CHARACTERIZATION OF, AND 5-HYDROXYMETHYLFURFURAL CONCENTRATION IN CAROB PEKMEZ

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Abstract
Carob pekmez, a traditional concentrate, has long been produced from *Ceratonia siliqua L*. for human consumption in Turkey. 5-hydroxymethylfurfural (5-HMF) is a quality parameter for carob pekmez and other concentrated food products. High levels of this compound are not desirable in pekmez because this may cause mutagenic effects on human metabolism. 5-HMF formation depends on both the sugar content and the acidity of the product. The results of this study show that although a the total sugar concentration was high in carob pekmez (62.80 g/100g) the 5-HMF concentration after the thermal process was only 12.25 mg/kg, which is also lower than Codex Standard, and may possibly be due to its low acidity. The data revealed that carob pekmez is a high source of minerals such as; potassium (1057.3 mg/100g), phosphorus (77.8 mg/100g), magnesium (55.6 mg/100g), and calcium (314.9 mg/100g as well as sugars. The aim of the study was to determine the composition of carob pekmez and content of 5-HMF during its production in the Mediterranean and Aegean basin of Turkey.

Anahtar kelimeler: Carob bean, pekmez, minerals, 5-Hydroxymethylfurfural, carob extract

KEÇİBOYNUZU PEKMEZİNİN KARAKTERİSTİĞİ VE 5-HİDROKSİMETİLFURFURAL İÇERİĞİ

Özet
Türkiye'de keçiboynuzu pekmezi *Ceratonia siliqua* L. meyvelerinden elde edilen ve insanların beslenmesi amacıyla uzun yıllardır kullanılan geleneksel bir üründür. 5-hidroksimetilfurfural (5-HMF) keçiboynuzu ve diğer konsantre edilmiş gıda ürünlerinde bir kalite parametresidir. İnsan metabolizması üzerine mutajenik etkilere sebep olabilmesi nedeniyle pekmezlerde bu bileşiğin konsantrasyonunun yüksek olması istenmemektedir. 5-HMF oluşumu ürünün şeker içeriği ve konsantrasyonuna bağlıdır. Bu çalışmanın sonuçları keçiboynuzu pekmezinde ısıl işlem sonrasında 5-HMF konsantrasyonunun, yüksek şeker konsantrasyonuna (62.80g/100g) rağmen Gıda Kodeksinde belirlenen değerden daha düşük bir değer olan 12.25 mg/kg olduğunu göstermiştir. Keçiboynuzu pekmezinin düşük asit içeriği nedeniyle az miktarda 5-HMF oluşumun gerçekleştiği düşünülmektedir. Bunun yanında çalışmada elde edilen veriler keçiboynuzu pekmezinin içerdiği zengin şeker içeriğinin yanında potasyum (1057.3 mg/100g), fosfor (77.8 mg/100g), magnezyum (55.6 mg/100g) ve kalsiyum (314.9 mg/100g) gibi mineraller açısından da zengin bir kaynak olduğunu ortaya çıkarmıştır. Bu çalışmanın amacı Türkiye'de Akdeniz ve Ege bölgesinde üretimi yapılan keçiboynuzu pekmezinin bileşimini ve 5-HMF içeriğini belirlemektir.

Keywords: Keçiboynuzu, pekmez, mineral, 5-Hidroksimetilfrufural, keçiboynuzu ekstraktı

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INTRODUCTION

Carob bean is the fruit of *Ceratonia siliqua* L., which belongs to the Leguminosae family. The tree has been extensively cultivated in most countries of the Mediterranean for years (1, 2). The carob bean is composed of two main parts: the pod and the seeds (3). Seeds are used predominantly in the food industry for locust bean gum, which contains approximately 90% galactomannans. Deseeded pods are also used as a raw material for the manufacture of pekmez, which is a traditional concentrated syrup produced in Turkey. Carob pods contain high concentrations of sugar (particularly sucrose) and tannins and low concentrations of protein and fat (4, 5). Pekmez can also be produced from mulberry and grape, as well as carob (6, 7, 8).

Traditional production of carob pekmez consists mainly the stages of extraction, clarification and concentration. Carob pods, therefore, are extracted with hot water at 85 °C after the pods are sieved using 5 and 7 mm sieves, and portions between 5 and 7 mm dimensions selected as the material. The carob pods are then extracted by reverse flow for almost 3 h and the soluble solids in the pod gradually reduced. After the extraction process, the extract is clarified using filter aid agents such as perlite or bentonite and concentrated using a vacuum evaporator at temperatures between 50-60 °C. The clear extract is concentrated up to 65-70 brix and pasteurized at 85 °C in order to prevent microbial growth and spoilage. Carob pekmez is filled into glass jars hermetically (Figure 1).

