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Determination of bioactivity of seed protein hydrolysates and amygdalin content for some apricot (*Prunus armeniaca* L.) varieties grown in Malatya, Turkey

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Abstract

In this study, the aim was to determine the bioactivity of seed proteins from different apricot varieties, namely Hacıhaliloğlu (a PDO product protected by the EU), Hasanbey, Kabaaşı and Zerdali. Bioactivities of isolates obtained by precipitation at the isoelectric point of seeds and hydrolysates obtained by enzymatic hydrolysis with alcalase were examined. In terms of angiotensin converting enzyme (ACE)-inhibitory activity, isolate and hydrolysate from Kabaaşı variety showed the highest activity, while isolate from Hasanbey and hydrolysate from Zerdali had the lowest activity (P<0.05). Kabaaşı isolate and hydrolysate had the lowest antioxidant activity (P<0.05). The amount of amygdalin detected in sweet apricot seeds (Hacıhaliloğlu, Hasanbey, Kabaaşı) is below the toxic level specified by the EFSA. The results suggest that apricot seed protein may be a potential source of plant protein for human nutrition, especially for vegan consumers. Apricot seeds were also rich in terms of ACE-inhibitory and antioxidant activities.

Keywords: *Apricot seed; Prunus armeniaca L; Apricot kernel; ACE-inhibition; Antioxidant activity*

1. INTRODUCTION

Turkey is one of the biggest producers of apricot fruit, which is an important export product for Turkey. Apricot is mostly produced in Malatya (Turkey) which supplies approximately 85% of the world's dried apricot exports. Therefore, Malatya has become a global brand for apricots. When apricots are mentioned in Turkey, Malatya comes to mind (Öztürk and Karakaş, 2017). Apricot contains high levels of antioxidants and phenolic substances and these substances play a protective role in preventing many diseases. It is estimated that apricots are beneficial for a healthy and high-quality life by strengthening the immune system against damage caused by free radicals in the body, protecting against diseases, and delaying aging (Vardı et al., 2008). In addition to the apricot flesh, its seeds are also important export products. It was reported that a total of 5419 tons of apricot kernels or seeds were exported from Malatya in January-December 2020 (AA, 2022). Apricot seeds contain a significant amount of protein, minerals and oil. Apricot seeds (sweet or bitter type) are used for different purposes. Sweet seeds are consumed as snacks or in the production of confectionery, and bitter seeds are also used in the cosmetics and pharmaceutical industry (Hasdemir, 2018).
Protein requirements for humans are met mostly by animal-derived proteins; however, in recent years, the interest and need for vegetable proteins have increased due to factors such as heart disease, diabetes, obesity, animal-borne diseases, the increase in the number of animals fed with antibiotics and desire for vegan nutrition. In addition, the increase in meat prices has restricted people's consumption of animal proteins to some extent. Also, vegetable protein sources are cheaper and more accessible for people who prefer a vegan and vegetarian diet, which has made plant proteins

an alternative source of food (Çetiner and Bilek, 2018). When the studies about protein-containing food sources are examined, bioactive peptides with positive effects on body functions come to the forefront and thesepeptides are also present in the animal, vegetable and marine sources (Albenzio et al., 2017; Admassu et al., 2018).

Studies about plant protein-based peptides have gained importance in recent years due to the limited and expensive nature of animal foods as protein sources (Çetiner and Bilek, 2018). Thus, it was considered that the seeds of the apricot, which is mostly produced in Malatya (Turkey), could be an alternative plant protein source.

In the present study, four different seeds of apricot cultivars, namely Hacıhaliloğlu, Hasanbey, Kabaaşı and Zerdali, were used. The aim was to determine the protein isolates obtained by precipitation at the isoelectric point using oil from the apricot seed cultivars. The hydrolysates were obtained by enzymatic hydrolysis from protein isolates with bioactive properties such as ACE-inhibition and antioxidant activity. Also, it is believed that apricot seeds contain the cyanogenic glycoside, amygdalin, at a high level which is hazardous for consumption. However, the apricot varieties grown in Malatya and exported around the world and these are sweet taste at a ratio of ca. 99% when compared to seed with bitter taste. Only 1% or less is the Zerdali variety, which is bitter and contains a high level of amygdalin (Asma, 2000; Ünal, 2010). The secondary aim was to determine the levels of amygdalin, while the primary aim was to determine the bioactivity of hydrolysates or isolates from the plant proteins.

