



# Determination of Acrylamide and 5-hydroxymethyl-2-furfural (HMF) Levels and Related Parameters in Turkish Pekmez (A Traditional Fruit Product)

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## HIGHLIGHTS

- 5-hydroxymethyl-2-furfural (HMF) level in Turkish Pekmez was lower than legal regulation.
- There is a moderate positive linear correlation between acrylamide with HMF and total phenolic.
- There is a moderate negative linear correlation between acrylamide and L\*a\*b.

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### Acronyms and abbreviations

AA=Acrylamide  
GAE=Gallic Acid Equivalent  
HMF=5-hydroxymethyl-2-furfural  
HPLC=High Performance Liquid Chromatography  
TP=Total Phenolic  
TRS=Total Reducing Sugar

## ABSTRACT

**Background:** Pekmez is an important fruit-based food of Turkish culinary culture. The aim of this study is to determine the levels of acrylamide (AA), 5-hydroxymethyl-2-furfural (HMF) and other selected parameters in grape, mulberry and carob Pekmez.

**Methods:** AA and HMF were analyzed by Liquid Chromatography and High Performance Liquid Chromatography, respectively. Also, glucose, fructose, pH, protein, total phenolic, and color (L\*a\*b\*) were analyzed. The analyses were done by IBM SPSS Statistics 26 software.

**Results:** The average AA, HMF, glucose, fructose, total reducing sugar, pH, protein, total phenolic, and colour (L\*a\*b\*) values of Pekmez were 302 µg/kg, 25.7 mg/kg, 13.2%, 14.0%, 27.2%, 5.27, 1.16%, 4.64 mg GAE/g, and 4.83\*5.60\*1.52, respectively. AA indicates a moderate positive linear correlation with HMF, protein, total phenolic; whereas AA indicates a moderate negative linear correlation with glucose, fructose, total reducing sugar, pH, and L\*a\*b.

**Conclusion:** It is presumed that heat treatment is a determinant in AA and HMF formulation.

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## Introduction

Pekmez (molasses) is one of the most important traditional foods in the eastern culture (Heshmati et al., 2019). The main reason for producing Pekmez is to make fruits

and vegetables, which can easily go bad, non-perishable by using various techniques. While the process of Pekmez production may differ according to the main

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ingredient, a general outline of the steps (for producing liquid Pekmez from fresh fruits and vegetables) is as follows: (i) the raw material is cleaned and washed, (ii) the raw material is chopped and mashed, (iii) it is compressed and a muddy wort is extracted, (iv) it is run through a separator in order to separate the fruit pulp, (v) marl or technical  $\text{CaCO}_3$  is added in order to reduce acidity, (vi) it is heated up to 60-70 °C, (vii) it is cooled down to 45-50 °C and rested, (viii) in order to clarify the wort it is settled and filtrated, (ix) it is densified by boiling it at high temperature (65-70 °C) in open lid boilers (above 100 °C) or in vacuum (65-70 °C) in order to concentrate the pekmez (Brix: 68-80%), (x) it is cooled down and rested, (xi) it is packaged and stored (Batu, 2005; Karababa and Develi Isikli, 2005).

Pekmez, which is an important source of energy (246-273 kcal; 1,030-1,141 kJ) due to its high carbohydrate level (60-65%), is also rich in vitamins ( $\text{B}_1$ ,  $\text{B}_2$ ), minerals (Ca, Fe, K, Mg, Na), organic acids, and phenolic compounds (Özhan et al., 2010; Tüzün et al., 2020). Pekmez has a rich content regarding the reducing sugars such as glucose and fructose, and it is heat treated during its production. Therefore, it stands out as a food with a relatively high potential for the formation of 5-hydroxymethyl-2-furfural (HMF) and acrylamide (AA) whose main formation mechanism is considered to be caramelisation and Maillard Reaction (Nguyen et al., 2016; Stadler et al., 2002).

AA ( $\text{C}_3\text{H}_5\text{NO}$ , CAS No: 79-06-1), which was first published to be discovered in food in 2002 (Tareke et al., 2002), is a colourless, odourless compound in a crystal powder form which can easily be solved in water, methanol and acetone (National Center for Biotechnology Information, 2021). The amount of AA found in food is 24-1,499  $\mu\text{g}/\text{kg}$  and it has a relatively wide range depending on various factors such as the type and composition, the processing technique, and storage conditions of the food. AA is found at a high level in French fries, chips, bread, biscuits, breakfast cereals, baby food, and coffee (European Food Safety Authority, 2015). AA, as an extremely toxic compound, has been defined in Group 2A, which is a probable carcinogenic for humans, by International Agency for Research on Cancer (1994). It has been reported that nutritional AA intake increases the risk of getting cancer (Adani et al., 2020), affects foetal development negatively (Duarte-Salles et al., 2013), and may cause damage on nervous system (Kopanska et al., 2018). Joint FAO/WHO Expert Committee on Food Additives pointed out in 2011 that the neurotoxic NOAEL level in mice is 0.2 mg/kg bw per day (Joint FAO/WHO Expert Committee on Food Additives, 2011).

