar, cultural practices, climate, soil characteristics, and also using whole apple seeds and 80% ethanol in the extraction process applied during recovery of phenolics. In another study, it was determined that the total phenolic content of apple seeds prepared with acetone solution (30:70, v/v) varied between 2 and 16 mg GAE/g defatted matter (Fromm et al., 2013). The results obtained for defatted apple seed flours in our research are close to these values.

Regarding antioxidant activities, Xu et al. (2016) found it in whole seeds of different apple cultivars as 37.56–64.31 μM TE/g fresh weight for DPPH, and 220.52–708.02 μM TE/g fresh weight for ABTS method. In our study, the $TEAC_{ABTS}$ and $TEAC_{DPPH}$ values of defatted seed flours were found between these values. Furthermore, in another study, $TEAC_{ABTS}$ values of apple peel and flesh were determined to be 29.9–72.1 μmol TE/g fresh weight and 3.6–22.4 μmol TE/g fresh weight, respectively (Vieira et al., 2011). Hence, these results indicated that apple seeds exhibited stronger antioxidant activity than the peel and flesh.

In defatted seed flours, total phenolic contents were significantly correlated with DPPH assay ($p < 0.01$, $r = 0.846$). By contrast, there was no correlation between the total phenolic content of defatted seed flours and their ABTS values ($p < 0.01$, $r = -0.091$). Also, it was determined that ABTS values of defatted apple seed flours were not correlated with DPPH values ($p < 0.01$, $r = -0.513$). In the literature, there are controversial findings about positive or negative correlations between total phenolic content and antioxidant activity (Fidrianny et al., 2015; Sousa and Correia, 2012). This could be explained by the different kinetic profiles of phenolic compounds against ABTS radical, the need of a longer reaction time, other unspecified phenolic compounds and the synergism between them, and also other parameters such as, pH, temperature, choice of solvent, aging, and storage conditions of the radical reagent (Apak et al., 2016; Imeh and Khokhar, 2002).

3.2. Phenolic profile of defatted apple seed flours

Table 2 represents the general chromatogram for defatted apple seed flours. In our research, phloridzin, a characteristic phenolic compound present in apple seeds, was identified as the highest phenolic compound (1748.7–3462.2 mg/kg dry matter) in all cultivars. As shown in Table 2 and Fig. 1, phloridzin represents 75–83% of total phenolics determined by HPLC and 52–67% of total phenolics according to the Folin-Ciocalteu method. In addition, a significant correlation

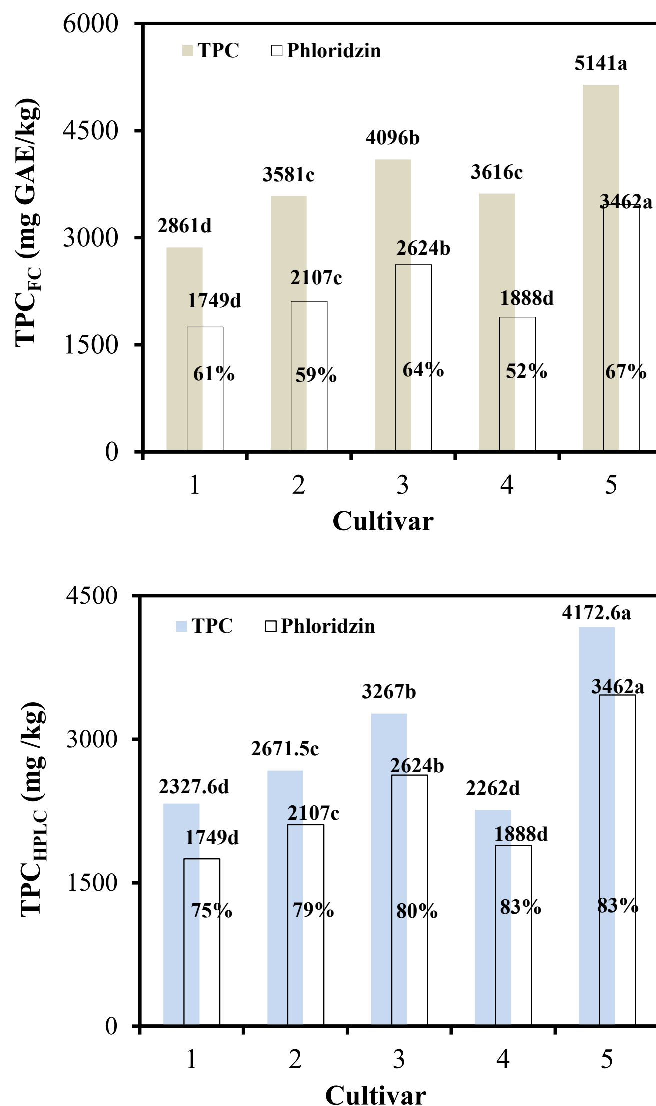

Fig. 1. Percentages of phloridzin monomer in total phenolic contents determined by Folin-Ciocalteu and HPLC method. Numbers on the x-axis represent apple cultivars: 1, Fuji Zhen Aztec; 2, Granny Smith; 3, Jeromine; 4, Pink Lady; 5, Super Chief. There is no statistical difference between the results indicated by the same letter on the same colored columns ($p > 0.01$).

