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Furfural Contents and Some Physical and Chemical Properties of Raisins

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ABSTRACT

Furfural contents of packaged / unpackaged red (red globe) and white (sultanas) raisins were examined by RP-HPLC-DAD and some physical and chemical characteristics of raisins were also investigated. Titratable acidity and L*, a* and b* color values of white raisins were higher than those of red raisins (P<0.05) but a_w, fructose and glucose contents of red and white raisins were similar. The average 5-hydroxymethyfurfural (HMF) and 2-furylmethylketone (FMC) contents of unpackaged raisins were significantly higher than those of packaged raisins (P<0.05). Insignificant difference was found between the raisin cultivars in terms of contents of each furfural compounds (P>0.05); however, the furfural level in red raisins was the highest. Furfural contents of raisins were found lower than the limit value of grape molasses in Turkish Food Codex. Estimated Daily Intake of furfurals was calculated as <0.05 mg/kg body weight, and this value was lower than the Acceptable Daily Intake value (0.5 mg/kg body weight) established by European Food Safety Authority.

Keywords: Furfurals, 5-Hydroxymethyfurfural, Monosaccharide, Packaging, Raisin

Kuru Üzümlerin Furfural İçerikleri ile Bazı Fiziksel ve Kimyasal Özellikleri

ÖZ

Bu çalışmada paketlenmiş / paketlenmemiş kırmızı (red globe) ve beyaz (sultana) kuru üzümlerin furfural içerikleri RP-HPLC-DAD ile araştırılmış ve ayrıca furfural oluşumu ile ilgili bazı fiziksel ve kimyasal özellikler de belirlenmiştir. Beyaz kuru üzümlerde titrasyon asitliği ile L*, a* ve b* renk değerleri daha yüksek bulunurken (P<0.05), a_w, fruktoz ve glikoz içerikleri iki kuru üzüm türünde de benzer çıkmıştır. Paketlenmemiş kuru üzümlerin 5-hidroksimetilfurfural (HMF) ve 2-furilmetilketon (FMC) içerikleri paketli örneklere göre daha yüksek bulunmuştur (P<0.05). Kırmızı kuru üzümlerin furfural içerikleri daha yüksek olmasına karşın istatistiksel olarak beyaz kuru üzümlerin içerdiği furfural değerlerinden farklılık göstermemektedir (P>0.05). Türk Gıda Kodeksi'nde katı üzüm pekmezi için belirtilen limit değer ile kıyaslandığında kuru üzümlerin furfural içerikleri oldukça düşüktür. Bununla birlikte kuru üzümler için hesaplanan Tahmini Günlük Alım miktarı (<0.05 mg/kg vücut ağırlığı) Avrupa Gıda Güvenliği Otoritesi tarafından belirlenen Kabul Edilebilir Günlük Alım değerinden (0.5 mg/kg vücut ağırlığı) daha düşük bulunmuştur.

Anahtar Kelimeler: Furfural, 5-Hidroksimetilfurfural (HMF), Monosakkarit, Paketleme, Kuru üzüm

INTRODUCTION

Heat applications are widely used in food industry for the purpose of prolonged shelf-life, better final quality and safety of food products [1]. Roasting, frying, drying, baking, toasting and sterilization are some of the heat treatments used in food industry for production processes [2]; however, some chemical reactions can take place during heat processing of carbohydrate rich foods [3]. Maillard Reaction (MR) is a reaction between reducing sugars and amino acids during thermal processing of foods. The reaction is influenced by process and storage conditions as temperature and food contents [4, 5]. The reaction give rise to forming new compounds called Maillard Reaction Products (MRPs), which contain different molecular weight compounds as aldehydes, dicarbonyls, heterocyclic amines, ketones, melanoidins and some further stage products [6]. 5-Hydroxymethyfurfural (HMF), 2-furoic acid (FA), 2-furylmethylketone (FMC) are some of these compounds as a results of MR, and they can be also formed as a result of acid-catalyzed sugar degradation [7, 8]. Generally, HMF is used as a quality factor in several food products applied thermal treatment, and its concentration is adversely associated with the healthiness of the product because of high concentration of HMF reported to have harmful effects for human [1, 9]. Moreover, there is limitation of HMF for some foods as fruit juices and grape molasses because of its dangerous effects on human health like genotoxic, cytotoxic, mutagenic and carcinogenic reported by some authors [10-13] although the International Agency for Research on Cancer (IARC) has classified furfural as a Group 3, not classifiable as to its carcinogenicity to humans [14]. For these reasons, some limitations are defined in different foods like grape molasses (75 mg/kg) and honey (40 mg/kg) [15, 16]. In addition, it is important that carrying out further researches about another carbohydrate based foods in terms of their effects for human body on account of HMF levels. Raisins, one of the carbohydrate rich foods and applied thermal process for its production from grapes, should be investigated in terms of their furfural levels.

Raisin is important agricultural product and Turkey is the largest producer of them in the world with 286.58 thousand tons in 2012 [17]. Although drying processes show differences from each other in terms of geographical locality and variety of grapes, there are three main drying methods as sun, shade and mechanical drying [18]. An oil emulsion or a dilute alkaline solution is applied to grape before drying stage in terms of acceleration of drying process by reducing the resistance to moisture transfer of the surface skin of grapes [19]. At the end of the drying process, raisins can be stored at non-controlled conditions for up to 10 months for further process as a semi-finished product. Moreover, some quality parameters as color, texture, microbial stability and furfural content of raisins can change with storage conditions and physicochemical specifications of grapes such as water activity and skin damage during the storage period [20]. In addition, quality parameters are important both semi-finished products and their final products because quality issue start from raw material to end product.