HMF ( $\text{C}_6\text{H}_6\text{O}_3$ , CAS No: 67-47-0) is a water-soluble, heterocyclic organic compound which has been reported to be found in food since 1950s and it is also a furan

derivative. HMF, which is found in many of the foods we consume daily with a high concentration of 0-1,900 mg/kg (Capuano and Fogliano, 2011), is also considered to be a sign of quality in many other foods (honey, juice etc.) as well (Gökmen and Şenyuva, 2006). Toxicological features of HMF have not been detailed so far. It has been concluded in a number of studies on animals that there is no adverse effect of 80-100 mg/kg body weight per day (Abraham et al., 2011). Some researchers stated that nutritional HMF consumption in high concentrations may show cytotoxic traits and that HMF is an indirect mutagen (Capuano and Fogliano, 2011). Dietary intake of HMF was estimated at 1.6 mg/person/day by the European Food Safety Authority (2011).

HMF and AA are found in many of the foods in our daily nutrition at different levels. Exposures start with consuming these foods and continue throughout our lifetime. Therefore, many researchers develop strategies regarding the production and consumption of ideal nutrition by studying risky foods which are rich in HMF and AA and by doing that, they contribute to the public health. In this regard, European Commission has been advising EU countries since 2007 that they monitor the level and the exposure of AA systematically (European Commission, 2019). Moreover, Joint FAO/WHO Expert Committee on Food Additives emphasised that there is very little information regarding the level and formation of AA in foods in developing countries and that the research in this field is very important (Joint FAO/WHO Expert Committee on Food Additives, 2011). If the literature is to be reviewed, it is possible to come across studies which examine the levels of HMF and AA in different kinds of Pekmez. However, no research, which studies the correlation of HMF and AA with sugar, protein, pH, phenolic compound and the colour values, has been found. The aim of this study is to determine the levels of AA, HMF, glucose, fructose, Total Reducing Sugar (TRS), pH, protein, Total Phenolic (TP), and colour ( $L^*a^*b$ ) in grape, mulberry and carob Pekmez, and to examine their correlation statistically.

## Materials and methods

### Samples

In Turkey, Pekmez is called by the name of the raw material from which they are produced. In this research, grape, mulberry, and carob Pekmez, which are sold and known to be consumed widely in Turkey, were studied between December 2020 to January 2021. Within this scope, a total of 24 Pekmez samples in their original packaging (glass jar) were bought from a supermarket, consisting of 2 products from each brand with different

expiry dates (grape Pekmez: 3 brands×3 products; mulberry Pekmez: 3 brands×3 products; carob Pekmez: 2 brands×3 products).

#### Reagents

Acetonitrile, methanol, FeSO<sub>4</sub>·7H<sub>2</sub>O, glacial acetic acid (CH<sub>3</sub>COOH), hydrochloric acid (HCl %37) (Merck, Germany), formic acid (ISOLAB; Wertheim, Germany), FeCl<sub>3</sub> (Carlo Erba, Spain), ethanol (C<sub>2</sub>H<sub>5</sub>OH) (Symras, Turkey), sodium carbonate (Na<sub>2</sub>CO<sub>3</sub>), Folin Ciocalteu's phenol reactive, 2,2-Diphenyl-1-picrylhydrazyl (DPPH), 6-hydroxy-2,5,7,8-tetramethylchromane-2-carboxylic acid (Trolox), gallic acid, sodium acetate trihydrate (CH<sub>3</sub>COONa·3H<sub>2</sub>O), 2,4,6-Tris(2-pyridyl)-s-triazine (TPTZ), fructose, glucose, HMF, AA-d<sub>3</sub> standards (Sigma-Aldrich; St. Louis, MO, US), 0.45 μm PTFE and 0.45 μm PVDF filters (ISOLAB; Wertheim, Germany).