2. MATERIALS AND METHODS

2.1. Materials

The seeds of Hacıhaliloğlu, Hasanbey, Kabaaşı and Zerdali apricot varieties (non-sulfured) used in the present study were supplied by the Apricot Research Institute (Malatya, Turkey) in 2018. Apricot seeds were collected from at least ten different apricot trees and then a homogenous mix was used as material. Before sample preparation, at least 50 g of each apricot seed was ground and used corresponding analysis in required amount.

Chemicals or standards used during experiments were purchased as follows; amygdalin (from the apricot kernel) from Chem-Impex, International, USA), 2,2´-azino-bis(3-ethylbenzothiazoline-6-sulfonacid (ABTS) from Sigma-Aldrich Co., (Ontario, Canada), angiotensin-converting enzyme (ACE) (from rabbit lung, 1.0 U/mg), bovine serum albumin (BSA), and sodium dodecyl sulfate (SDS), Hippuryl-L-histidyl-L-leucine (HHL) from Sigma-Aldrich Co.,(Missouri, ABD), alcalase (obtained from *Bacillus licheniniforms*, activity ≥ 5 U/g) from Sigma-Aldrich Co., (Zwijndrecht, Netherlands) and Coomassie Brilliant Blue, β-mercaptoethanol from Merck KgaA Co., (Darmstadt, Germany). All chemicals and reagents were analytical grades.

2.2. Determination of protein and amygdalin contents

Total nitrogen content of the apricot seeds was determined by micro Kjeldahl method as described in IDF (1993). Percentage of protein in the samples was calculated by multiplying of the total nitrogen with 6.25. Determination of amygdalin was performed according to the method of Cortés et al. (2018) with slight modifications. Each variety of seed was broken and ground in a mortar. Then 0.5 g of seed flour was removed from the samples and 20 mL of methanol was added to this and mixed by a magnetic stirrer for 24 h. Then, the mixture was centrifuged (Hettich 320, Tuttligen, Germany) at 3500 × *g* for 10 min at 4 °C. The supernatants obtained were filtered through 0.22 μm PTFE syringe filter and transferred to vials and, 20 μL was injected into RP-HLPC (Shimadzu, LC 20 AD Prominence, Kyoto, Japan). The HPLC conditions were: isocratic flow rate 0.8 mL/min at 218 nm in a photo diode array (PDA) detector (Shimadzu, LC 20 AD Prominence, Kyoto, Japan), mobile phase ultrapure water and acetonitrile (80:20 v/v), and Agilent Zorbax (USA) C18 column (4.6 × 250 mm × 5μm).

2.3. Preparation of apricot seed protein extract

The apricot seed protein isolate was prepared by alkaline extraction and acid precipitation method (Mirzapour et al., 2015). The defatted seed powder was mixed with distilled water at 1/10 (w/v) ratio. The pH of the mixture was adjusted to 9.0 by adding in drops of 1 N NaOH then mixed by a magnetic stirrer until a homogeneous mixture was obtained. After mixing, it was centrifuged at 6500 × *g* at 4 °C for 25 min and the precipitate was separated.

The upper liquid phase was removed and its pH was adjusted to 4.5 with 1 N HCl, then mixed by a magnetic stirrer for 30 min. After mixing it was centrifuged at 8000×*g* 4 °C for 25 min. The precipitate obtained after the process was neutralized with 1 N NaOH and then freeze-dried for further analysis.

2. 4. Enzymatic hydrolysis

Enzymatic hydrolysis was performed using the method of Mirzapour et. al. (2015) with some modifications. Apricot seed proteins were hydrolyzed using alcalase enzyme. Briefly, apricot seed protein isolates and alcalase enzyme were dissolved in sodium phosphate buffer (pH 8.0) The seed proteins were hydrolyzed for 3 h (enzyme/substrate ratio of 1/100 (w/w) at 50 °C) in a shaking water bath. The hydrolysis was stopped at 85 °C for 10 min to inactivate the enzyme. After centrifugation (3300 × g for 10 min at 4 °C), supernatants were collected and freeze-dried for analysis.