In recent years, consumption of carob pekmez has been rapidly increased because of its beneficial compounds such as minerals K, Ca, Fe, P and Mg (9, 10, 11, 12). Moreover, carob pekmez can be considered a functional food due to its rich phenolic content (4, 13, 14). In addition, roasted carob pods are used for carob powder production, which is a chocolate or cocoa substitute (15). 5-Hydroxymethylfurfural (5-HMF) is a major by-product of thermal processing and results from overheating during sterilization. Upon storage it is formed by hexose dehydration especially at pH 5 or lower, or by the Maillard reaction (16, 17). Reports have shown 5-HMF to be mutagenic (18). Therefore, 5-HMF concentration can be used a quality parameter for concentrated food products because high concentrations of 5-HMF are not desirable in pekmez (8, 19, 20). It indicates the degree of heating of the treated products in various foods containing carbohydrates such as fruit juice and pekmez (19). The presence of this compound indicates the effects of overheating of the product during manufacture and storage on the quality of pekmez. In the Turkish Standard of Grape Pekmez (21), the maximum 5-HMF concentrations of liquid and hard pekmez are 75 mg/kg and 100 mg/kg, respectively. Therefore, the purpose of this study was to define the characteristics and 5-HMF concentration of carob pekmez.

MATERIAL AND METHODS

Material

Four different commercially available carob pekmez samples were purchased from local markets for the assay.

Methods

Descriptive analyses

Each analysis was replicated twice and there were two analyses per sample. Total dry matter was determined by drying the samples to a constant weight at 65 °C using a vacuum drier (22). Soluble solids were determined using an Abbe refractometer. Total acidity (dry citric acid), pH and formol index were measured using a pH meter (22).

Crude protein was determined using the Kjehdahl method (22). Mineral concentrations (K, P, Mg, Ca, Na, Fe, Cu, Zn, and Mn) were determined using an Atomic Absorption Spectrophotometer (Varian Spectra A-550 plus) and calculated using a standard curve. The phosphorus concentration of carob pekmez was analyzed colorimetrically in the form of vanadium phosphomolybdate by spectrophotometer (Shimadzu UV-160A, Tokyo, Japan) at 430 nm wavelength (23).

Concentrations of total sugar, inverted sugar and sucrose were determined by the Lane-Eynon method (22). Total invert (reducing) sugar was determined after inversion at 67-70 °C for 10 minutes

Grinding Dry Carob Pod ==> Washing ==> Extraction (In tap water for 3h at 85 °C) ==> Clarification ==> Evaporation ==> Carob Pekmez (65-70 °Bx) ==> Pasteurization ==> Packaging

Figure 1. Main manufacturing stages for Carob pekmez

by adding HCl and with and a NaOH adjusted pH of 7. Reducing sugar concentration was measured before inversion. The difference between total invert (reducing) sugar content and reducing sugar content gives non-reducing sugar. Then the reducing sugar and non-reducing sugar (sucrose) concentrations were taken together as total sugar concentration.The color of carob pekmez was evaluated by measuring the Hunter L, (Brightness; 100: white, 0: black), a (+: red, -: green) and b (+: yellow, -: blue) parameters with a colorimeter (Model CR 200, Konica Minolta, Japan). The instrument was calibrated with a white tile before measurements.

The total phenolic content of carob pekmez was determined according to Spanos and Wrolstad (24). Briefly, 5 ml of Folin-Ciocalteu's reagent (0.2 N) and 4 ml of Na_2CO_3 (7.5% w/v) were respectively added to 100 ml of each sample distilled with 900 ml water and incubated under ambient conditions for 2 hours. Folin-Ciocalteu reagent consists of sodium tungstate, sodium molybdate, lithium sulfate, bromine, and other acids. The reduction of phenolic substances by the Folin Ciocelteau's reagent involves reaction with a mixture of phosphotungstic acid and phosphomolibdic acid. Phenolic substances in carob oxidized with Folin-Ciocalteu reagent and produced a blue color upon reaction, which is measured spectrophotometrically. The Absorbance of each solution was measured at 765 nm along with the reagent blank and all standards using a UV-VIS spectrophotometer (Shimadzu UV-160A, Tokyo, Japan). A regression analysis was performed on the standards (Absorbance vs. Concentration) and the final result for each sample was determined from that regression curve. Results were expressed as gallic acid equivalent per gram of dry weight (mg GAE/g DW).