#### Determination of HMF content

The HMF analysis Makawi et al. (2009) was carried out by making some modifications to the study. Ten g sample was weighed into a beaker and diluted with distilled water, put into a 50 ml volumetric flask and completed. The solution was taken through the syringe filter into vials and injected into the conditioned High Performance Liquid Chromatography (HPLC) system with UV detector (Shimadzu, Japan). The mobile phase used was methanol: water (10:90) (v/v). A 20 μl sample was injected into a 5 μm C18 reversed phase column (150×4.66 mm; Nanologica, Sweden). Measurements were performed at 30 °C column temperature and at a flow rate of 1 ml/min. Samples of each extract were analysed in duplicate.

The method for HMF analysis was evaluated using a spiked sample. The spiked samples were mixed and allowed to stand for 15 min before extraction. Mean recoveries and relative standard deviations were determined at four spiking levels of 10, 20, 30 and 40 mg/l to the prepared samples, six replicates at each level. Mean recoveries ranged from 98.6 to 103.4 with Relative Standard Deviations (RSD) ranging from 3.15% to 5.35%. The calibration data fitted a linear regression model with a good value (R<sup>2</sup>: 0.9999700). The Limits Of Detection (LOD) and Limit Of Quantification (LOQ) were determined as 0.81 mg/kg and 2.12 mg/kg, respectively.

#### Determination of AA content

Stock and working standards of AA (99%) and AA-d<sub>3</sub> were prepared in HPLC-grade water with 0.1% formic acid. Working standard solutions were prepared by diluting the stock solution of AA.

One-ml samples were weighed into 50 ml centrifuge tube, and 9 ml water, 1 ml (100 ng/ml) AA-d<sub>3</sub> were added. The centrifuge tubes were capped and shaken or vortexed for 5 min to mix contents. The tubes were centrifuged at 9,000 rpm for 15 min by an Allegra X-30R centrifuge equipped with a C0650 head (Beckman Coulter; Palo Alto, CA). A pipette was used to transfer a 5 ml aliquot of the clarified aqueous layer to a Maxi-Spin, 0.45 μm PVDF filtration tube, and this tube was centrifuged at 9,000 rpm for 3 min. Oasis HLB cartridges (Waters; Milford, MA) were preconditioned with first 3.5 ml MeOH and then 3.5 ml water. The solvents used for column conditioning were discarded. Afterwards, 1.5 ml of filtered extract was added to the cartridge. 0.5 ml water was used to wash the cartridge. The column eluent was discarded. Then, 1.5 ml of water was loaded onto the cartridge and the eluant was collected for the second clean-up. A Bond Elut Accucat SPE cartridge (Agilent Technologies; Inc. Folsom, CA, USA) was preconditioned with first 2.5 ml MeOH and then 2.5 ml of water. The solvents used for column conditioning were discarded. All of the eluant was loaded with the obtained extract. In this step, the first 0.5 ml of the eluate was discarded, and the remaining portion was collected into vials (Roach et al., 2003). Samples of each extract were analysed in duplicate.

Liquid Chromatography (LC) was carried out using a UPLC system (Agilent Technologies, model LC-1200 Infinity Series, Englewood, CO, USA). The analytical column used was a Zorbax Eclipse XDB-C18 (4.6 mm, 150 mm, 5-Micron) (Agilent Technologies, Loveland, CO, USA). The method was operated for 10 min using gradient elution with 0.1% formic acid in water (mobile phase A) and 0.1% formic acid in acetonitril (mobile Phase B). Source gas flow: 10 l/min; sheath gas flow: 10 l/min; capillary voltage, 4.0 kV gas temperature: 350 °C, sheath gas temperature: 325 °C, nebulizer: 40 psi and the column temperature: 30 °C. Flow rate was 0.3 ml/min. Sample injection volume was 10 μl.

Linearity of the method was constructed by building at seven standard solutions (10, 25, 50, 100, 250, 500, 1,000 ng/ml) with 100 ng/ml of AA-d<sub>3</sub>. The calibration data fitted a linear regression model with a good value (R<sup>2</sup>: 0.9997209). The LOD and the LOQ values were calculated as 3.0 and 10.0 ng/ml, respectively. Method performance was evaluated by means of recovery experiments at different spiking levels (50, 100 ng/g). AA recovery (R±RSD %) was 98.6±4.2% for grape Pekmez. The chromatograms of the fragment ions m/z 58.20 and 44.10 of the internal standard and m/z 55.10 and 44.10 for AA in Pekmez were used for quantification. AA-d<sub>3</sub> and AA showed a peak at 5.39 and 4.63 min, respectively. Retention time may change slightly for

samples which contain a complex matrix. It was also seen that substances such as lipids in the traditional foods could not be completely removed in spite of the clean-up step (Akgün and Arıcı, 2019).