2.5. ACE inhibition (ACE-i) activity

The ACE-i activity was measured by a method (Rawendra et al., 2013) with some modifications. Firstly, a mixture containing 100 µL apricot protein isolate or hydrolysate sample and 25 µL of 5 mM hippuric acid-histidine-leucine (HHL) was prepared using 25 µL of 10 mM sodium phosphate buffer (containing 400 mM sodium chloride pH 8.9) and vortexed after the addition of 25 µL (200 mU) ACE to initiate the reaction at 37 °C for 30 min. Finally, the reaction was terminated by the addition of 6.75 µL 1 M HCl and then 10 µL of this solution was injected into a C18 column (4.6 × 250 mm, 5 µm) using an HPLC (Shimadzu LC 20 AD Prominence, Kyoto, Japan). The samples were eluted using 5% (v/v) acetonitrile in ultrapure water with 0.1% trifluoroacetic acid (TFA) at a rate of 1 mL/min. The elution was monitored at 228 nm and inhibition activity was calculated as described in the method (Rawendra et al., 2013).

2.6. Antioxidant activity

ABTS radical-scavenging capacity of seed protein isolates and hydrolysates was examined using the method of Kocabey et al. (2016) with some modifications. A stock solution was prepared by mixing 7 mM ABTS with 2.45 mM potassium persulfate and the stock solution was kept in the dark at room temperature for 16 h. Before the test, a fresh solution of ABTS was prepared by diluting with ethanol to obtain an absorbance of 0.70±0.02 at 734 nm. Then samples (100 µL) were reacted with 1 mL of ABTS solution, followed by incubation in the dark at room temperature for 10 min, and then the absorbance was measured at 734 nm by UV/VIS spectrophotometer (Shimadzu, model UV-1800, Kyoto, Japan). ABTS radical scavenging capacity was calculated using the following equation:

$$\% \text{ inhibition} = (A\text{control}-A\text{Sample}) / A\text{control} \times 100$$

$A_{control}$ = Absorbance of the control

A_{sample} = Absorbance of the sample (seed protein isolates or hydrolysates).

2.7. SDS-polyacrylamide gel electrophoresis (SDS-PAGE)

Evaluation of the molecular size distribution of apricot seed protein isolates and hydrolysates was performed with SDS-PAGE using the method described by Schägger (2006) with some modifications. Firstly, the protein samples were mixed with buffer containing 2 mL Tris-HCl / SDS (pH 6.8), 3 g glycerol, 0.8 g SDS, 2 mL β-mercaptoethanol, and 2 mg Coomassie Brilliant Blue G-250. Coomassie blue staining gel was prepared with the following: 10% acetic acid + 0.025% Coomassie Brilliant Blue mixture was kept in the dark at room temperature and then used after electrophoresis. The polymerized separating gel (16%) and stacking (4%) gel was prepared as indicated below:

	Separating gel %16	**Stacking gel %4**
AB*	10 mL	1 mL
Gel buffer	10 mL	3 mL
Glycerol	3 g	-
APS (10%)	100 µL	90 µL
TEMED	10 µL	9 µL

**AB (49,5% acrylamide + 3% bisacrylamide)*

During gel running, β-lactoglobulin (18 kDa), BSA (66.5 kDa), lysozyme (14.3 kDa) and sodium caseinate (κ-casein 19 kDa, αs1-casein 23 kDa, β-casein 24 kDa, αs2-casein 25 kDa) were used as standards.

After all the samples and standards were mixed with SDS buffer, they were heated at 95 °C for 4 min, vortexed for 1 min and left for 15 min at room temperature. After cooling, 5 µL standard and 10 µL samples were loaded carefully onto the gel. Electrophoresis was carried out (a Bio-Rad mini Protein III) at 80 mA for about 90-120 min.