($r = 0.965$, $p < 0.01$) was found between the amount of total phenolics determined by the Folin-Ciocalteu method and the amount of phloridzin determined by HPLC. At the same time, there was a very high correlation ($r = 0.934$, $p < 0.01$) between the total phenolics determined by both the Folin-Ciocalteu method and HPLC.

Table 2
Individual phenolic amount of defatted seed flours of five apple cultivars.

Phenolic Compounds	Fuji Zhen Aztec	Granny Smith	Jeromine	Pink Lady	Super Chief
Phloridzin	1748.7 ± 52.4^{d}	2106.6 ± 15.0^{c}	2623.5 ± 30.5^{b}	1888.4 ± 17.4^{d}	3462.2 ± 62.7^{a}
Ellagic Acid	189.5 ± 1.2^{c}	275.3 ± 18.4^{a}	286.7 ± 0.8^{a}	216.5 ± 9.7^{bc}	230.7 ± 10.7^{b}
(−)-Epicatechin	76.3 ± 6.7^{b}	77.2 ± 1.0^{b}	164.6 ± 12.2^{a}	73.9 ± 3.4^{b}	69.0 ± 4.6^{b}
Caffeic Acid	114.2 ± 2.8^{a}	9.1 ± 0.1^{c}	11.8 ± 0.4^{bc}	10.4 ± 0.2^{bc}	14.2 ± 0.0^{b}
(+)-Catechin	90.1 ± 2.0^{b}	5.0 ± 0.1^{cd}	ND	21.0 ± 1.3^{c}	191.0 ± 11.8^{a}
Ferulic Acid	57.8 ± 1.8^{b}	142.2 ± 1.1^{a}	21.2 ± 0.1^{d}	8.2 ± 0.2^{e}	36.4 ± 0.3^{c}
Protocatechuic Acid	46.8 ± 1.6^{b}	48.7 ± 0.6^{b}	153.8 ± 6.1^{a}	39.6 ± 1.3^{b}	161.3 ± 2.1^{a}
Gallic Acid	4.2 ± 0.2^{c}	7.5 ± 0.2^{a}	5.6 ± 0.1^{b}	4.2 ± 0.6^{c}	7.9 ± 0.0^{a}

Results were expressed as mg/kg dry matter.
There is no statistical difference between the results indicated by the same letter on the same line ($p > 0.01$).
ND: Not detected.

Furthermore, ellagic acid, attributed as an important antimutagenic, antimicrobial and antioxidant compound, was recorded as the second highest phenolic in the seeds (189.5–286.7 mg/kg dry matter) and it accounted for 5–10% of the total phenolics determined by HPLC. In previous studies, the amount and percentage of phloridzin compound were similar or in line with our results (Fromm et al., 2012; Xu et al., 2016).

According to Fig. 1, the sum of 8 different phenolic compounds in each cultivar that determined by HPLC (TPC_{HPLC}) was lower than the total amount of phenolics determined by Folin-Ciocalteu assay (TPC_{FC}). It is thought to be caused by the fact that only low molecular weight phenolics can be extracted with methanol as reported by Xu et al. (2016). Also, it was stated that thiolysis of apple seed extracts prior to the quantitative determination of phenolics ensuring the conversion of the polymers such as procyanidins to their structural monomeric units ((+)-catechin or (−)-epicatechin) which can be identified by HPLC (Fromm et al., 2012).

3.3. Dissolution of phloridzin from chewing gum system

In the present study, the dissolution rate of the phloridzin was determined as a function of time by the centrifugation method. As is known, chewing process includes mechanical forces affecting dissolution behavior of the corresponding compound. However, forces applied during chewing and saliva composition change from person to person, which can remarkably influence dissolution behavior. Therefore, a standard method is required for this aim. In the present study, the centrifugation method was applied where samples are exposed to mechanical forces. The dissolution rate of phloridzin was determined with respect to the centrifugation time.

Fig. 2 indicated the dissolution kinetics of the phloridzin compound. According to the results, it was clear that the amount of phloridzin dissolved from the chewing gum matrix was proportional to the centrifugation time. After 1 min, 13.18–33.89% of the phloridzin was dissolved and there was a sharp increase in the amount of phloridzin (88.43–96%) dissolution at the end of 5 min centrifugation. Driving force determining the dissolution rate of phloridzin was the concentration differences between chewing gum and centrifugation media. After dissolving phloridzin, the concentration difference decreases and therefore, the dissolution rate reduces. Considering some consumers who chew the gum for only a few minutes, it could be said that almost all of the phloridzin could be dissolved from the gum material before throwing the gum away. However, it should be taken into account that individual differences such as changes in chewing speed, strength and mouth microflora may also affect these dissolution times.