#### Determination of fructose and glucose

Glucose and fructose content was determined according to Turkish Standard (2008) method with some modifications (Demir Kanbur et al., 2021). Five g sample of Pekmez was weighed into a beaker and dissolved in approximately 40 ml of distilled water. Twenty-five ml of methanol was added, and 100 ml was transferred to a graduated flask and completed to the marking line. Fructose and glucose were extracted with water, centrifuged, filtered through 0.45 µm PTFE filters, and injected to HPLC device by a refractive index detector (Shimadzu RID-10A). A 20 µl sample was injected into a 5 µm HN<sub>2</sub> column (250×4.66 mm; GL Sciences, Japan). The mobile phase used was acetonitrile:water (80:20) (v/v). Measurements were performed at 30 °C column temperature and at a flow rate of 1.3 ml/min. Samples of each extract were analysed in duplicate.

#### Determination of TP

Determination of TP was carried out in accordance with Singleton et al. (1999) procedure. The TP of the extracts was determined by using Folin-Ciocalteu reactive. To begin with, 400 µl freshly prepared Folin-Ciocalteu reactive was added to 680 µl distilled water (1:10, v/v with distilled water). Then, 20 µl from the Pekmez sample extracts was added to this mixture, and it was vortexed. Afterwards, 400 µl 10% Na<sub>2</sub>CO<sub>3</sub> was added to the mixture. It was incubated in room temperature for 120 minutes. After the incubation, the absorbance was read to be 760 nm, using UV-Vis spectrophotometer (Thermo Scientific Evolution 201, China). TP of the extracts was stated to be mg Gallic Acid Equivalent (GAE) per sample g. Samples of each extract were analysed in duplicate.

#### Determination of colour parameters

Colour measuring of the Pekmez samples was carried out with three repeats by using Minolta CR-400 (Osaka, Japan) in accordance with Hunter (L, a, b) system. In Hunter system the colour values are represented as L\* value for lightness (0: black; 100: white); a\* value (-a: green; +a: red); and b\* value (-b: blue; +b: yellow). Samples of each extract were analysed in duplicate.

#### Determination of protein

Protein content of Pekmez samples was determined by using nitrogen/protein detecting device (Thermo Scien-

tific Flash 4000) which works in accordance with Dumas method (N×6.25) (Cañas et al., 2020). Samples of each extract were analysed in duplicate.

#### Determination of pH

Ten g of the Pekmez sample was weighed, and the pH was measured using a pH meter (Malvern Panalytical, UK). Samples of each extract were analysed in duplicate.

#### Statistical analysis

The analyses were completed by transferring the study data to IBM SPSS Statistics 26 software. While evaluating the data, firstly Shapiro Wilk Normality Test ( $\leq 50$ ) was applied to the variables. Test results showed that the variables other than pH and proteins did not comply with the normality hypothesis. Therefore, Analysis of Variance (ANOVA) and Kruskal Wallis Test ( $\chi^2$ ) were used in analyses (median). On the other hand, the differences among the groups were evaluated by using Tukey and Bonferroni Test. Spearman's rho Correlation Coefficient was used to determine the anticipatory correlations between two numerical variables.  $p < 0.01$  is statistically significant.

## Results

AA, HMF, glucose, fructose, TRS, pH, protein, TP, and colour (L\*a\*b\*) levels detected in grape, mulberry and carob Pekmez are shown in Table 1.

The average AA levels of grape, mulberry and carob Pekmez are 130, 435 and 430 µg/kg, respectively. AA level of grape Pekmez is statistically and significantly lower than the AA level of mulberry and carob Pekmez ( $p < 0.01$ ). The average HMF levels of grape, mulberry and carob Pekmez are 6.84, 34.5 and 40.7 mg/kg, respectively while the average HMF level of all types of Pekmez was determined to be 25.7 mg/kg. HMF level measured in grape Pekmez is statistically and significantly lower than the HMF levels in mulberry and carob Pekmez ( $p < 0.01$ ).

The protein levels of grape, mulberry and carob Pekmez were reported to be in the range of 0.38-1.01, 0.71-2.12 and 0.99-2.30%, respectively. The average protein detected in grape Pekmez is significantly lower than the average protein in mulberry and carob Pekmez ( $p < 0.01$ ).