After separation, samples were fixed in staining solution (10% acetic acid, 0.025% Coomassie Brilliant Blue G-250) by shaking for 30 min. After staining, the gel was fixed in 10% acetic acid solution for 10 min and then left in pure water for destaining. Finally, the gel obtained was digitized using a scanner and shown in a figure.

2.8. Statistical analysis

The data obtained were evaluated using analysis of variance (ANOVA) and differences were compared at 95% ($P<0.05$) using Duncan's multiple range test (SPSS for Windows version 16.0).

3. RESULTS AND DISCUSSION

3.1. Levels of protein and amygdalin

Table 1 shows the protein content of apricot seeds from Hacıhaliloğlu, Hasanbey, Kabaaşı and Zerdali varieties. The results showed that the levels of protein in the samples varied from 25.49% to 30.87% (Hacıhaliloğlu < Zerdali < Hasanbey << Kabaaşı). The Kabaaşı samples exhibited a significant difference in terms of protein level compared to the other samples, which were almost at the same level (Table 1). Sharma et al. (2010) reported that the levels of protein in apricot seeds were 23.6-26.2%. Also, similar levels of protein content were observed in apricot kernels grown in Turkey (23.58-27.70%), as reported by other researchers (Özcan, 2000; Özcan et al., 2010).

The amount of amygdalin had the highest levels in the Zerdali apricot seed and the lowest amount of amygdalin was in the Kabaaşı variety (Table 1). Hacıhaliloglu (0.82 mg/g), Hasanbey (1.27 mg/g) and Kabaaşı (0.74 mg/g) seeds are sweet; however, Zerdali (25.90 mg/g) seeds have a bitter taste, which originates from amygdalin. There was no significant difference between the amygdalin contents of Hacıhaliloğlu and Kabaaşı cultivars ($P>0.05$); however, the amygdalin content in Zerdali was higher than the other cultivars ($P<0.05$). Karsavuran et al. (2015) reported that the average amygdalin contents were: 0.25 mg/g for Hacıhaliloğlu, 0.40 mg/g for Hasanbey and 0.10 mg/g for Kabaaşı. Yıldırım et al. (2010) stated that the amygdalin content was different in samples taken from the same seed variety between years. In another study, Hasanbey and Kabaaşı cultivars contained 0.376 mg/g and 0.214 mg/g amygdalin, respectively (Mısırlı et al., 2006). The EFSA (2016) report implies that the cyanide level in the apricot seed is approximately 0.5-3.54 mg/g. When calculated to find the cyanide concentration in our

Table 1. Moisture, oil, protein and amygdalin levels of apricot seeds.

Apricot Cultivar	Moisture (%)	Oil (%)	Protein (%)	Amygdalin (mg/g)
Hacıhaliloğlu	4.82±0.02a	49.46±0.09b	25.49±0.43a	0.82±0.11a
Hasanbey	4.81±0.02a	46.75±1.06a	26.09±2.24a	1.27±0.11b
Kabaaşı	4.86±0.01a	50.05±0.77b	30.87±0.53b	0.74±0.008a
Zerdali	4.88±0.03a	47.07±0.94a	25.52±0.48a	25.90±0.42c

The results were statistically evaluated by one-way analysis of variance (ANOVA). Statistical differences were compared by 95% ($P < 0.05$) Duncan multiple range test.

data, this value was about 20 times below the lowest toxic dose reported by the EFSA. All of the apricot seeds sold in markets or commercially, processed into another product or exported consist of Hacıhaliloğlu (PDO by EU), Kabaaşı and Hasanbey varieties, which have sweet apricot kernels.

3.2. ACE-i activity

Table 2 shows the ACE-i activities of protein isolates and hydrolysates obtained from apricot seeds. The highest ACE-i activity value was observed in the Kabaaşı cultivar with a rate of 89.80% and 94.05% for isolate and hydrolysate from the cultivar, respectively. The lowest ACE inhibition activity value was observed in the Zerdali cultivar with the level of 69%. Almost the same levels of ACE-i were recorded for Hasanbey and Hacıhaliloğlu hydrolysates ($P > 0.05$). As shown in Table 2, the hydrolysates of Kabaaşı and Hasanbey cultivars were more active than isolates; however, the isolates from Hacıhaliloğlu and Zerdali cultivars had higher ACE-i activity than their hydrolysates.