5-Hydroxymethylfurfural analysis

5-Hydroxymethylfurfural was analysed by reverse-phase high performance liquid chromatography (RP-HPLC). One millilitre of sample was mixed with 9 mL of distilled water. The mixture was centrifuged at 20.124xg for 15 min to remove any solid particles in the sample. The supernatant was filtered through a 0.45 mm membrane and 20 ml of filtrate was injected into the column. HPLC analysis was carried out with a Shimadzu LC-10AD VP series HPLC system, a UV-VIS detector and Nucleosil 5C18 (250 x 4.6 mm ID) column (HICHROM, Reading Berkshire, England). Mobile phase consisted of 10:90 (v/v) methanol-water by isocratic elution with a 1.5 ml/min flow rate at ambient temperature. The concentration of 5-HMF was determined using an external calibration curve (55690-5-HMF, Fluka Chemika), measuring the signal at 280 nm (25).

Thermal treatment in tubes

For commercial fruit juices, the processes such as evaporation and pasteurization are favoured for preserving the juice for a longer period of time. In order to mimic industrial pasteurization, the thermal treatment of carob pekmez samples was performed at 85 °C for up to 75 min. Each experiment was replicated twice and there were two determinations per sample. Twenty milliliters of samples were filled into thermal treatment tubes (100×7.5 mm i.d.) and sealed hermetically. A thermocouple was placed at the geometric centre of the tube to monitor the sample temperature. Zero point tubes were taken after a previously determined transient time, and three tubes which were used for the determination of 5-HMF were taken out of the water bath at 15 min intervals and cooled down to 4 °C by immediately plunging the tubes in an ice bath.

Statistical analysis

All analyses were duplicated. The Generalized Linear Model (GLM; with $P<0.05$) was used in order to evaluate the significance among results using SAS Statistical Software (release for Windows, SAS Institute Inc., Cary, NC, USA) (26).

RESULTS and DISCUSSION

This study was designed not only to indicate the chemical characteristics of carob pekmez but also to determine 5-HMF content. The data revealed that carob pekmez is a rich source of sugars and also contains a high concentration of minerals. However, it was determined that the concentration of 5-HMF was not significantly high following thermal processing.

Chemical characteristics

Carob pekmez was found to contain high concentrations of total sugar (62.80 g/100g), invert sugar (17.05 g/100g), and sucrose (45 g/100g), respectively (Table I). Şimşek (10) also reported that carob pekmez had 62.16-68.79 g/100g total sugars and 40.36-44.38 g/100g sucrose.

Table 1. Composition of carob pekmez (n=4)

Composition	Mean value	Standard deviation
Total dry matter (g/100 g)	72.18	0.57
Soluble solids (g/100 g)	71.50	0.60
pH value	5.18	0.08
Total acidity (g/100 g)	0.87	0.01
Protein (g/100 g)	1.18	0.02
Formol index	84.01	0.58
Total phenolic compounds (mg GAE/ g DW)	1.62	0.29
5-HMF (mg/kg)	1.53	0.41
Total sugar (g/100 g)	62.80	0.43
Invert sugar (g/100 g)	17.05	0.34
Sucrose (g/100 g)	45.00	0.43
L*	16.33	0.01
a*	1.29	0.01
b*	1.21	0.01
Minerals (mg/100g)		
Potassium (K)	1057.3	36.7
Phosphorus (P)	77.8	1.3
Magnesium (Mg)	55.6	0.4
Calcium (Ca)	314.9	5.1
Sodium (Na)	17.1	0.6
Iron (Fe)	1.4	0.3
Copper (Cu)	0.5	0.08
Zinc (Zn)	0.4	0.31
Manganese (Mn)	0.3	0.11

*L, (Brightness; 100: white, 0: black), a (+: red, -: green) and b (+: yellow, -: blue)