The average glucose levels of grape, mulberry and carob Pekmez were determined to be 15.9, 14.2 and 7.74%, respectively; the fructose levels were determined to be 16.2, 14.2 and 10.4%, respectively; and the TRS levels were determined to be 32.2, 28.4 and 18.2%, respectively. In the statistical analysis, the glucose level of carob Pekmez is significantly lower than the glucose

levels of grape and mulberry Pekmez ( $p < 0.01$ ). The fructose and TRS levels of grape Pekmez are statistically and significantly higher compared to the mulberry Pekmez, while the fructose and TRS levels of mulberry Pekmez are statistically and significantly higher than those of carob Pekmez ( $p < 0.01$ ).

The average pH levels of grape, mulberry, and carob Pekmez were recorded to be 5.4, 5.30 and 5.01, respectively while the average pH level of all types of Pekmez was found to be 5.27. pH average measured in carob Pekmez is significantly lower than the pH average measured in grape and mulberry Pekmez ( $p < 0.01$ ).

The highest TP level was found to be in carob Pekmez (9.40 mg GAE/g), whereas the lowest TP level was found to be in grape Pekmez (1.89 mg GAE/g). The TP level of grape Pekmez is statistically and significantly lower than the TP level of mulberry Pekmez, whereas the TP level of mulberry Pekmez is statistically and significantly lower than the TP level of carob Pekmez ( $p < 0.01$ ). The

average  $L^*a^*b^*$  levels of grape Pekmez were found to be 7.24, 12.28 and 5.62, respectively; the average  $L^*a^*b^*$  levels of mulberry Pekmez were determined to be 3.41, 12.28 and -0.92, respectively; the average  $L^*a^*b^*$  levels of carob Pekmez were reported to be 3.36, 1.45 and -0.94, respectively. The average  $L^*a^*b^*$  levels of all types of Pekmez are 4.83, 5.60 and 1.52, respectively. In the statistical evaluation,  $L^*a^*b^*$  levels measured in grape Pekmez are significantly higher than the  $L^*a^*b^*$  levels in mulberry and carob Pekmez ( $p < 0.01$ ).

The statistical correlation of AA and HMF levels, which were detected in different types of Pekmez, with glucose, fructose, TRS, pH, protein, TP and colour ( $L^*a^*b^*$ ) are given in Table 2. There is a meaningful, linear correlation between AA and HMF, protein, TP at a positively moderate level; and between AA and glucose, fructose, TRS, pH,  $L^*a^*b^*$  in a negatively moderate level; between HMF and TP at a positively moderate level; and between HMF and protein at a positively strong level.