Table 2. ACE-inhibition and antioxidant activities of apricot seed protein isolates and hydrolysates (%)

	ACE-i activity		**Antioxidant activity**	
Apricot Cultivar	**Isolate**	**Hydrolysate**	**Isolate**	**Hydrolysate**
Hacıhaliloğlu	83.20±0.27b	76.92±0.00b	53.43±0.15b	37.81±0.03ab
Hasanbey	66.00±0.66a	78.72±0.42b	55.57±2.48b	51.48±0.94c
Kabaaşı	89.80±0.27c	94.05±1.33c	46.04±2.03a	31.48±0.34a
Zerdali	88.96±0.21c	69.88±0.12a	54.63±2.83b	41.78±0.11b

The results were statistically evaluated by one-way analysis of variance (ANOVA). Statistical differences were compared by 95% ($P < 0.05$) Duncan multiple range test.

Zhu et al. (2010) analyzed the ACE-inhibition activity of apricot kernels by testing with different enzymes and hydrolysis times. The results showed that long hydrolysis times did not increase the ACE-i activity. It was argued that increasing the hydrolysis time may inactivate the small peptides or amino acids. These were divided into three different fractions and peptides smaller than 1 kDa were incubated at different temperatures by Wang et al. (2011). Mirzapour et al. (2017) found that ACE inhibition activity of hydrolysates obtained from wild almond proteins by hydrolysis with alcalase had the highest activity value with a rate of 99.9%. Bioactivity of isolates and hydrolysates (using alcalase and thermolysin) from pomegranate peel proteins was studied and isolates and hydrolysates had values of isolate. 82% ACE inhibition activity, with no significant difference between alcalase and thermolysin hydrolysates (Hernández-Corroto et al., 2019). Chia seeds were used in a study about fractionation based on amino acid type. Peptides belonging to albumin and globulin fractions showed ACE-i activity (Orona-Tamayo et al., 2015). The peptides with a high level of hydrophobic amino acids had higher activity, as peptide sequences and chain length significantly affected the ACE-inhibitory activity (Wang et al., 2014). Another study by Stark et al. (2008) revealed that there was no significant difference between the ACE-i activities of isolates or hydrolysates and these authors stated that the degree of hydrolysis does not substantially affect the ACE-i activity. Pablo-Osorio et al. (2019) reported that chia seed protein isolates had 50% ACE-inhibition activity value. Pedroche et al. (2002) suggested that the hydrolysis time and the enzyme type affect the ACE-inhibition activity, as the inhibitory peptides in the sample may emerge during hydrolysis and will become the target of the enzyme after a period. This may cause a decrease in their ACE-i activities.

The isolate ACE-i activity of the Kabaaşı cultivar was found to be higher when compared to the other cultivars; however, antioxidant activity did not exhibit the same profile for the Kabaaşı cultivar. It was observed that isolates belonging to the four cultivars had both ACE-i and antioxidant activities at certain rates. In addition, other components (such as phenolics) in the seed apart from protein may contribute to the ACE-i activity (Gomaa, 2013). This suggests that peptide properties such as amino acid type, sequence, and molecular weight have a greater effect on bioactivity than quantity (Maqsoudlou et al.,2018). The ACE-i activity of Kabaaşı and Hasanbey cultivars increased after the enzyme hydrolysis of protein.