As shown in Table I, the high concentrations of minerals in carob pekmez were potassium (1057.3 mg/100g), phosphorus (77.8 mg/100g), magnesium (55.06 mg/100g) and calcium (314.9 mg/100g). The Codex Alimentarius describes "source" of liquid foods in vitamins and minerals to be containing more than 7.5 percent of "Nutrient Reference Value" (NRV) per 100 ml. NRV indicates that recommended daily intake (RDI) or adequate intake (AI) needed by individuals (27). Thus, for the average adult it is recommended that 2800-3200 mg/day of potassium (K), 1000 mg/day of phosphorus (P), 310-420 mg/day of magnesium (Mg), 1000 mg/day of calcium (Ca), 460-920 mg/day (AI) of sodium, 8-18 mg/day of iron (Fe), 8-10 mg/day (AI) of copper, 8-14 mg/day of zinc (Zn) and 5-5.5 mg/day of manganese (Mn) be consumed. Carob pekmez contained levels of P, Zn, and Mn that met the NRV requirements and exceed by 2 times the value of "source" for K, Mg, Ca, and Fe; however, the value of Na in carob pekmez was less than the recommended AI. Because of the demonstrated high sugar and mineral content carob pekmez is very good source in human nutrition (12).

The total phenolics, which are susceptible to degradative reactions during various steps of processing, were lower in concentration in the pekmez (1.62 mg/g) than in the pod (17.5 mg/g) because of the high temperature applied during manufacture (28, 29).

Mean L, a, and b values were 16.33, + 1.295 and – 1.205, respectively. A high redness (a) value is not desired because it occurs as a result of excessive caramelization of sugars. Therefore, a low redness (a) and a high brightness (L) value indicate that the pekmez is of good quality. Şimşek (10) also determined that L, a, b values in carob pekmez, 18.28, 0.61, 0.51, respectively.

Determination of 5-HMF

Generally 5-HMF is known to form as a result of thermally processing acidic foods, especially those containing hexose sugars (30). Thermal processing is widely used for the pasteurization of carob pekmez in Turkey. However, there is no international standard for carob pekmez; it may be compared with the standards for honey, which can be considered to be a similar product because of its high sugar and mineral contents. According to the Codex Standard, the 5-HMF concentration of processed honey should not be more than 40 mg/kg (31). Samples of carob pekmez taken after the packaging step were analyzed for their 5-HMF concentration and results showed that none of the samples contained high concentrations of this compound. After thermally processing carob pekmez the maximum value of 5-HMF was 12.25 mg/kg (Figure 2) which is still lower than standard pasteurization time for foods even after a long thermal process and can be explained by the low acidity of the carob pod. Subsequently, the high temperature process did not lead to be exceeded formation of 5-HMF.

CONCLUSIONS

Carob pekmez has high concentrations of total sugars (62.80 g/100g), especially sucrose (45.00 g/100g). Minerals such as potassium (1057.3 mg/100g), phosphorus (77.8 mg/100g), magnesium (55.6 mg/100g), and calcium (314.9 mg/100g) were determined in carob pekmez. The addition of sugars and minerals and total phenolics resulted in lower concentration in the carob pekmez (1.62 mg/g) compared to the carob pod (17.5 mg/g) (29). However, 5-HMF concentration of carob pekmez after the thermal process was 12.25 mg/kg, which is lower than Codex Standard. Overall, this study clearly demonstrated that carob pekmez is a rich source of sugars and contains high concentrations of minerals. It has also been determined that the formation of 5-HMF is not significant during the thermal processing of the product. Furthermore, the long application of heat did not affect quality of the final product. This indicates that carob can be used as a good source of nutrients for humans.

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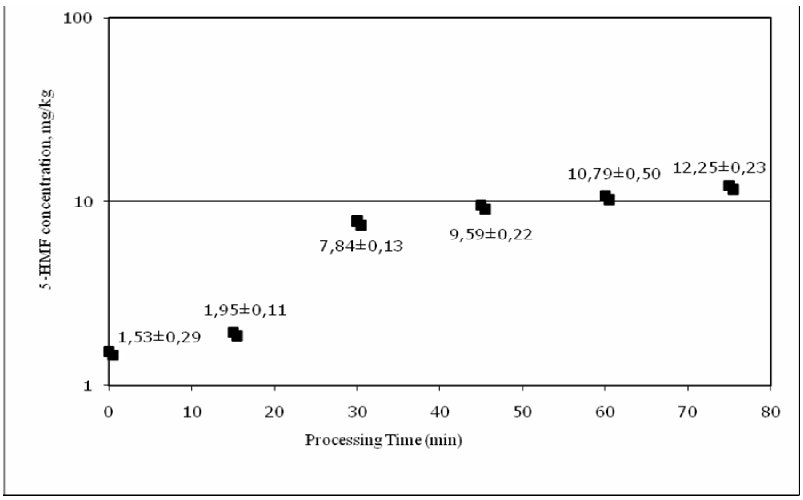

Figure 2. 5-HMF formation of carob pekmez during thermal treatment at 85 °C (n=4)

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