**Table 1:** AA, HMF, glucose, fructose, TRS, pH, protein, TP,  $L^*a^*b^*$  levels of grape, mulberry, and carob Pekmez

Brands	N	AA	HMF	Glucose	Fructose	TRS (%)	pH	Protein	TP	L*	a*	b*
		( $\mu\text{g/kg}$ )	( $\text{mg/kg}$ )	(%)	(%)	(%)		(%)	( $\text{mg GAE/g}$ )			
Mean $\pm$ SD												
<i>Grape Pekmez</i>												
Brand 1	3	77.0 $\pm$ 12.8	1.33 $\pm$ 0.49	16.9 $\pm$ 0.64	16.1 $\pm$ 0.55	33.0 $\pm$ 1.19	5.50 $\pm$ 0.02	0.41 $\pm$ 0.02	1.96 $\pm$ 0.24	3.44 $\pm$ 0.02	1.83 $\pm$ 0.02	-0.89 $\pm$ 0.04
Brand 2	3	104 $\pm$ 76.3	6.00 $\pm$ 0.77	14.7 $\pm$ 0.62	16.1 $\pm$ 0.68	30.8 $\pm$ 1.30	5.47 $\pm$ 0.01	0.51 $\pm$ 0.02	1.67 $\pm$ 0.30	14.7 $\pm$ 0.11	32.6 $\pm$ 0.02	18.5 $\pm$ 0.20
Brand 3	3	202 $\pm$ 104	13.4 $\pm$ 1.15	16.2 $\pm$ 0.37	16.5 $\pm$ 0.37	32.7 $\pm$ 0.74	5.25 $\pm$ 0.01	0.92 $\pm$ 0.09	2.04 $\pm$ 0.23	3.56 $\pm$ 0.06	2.43 $\pm$ 0.27	-0.78 $\pm$ 0.10
<i>Mulberry Pekmez</i>												
Brand 1	3	189 $\pm$ 103	10.1 $\pm$ 2.85	16.4 $\pm$ 1.03	14.2 $\pm$ 0.87	30.6 $\pm$ 1.90	5.40 $\pm$ 0.01	0.77 $\pm$ 0.06	2.02 $\pm$ 0.12	3.52 $\pm$ 0.04	2.15 $\pm$ 0.05	-0.80 $\pm$ 0.04
Brand 2	3	405 $\pm$ 159	44.3 $\pm$ 2.65	12.7 $\pm$ 1.26	14.3 $\pm$ 1.24	26.9 $\pm$ 2.49	5.31 $\pm$ 0.02	1.38 $\pm$ 0.05	4.78 $\pm$ 1.29	3.37 $\pm$ 0.03	1.49 $\pm$ 0.06	-0.98 $\pm$ 0.04
Brand 3	3	578 $\pm$ 336	48.9 $\pm$ 18.0	13.4 $\pm$ 0.82	14.2 $\pm$ 0.90	27.6 $\pm$ 1.71	5.20 $\pm$ 0.01	2.06 $\pm$ 0.05	5.87 $\pm$ 0.29	3.330.03 $\pm$	1.44 $\pm$ 0.05	-0.99 $\pm$ 0.06
<i>Carob Pekmez</i>												
Brand 1	3	282 $\pm$ 240	29.7 $\pm$ 4.79	7.21 $\pm$ 0.31	10.4 $\pm$ 0.45	17.6 $\pm$ 0.76	5.11 $\pm$ 0.01	1.09 $\pm$ 0.09	10.3 $\pm$ 1.44	3.36 $\pm$ 0.03	1.49 $\pm$ 0.05	-0.96 $\pm$ 0.06
Brand 2	3	579 $\pm$ 356	51.6 $\pm$ 5.50	8.27 $\pm$ 0.19	10.5 $\pm$ 0.24	18.8 $\pm$ 0.42	4.92 $\pm$ 0.01	2.10 $\pm$ 0.28	8.53 $\pm$ 1.38	3.37 $\pm$ 0.02	1.41 $\pm$ 0.03	-0.92 $\pm$ 0.04

AA=Acrylamide; HMF=5-hydroxymethyl-2-furfural; TP=Total Phenolic; TRS=Total Reducing Sugar

**Table 2:** Relationship of acrylamide (AA) and 5-hydroxymethyl-2-furfural (HMF) with selected parameters in grape, mulberry, and carob Pekmez

		AA	HMF
HMF	r	0.580	-
	p		0.000
Glucose	r	-0.448	-0.764
	p		0.001
Fructose	r	-0.400	-0.615
	p		0.005
TRS	r	-0.454	-0.737
	p		0.001
pH	r	-0.366	-0.800
	p		0.011
Protein	r	0.581	0.947
	p		0.000
TP	r	0.519	0.664
	p		0.000
L*	r	-0.524	-0.669
	p		0.000
a*	r	-0.505	-0.681
	p		0.000
b*	r	-0.451	-0.524
	p		0.001

r=Spearman's rho Correlation Coefficient, p=level of significance ( $p < 0.01$ )

AA=Acrylamide; HMF=5-hydroxymethyl-2-furfural; TP=Total Phenolic; TRS=Total Reducing Sugar

## Discussion

There is no legal regulation regarding the level of AA in Pekmez. Only one study on AA level of Pekmez products has been found in literature. AA level (whose resource is unknown) of Pekmez in Turkey was found to be 95 (<10-297) µg/kg in that study (Ölmez et al., 2008). HMF level of grape Pekmez is limited to be 75 mg/kg in Turkish Food Codex (2017). The HMF levels of Pekmez examined in this study did not exceed the limits.

In the previous similar researches in Turkey, HMF level in grape Pekmez samples has been reported as 18.5-23.4 mg/kg (Şimşek and Artık, 2002), 5.93-762 mg/kg (Türkben et al., 2016), and 3.31-6.34 mg/kg (Özcan et al., 2015). HMF level in mulberry Pekmez samples has been found as 5.69-135 mg/kg (Karataş and Şengül, 2018) and 18.8-105 mg/kg (Ergun et al., 2019). Also, HMF level in carob Pekmez samples has been indicated as 4.10-7.00 mg/kg (Şimşek and Artık, 2002) and 19.6-180 mg/kg (Özhan et al., 2010). In a study conducted in Istanbul (Turkey), HMF levels in grape, mulberry, and carob molasses were 11.7-219, 12.8-220, and 10.7-40 mg/kg, respectively (Erbil and Yeşilçubuk, 2020).