3.3. Antioxidant activity

Hydrolysates from Hasanbey and Kabaaşı cultivars had the highest and the lowest antioxidant activity at 51.48% and 31.48%, respectively. Differences between the samples for the antioxidant activity of the hydrolysate were significant ($P<0.05$). Table 2 shows the antioxidant capacity of the protein hydrolysates using alcalase and protein isolates. It was observed that the antioxidant capacity of the hydrolysates is lower than those of the isolates. Mirzapour et al. (2015) hydrolyzed wild almonds for 3 hours by using 5 different enzymes and alcalase hydrolysate presented the highest activity when compared with the other four enzymes. Hernández-Corroto et al. (2018) studied the antioxidant activities of some seeds and these varied between 75.3-86.5% for olive seeds (5 cultivars) and 40.1-72.6% for peach (10 cultivars) seeds. García et al. (2016) evaluated peptides in some fruit seeds including apricot, plum, peach and olive. They found that apricot seeds hydrolyzed with alcalase were active at 43%, and plum seeds had the highest activity value. Hernández-Corroto et al. (2019) reported that the antioxidant activity of isolates was higher than hydrolysates with a significant difference. The antioxidant activity values ranged between 10-80% depending on the peptide fractions as reported by Agrawal et al. (2016) and peptide activities changed by amino acid sequence (Feng et al., 2018). Alcalase, pepsin, papain and flavor enzymes were used for the hydrolysis process of rice grains and antioxidant activities of rice hydrolysates were in the range of 18.6-65.9% in the fractions (Yan et al., 2015). It was reported that the activity was dependent on the composition of the material and hydrophobic properties of the amino acids in the peptides, as well as the protein isolation conditions, hydrolysis temperature, enzyme type and peptide concentration (Wang et al., 2010; Silvestre et al., 2012). Antioxidant activity values of the isolates and hydrolysates obtained in this study were close to the above-mentioned studies. Only a few studies are comparing the bioactivity of isolates and hydrolysates. The antioxidant capacity of pomegranate peel protein isolates was higher than hydrolysates in results by Hernández-Corroto et al. (2019).

The ACE-i activities of Hacihaliloglu and Zerdali cultivars decreased after hydrolysis. This is thought to be caused by factors other than hydrolysis or factors affecting bioactivity during hydrolysis. The bioactivity (ACE-i and antioxidant activities) of the peptides resulting from hydrolysis may have changed depending on the amino acid sequences and types (Feng et al., 2018). Considering the studies suggesting hydrolysis conditions affect bioactivity, it is thought that the optimum hydrolysis conditions for Hacıhaliloğlu and Zerdali cultivars may be different from Kabaaşı and Hasanbey cultivars or the bioactivity of peptides emerging in Hacıhaliloğlu and Zerdali cultivars are lower. Many studies reported that bioactivity is not only dependent on hydrolysis but also depends on many factors including hydrolysis duration and enzyme type (Salampessy et al.,2017), the combined use of different enzymes in hydrolysis, heat treatment (Rui et al.,2012; Zheng et al., 2017), isolation method, amino acid sequence, composition and type in peptides, the molecular weight of peptides etc. (Yoshie-Stark et al.,2008; Wang et al.,2014; Maqsoudlou et al., 2018; Feng et al., 2018). It was stated that the relationship between the structures of ACE-inhibitory and antioxidant peptides could not be determined exactly and a complex mechanism was encountered. It is thought that the amino acid sequences of apricot kernel proteins and the amino acid types such as hydrophobic and aromatic ones are different. In addition, many factors such as climate and soil conditions in which fruits are grown, tree characteristics, ripening/harvesting times and components other than protein (e.g., phenolics) may affect the bioactivity of apricot kernel proteins (Gomaa, 2013; Karsavuran et al., 2015; Senica et al., 2016).

3.4.SDS-Polyacrylamide gel electrophoresis (SDS-PAGE)

Figure 1 illustrates the SDS-PAGE patterns of protein isolates or hydrolysates using alcalase from apricot seeds. The electrophoretic profile of isolates obtained from apricot seed proteins resulted in bands spanning mostly 19-66.5 kDa and these bands were different. While the band lines for Hasanbey, Hacıhaliloğlu and Kabaaşı cultivars (sweet taste) are close to each other, the band lines for the Zerdali cultivar (bitter taste) are thicker and more intense at certain points. The band intensity of hydrolysates decreased by hydrolysis, which was determined by the molecular weight loaded on the left side of the gel pattern. The molecular weights of hydrolysates are thought to be around 18-35 kDa for the samples. Similarly, Mirzapour et al. (2015) indicated that molecular weights of the band on the SDS-PAGE image for wild almond proteins varied between 18-55 kDa. Electrophoretic band lines for Hasanbey and Zerdali hydrolysates are considerably different from Hacıhaliloğlu and Kabaaşı cultivars. As in the isolate of Zerdali hydrolysate, the intensity of the band line is higher at certain points compared to other hydrolysates. The band appearance on the gel may have changed due to the difference in protein forms in the seeds