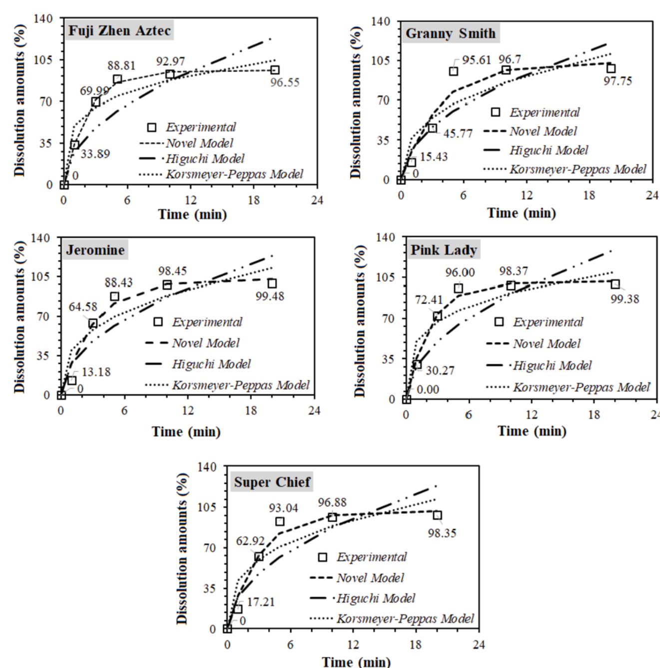

Fig. 2. Experimental and predicted values of dissolution rate of phloridzin from chewing gum enriched with different cultivar of defatted apple seeds. Dissolution concentrations shown in figures belong to experimental values.

3.4. Modelling of dissolution kinetics of phloridzin

The phloridzin concentration versus centrifugation time data was modeled with different models, namely, the Higuchi model, the Korsmeyer-Peppas model, and the novel model to determine the dissolution mechanism. Corresponding model parameters were tabulated in Table 3 where regarding R^2 values, the novel model used for dissolution of phloridzin from chewing gum was more suitable when compared with the other two models. As can be seen in Table 3, R^2 values were found to between 0.94 and 0.99. The Higuchi and Korsmeyer-Peppas models were previously used for drug release kinetics by dissolution mechanism from dosage forms (Camelo et al., 2016; Yang et al., 2004). Yang et al. (2004) found catechin release from chewing gum fitted the best with the Higuchi equation and the results suggested that the release time of catechins from the formulations was distinctly prolonged according to the coating agent. Therefore, coating material changed phenolic release mechanism from mechanical release to dissolution release.

In our study, a novel model was developed to describe the mechanical removal process. Among novel model parameters, a represents the maximum concentration of the compound dissolved during chewing and b represents the dissolution rate. Except for chewing gum prepared with the seed of Fuji Zhen Aztec cultivar, almost all of the phloridzin can be dissolved. b value changed between 0.64 and 0.76, indicating that phloridzin present in Granny Smith cultivar had the highest dissolution rate among all samples. The differences between dissolution rate can result from the composition of seed flours, which can affect chewing gum structure or interaction of phloridzin with the other compounds. Depending on the usage aim of the phloridzin, the chewing gum formulation can be optimized for providing controlled dissolution such as the fast or slow.

4. Conclusions

In our study, apple seeds were found to contain significant levels of phenolic compounds and phloridzin was proven to be the characteristic polyphenol of these seeds. According to the results, it was found that the antioxidant activity of defatted apple seed extracts was comparable to many other fruits seeds. Therefore, seeds of apples could be a potential source of natural antioxidants which can increase the overall quality of foods and can extend their shelf life. Also, using these seeds in various sectors such as food, pharmacy or cosmetic may lead to significant economic gains and prevent or decrease environmental problems caused by the accumulation of apple pomace. Moreover, according to the results of dissolution rates of phloridzin from chewing gum and considering of pharmacological effects of phloridzin, it could be inferred that chewing gum could be a suitable delivering material for the uptake of phloridzin. The present study was the first to use defatted apple seeds in chewing gum formulation, and compare kinetic models to reveal the dissolution mechanism. Therefore, further studies should be done with different formulation ratios to investigate dissolution behavior.

Conflicts of interest

The authors have no conflicts of interest to disclose.

Table 3
Releasing model parameters of phloridzin from chewing gums including different defatted apple seed source.

Models	Model equations	Parameters	Cultivars				
			Fuji Zhen Aztec	Granny Smith	Jeromine	Pink Lady	Super Chief
Novel model for chewing gum	$y = a \times (1\text{-}b^x)$	*a*	96.1	103.1	103.1	101.5	101.9
		b	0.64	0.76	0.73	0.65	0.72
		R^2	0.99	0.94	0.97	0.99	0.97
Higuchi model	$y = a \times x^{0.5}$	*a*	27.68	26.96	27.66	28.87	27.7
		R^2	0.86	0.89	0.90	0.85	0.88
Korsmeyer-Peppas Model	$y = a \times x^b$	*a*	49.5	36.6	39.6	50.4	41.6
		b	0.25	0.37	0.35	0.26	0.33
		R^2	0.92	0.83	0.86	0.89	0.86

a: represents the maximum concentration of the compound (phloridzin) released during chewing.
[b]: represents the releasing rate.

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