Considering the compound and production process of Pekmez, it can be claimed that AA and HMF levels obtained from this study are less than expected. It is considered that two factors have mainly played a role in this outcome. The fact that Pekmez production in vacuum is carried out at 65-75 °C is the first factor, while the fact that the protein level of Pekmez has a relatively lower value compared to the reducing sugar level is the second factor. It is considered that both of these two factors hinder the caramelisation and Maillard Reaction, and significantly affect the AA and HMF level.

It has been noted in literature that the protein levels of grape, mulberry and carob Pekmez samples of Turkey were 0.21-1.64, 0.698-2.797 and 0.75-2.47%, respectively (Erbil and Yeşilçubuk, 2020). According to the National Food Composition, the protein levels of grape, mulberry and carob are 2.35, 2.76 and 4.18%, respectively (TÜRKOMP, 2021a; 2021b; 2021c). The protein levels which were obtained in this research comply with the literature to a great extent.

Karababa and Develi Isikli (2005) stated that the glucose and fructose levels of other types of Pekmez are higher than the glucose and fructose levels in carob Pekmez, whereas the amount is more or less the same. The levels of glucose, fructose and TRS in grape Pekmez were recorded to be 27.6-41.1, 22.3-34.6, and 49.9-75.8% (Türkben et al., 2016), and 23.4-33.8, 21.8-35.9, and 45.2-68.9%, respectively (Erbil and Yeşilçubuk, 2020). The glucose, fructose, and TRS levels in mulberry Pekmez were determined to be 29.1-36.5, 25.7-32.2, and 54.8-68.7% (Ergun et al., 2019). The glucose, fructose,

and TRS levels in carob Pekmez were reported to be 9.12-29.4, 16.4-32.6 and 25.6-62.1%, respectively (Erbil and Yeşilçubuk, 2020). The glucose, fructose and TRS levels obtained from different kinds of Pekmez in this study correspond to a lower value compared to the results from other research. It is believed that various factors such as climate, soil, type of the fruit and its composition, the type of production, and storage have an effect on the results.

In some previous studies, pH levels were recorded to be 5.22 (Toker et al., 2013) and 3.59-5.23 (Türkben et al., 2016) in grape Pekmez; and 4.65-5.30 (Karataş and Şengül, 2018) in mulberry Pekmez. It is known that the optimal pH level for AA formation is between 7 and 8 (Stadler et al., 2002). HMF in food, on the other hand, mainly depends on pH and it has been proven by many researchers that it can easily be formed at low temperature and pH conditions (Capuano and Fogliano, 2011). Determined pH levels in Pekmez products are lower than the optimal value, which is essential for AA, however, it can be said that it is partially adequate for HMF formation.

Aliyazicioglu et al. (2009) reported the highest TP level in carob Pekmez and the lowest TP level in grape Pekmez among all types of Pekmez. Tüzün et al. (2020) reported the TP level in carob Pekmez from Tunceli City in Turkey to be 7.48 mg GAE/g. TP levels in mulberry Pekmez were reported to be 9.76-15.3 mg GAE/g (Karataş and Şengül, 2018), and 2.19 mg GAE/g (Tüzün et al., 2020).

In the study of Toker et al. (2013), L\*a\*b\* levels in grape Pekmez were determined to be 1.41-3.21, 0.73-2.25, and 0.85-1.85, respectively. Also, L\*, a\* and b\* levels in mulberry Pekmez were recorded to be 18.1-2.15-2.80, 0.98-2.93 and 1.35-2.40, respectively. TP and L\*a\*b\* levels which were obtained from different Pekmez samples in this study correspond to lower values compared to the other studies. It is believed that in addition to the aforementioned factors, such as the type and composition of the fruit, the preferred methods for analyses also have a role in these results.

No research on the correlation of different parameters with AA level in Pekmez products was found based on our knowledge. Therefore, the results were compared to similar research which was carried out on different products. Hamzalıoğlu and Gökmen (2020) stated that AA formation continues along with HMF formation in coffee. Alpözen and Üren (2013) stated that there is a statistically significant positive correlation of AA with glucose, fructose, TRS, and a\* and b\*; a negative correlation of AA with protein and L\*; and no statistically significant correlation between AA and HMF in İzmir Gevrek (a traditional Turkish bagel). Boz et al. (2016) reported that there is a negative correlation between AA and L\*a\*b\* in

pestil (fruit leather produced from mulberry pulp). Nguyen et al. (2016) reported that in AA formation in biscuits, glucose plays a negligible role, whereas fructose contributes to AA formation significantly. Akgün and Arıcı (2019) reported that there is a statistically positive strong correlation between AA and HMF, a statistically positive low correlation between TRS and L, and no significant correlation among protein, fructose, pH and a\* and b\* in coffee. Shakeri et al. (2019) noted that there is a positive correlation between pH and AA. It was stated that phenolic compounds make both positive and negative contribution to AA formation. It has been proven by a number of researchers that there is a negative (Sordini et al., 2019), positive (Oral et al., 2014), and no (Bas-sama et al., 2010) correlation of phenolic compounds with AA in model systems prepared with various foods.