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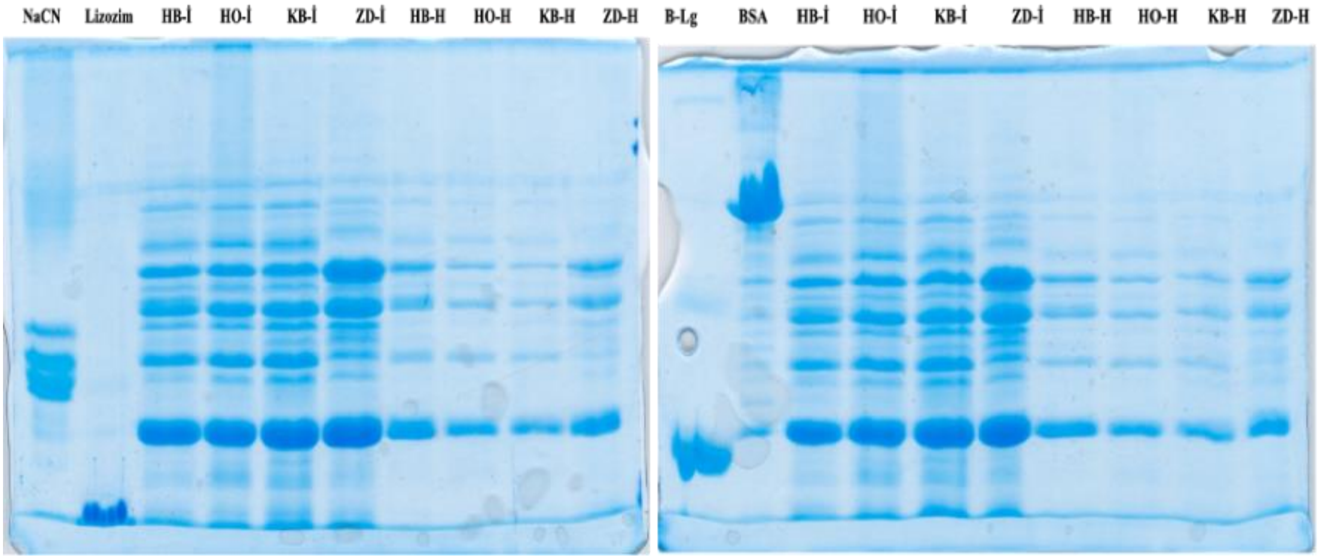

Figure 1. *SDS-PAGE images of seed protein isolate (I) and hydrolysates (H). HB: Hasanbey, HO: Hacıhaliloğlu, KB: Kabaaşı, ZD: Zerdali*

4. CONCLUSIONS

This study is the first attempt to investigate the ACE-i and antioxidant activities of seed proteins from apricot cultivars grown in Malatya. In this context, protein and amygdalin contents were also studied and compared to seeds from other plants. As expected, the Zerdali cultivar (bitter type seeds) contained a higher level of amygdalin than other cultivars including Hasanbey, Hacıhaliloğlu and Kabaaşı (sweet type seeds). The isolates and hydrolysates from apricot kernel proteins had a certain level of bioactive properties (ACE-i and antioxidant activities); however, no correlations were observed between the levels of amygdalin and bioactive properties of the seeds. Further analyses are needed by testing peptide sequences, amino acids and their compositions using different enzyme types and hydrolysis times. Peptide sequences with high activity values can be obtained by advanced chromatographic analyses. The protein isolates and hydrolysates of seeds should be examined in further studies and used as a source of plant protein.

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CONFLICT OF INTEREST STATEMENT

There are no conflicts of interest to report

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