It has been determined that there is a significant linear correlation of HMF with fructose and L\*a\*b\* at a negatively moderate level; of HMF with glucose, TRS and pH at a negatively strong level. It is possible to find many published articles in which HMF levels in Pekmez were studied, however, no study on the direct correlation between HMF and the other parameters could be found. Therefore, the results were compared to similar studies which were carried out on different types of food. Lee and Nagy (1990) stated that fructose/glucose ratio in low pH accelerates the HMF formation reaction. Nguyen et al. (2016) stated that among four types of sugar (sucrose, glucose and fructose, only glucose, only fructose), HMF formation level is maximum in glucose and fructose mixture in biscuits. Yıldız (2013) noted that there is a positive and strong correlation of HMF with invert sugar, total sugar, TP and L\*a\*b\* in pestil and churchkhela (fruit leather produced from fruit pulp and walnuts on a string dipped in starch grape Pekmez). Boz et al. (2016) reported that there is a negative correlation between HMF and L\*a\*b\* in pestil (fruit leather produced from fruit pulp). Gökmen et al. (2007) recorded that there is a reverse correlation between pH and HMF in bakery products.

The fact that AA and HMF are both a product of Maillard Reaction can explain their positive correlation. The negative correlation of AA and HMF with glucose, fructose, TRS, and the positive correlation of AA and HMF with TP is pretty surprising compared to the research in literature. Generally speaking, while an increase in AA and HMF depending on the increase in sugar concentration, a decrease in TP levels in parallel to the increase, and inhibition of formation in low pH (except for HMF) are expected, the reverse is expected for Pekmez. Increase in HMF levels in low pH values complies with the literature. Increase in AA and HMF levels in parallel to the increase in protein concentration also complies with literature.

As a result, when all findings are evaluated together, it is indicated that the heat treatment, protein/ amino acid and the types and concentrations of phenolic compound are more determinative in AA and HMF formation in Pekmez. It is known that both AA and HMF form in high levels above 120 °C, the type and concentration of protein/amino acid have an important role in formation (Stadler et al., 2002; Tareke et al., 2002), and the type of phenolic compounds, high polyphenol concentrations, the number and position of phenol hydroxyls of flavonoid compounds have an effect on the formation (Zhang and Zhang, 2008; Zhang et al., 2016). Even though the negative correlation of AA and HMF with L\*a\*b\* found in this study differs partially from the literature, there are also some researchers (Boz et al., 2016) who obtained similar results.

## Conclusion

In this study, the relationship between AA and HMF and glucose, fructose, TRS, pH, protein, TP, L\*a\*b\* in different Pekmez products was evaluated for the first time by using analytical methods. Although some results are similar to those of other studies in the literature, important differences were also noted. In this study, the HMF levels detected in all Pekmez samples were well below the legal limits. There is no legal regulation on AA levels in Pekmez. However, the potential risks of AA and HMF should be considered in Pekmez consumption.

The variety and composition of the fruit/vegetables used in Pekmez production, the conditions in which the fruit/vegetables are grown (climate, soil, fertilizer, harvest, etc.), and the fact that Pekmez production includes non-standard techniques in both traditional and industrial scale, and storage conditions constitute the basis of these differences. Strong findings indicating that the type and concentration of amino acid and phenolic compounds and the heat treatment applied are the main determining factors in the formation of AA and HMF in Pekmez. So, the need for more research on this issue has also emerged.

## Author contributions

B.B. did the conceptualization, methodology, investigation, validation, formal analysis, data curation, writing, visualization, writing- original draft preparation, resources, and supervision; E.D.K. and C.B. carried out the conceptualization, methodology, investigation, validation, formal analysis, and data curation; F.A. did the conceptualization, methodology, resources, supervision, reviewing and editing, project administration. All authors read and approved the final manuscript.

## Conflicts of interest

No potential conflict of interest was reported by the authors.

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