Raisins are used for producing of grape molasses and also consumed as a snack food in daily life. Additionally, it can be used as ingredients in patisserie products as cakes and cookies. Although there are some limitations about HMF for grape molasses and grape juices, there are not any HMF, FA or FMC limitations for their raw material (dried grapes). In addition that, there is not any research about furfural contents in white and red raisins in terms of packaging. Furfural levels of raisins based products can increase because of having heat treatment step in production and including high amounts of sugar. For this reason, furfural contents of raisins using for raw material in mentioned products are very important because of initial furfural levels. From this point of view, in this study, it was aimed to determine the potential furfural compounds (HMF, FA and FMC) and their levels of packaged or unpackaged both red (red globe) and white (sultanas) raisins, using RP-HPLC-DAD monitoring. Some physical and chemical properties of raisins were also evaluated.

MATERIALS and METHODS

Materials and Reagents

A total of 62 packaged (19 samples) and unpackaged (41 samples) raisin samples (23 red raisin samples, 39 white raisin samples) were collected from Turkish supermarkets, herbal store or bazaar. The raisins were collected from different 9 cities (Denizli, Eskişehir, Gaziantep, İzmir, Kahramanmaraş, Konya, Malatya, Manisa, and Mersin). The collected samples were taken with an amount of 250-500 g and after homogenization of the samples using with warring blender, they were kept in polyethylene cups and stored at 4°C.

The standards of fructose and glucose with >99% and ≥99.5% purity respectively, and 5-hydroxymethyfurfural, 2-furoic acid, 2-furylmethylketone with >99% purity were purchased from Sigma-Aldrich (St. Louis, MO, USA). Glacial acetic acid, zinc acetate, potassium ferrocyanide and sodium hydroxide were obtained from Merck (Darmstadt, Germany). The chromatographic mobile phase was prepared with ultra-pure water purified with a Milli–Q ultra–pure water system (Millipore, Bedford, MA, USA), and acetonitrile (HPLC grade) was supplied by Sigma-Aldrich (St. Louis, MO, USA).

Physical and Chemical Analysis

Titratable acidity was determined by titrating a known amount of homogenized raisin with a solution of 0.1 N NaOH until a pH 8.2 using a pH meter [21] and expressed as percent tartaric acid. The water activity (a_w) was measured at 24°C using an AquaLab a_w meter (Decagon Devices, Inc., Pullman, Wash.). The CIE *L** (lightness), *a** (redness) and *b** (yellowness) values of the sample were measured with a Minolta Colorimeter (CR 300, Chromometer, Minolta, Japan).

Determination of Monosaccharides and Chromatographic Procedure

The method of Muir et al. [22] for the determination of monosaccharides had been modified. Briefly, 5 g of raisin sample was accurately weighted into a beaker and 80 mL of distilled water at 80°C was added. The beaker was placed in a shaking water bath (JP Selecta SA, Barcelona, Spain) at 80°C for 15 min with agitation at 100 rev/min and the volume was adjusted to 100 mL with distilled water into volumetric flask after cooled to room temperature. Nine mL of solutions were taken and 0.5 mL of Carrez I and 0.5 mL of Carrez II were added to it. The mix was stirred and centrifuged at 2500 rpm for 10 minutes (NF 200, Nuve, Ankara, Turkey). After discarding the supernatants, 0.22 µm disc filter (Millex GV, Millipore, Molsheim, France) was used for filtration to remove the particles including bacteria and then the filtrate was analysed with HPLC.

Shimadzu (Tokyo, Japan) chromatographic system equipped with a LC-20 AT model pump, a RID-10A model refractive index detector, CTO-10AS VP model column oven, SIL-10A model auto sampler and LC-20AT model data station was used in this study. For separation of glucose and fructose, an Inertsil NH_2 column (5 µm, 250 mm x 4.6 mm; GL Sciences, Torrence, CA, USA) equipped a guard column (5 µm, 50 mm x 4.6 mm; GL Sciences, Torrence, CA, USA) was used, the solvent flow rate was 1 mL/min, the column temperature was 40°C and the injection volume was 20 µL. The mobile phase of HPLC was consisted of a solution of acetonitrile:water (75:25 v/v) mixture.

Determination of Furfurals and Chromatographic Procedure

Firstly, 20 g homogenized raisin sample was weighted in a flask and 40 mL of water was added on. The mix was homogenized using an Ultra-Turrax homogenizer (WiseTis HG-15D, Daihan Scientific Co., Seoul, Korea) about 2 min and then the homogenate was filtered through filter paper. Two mL of filtrate was mixed with 8 mL of distilled water, 0.5 mL of Carrez I (dissolving 21.9 g of crystallized zinc acetate and 3 mL of glacial acetic acid in 100 mL of distilled water) and 0.5 mL of Carrez II (dissolving 10.6 g of potassium hexacyanoferrate (Fe^{+2}) in 100 mL of distilled water). Then the mix was centrifuged at 4000 rpm at 20°C for 15 minutes (Sigma, 3K-30, UK) to remove the particles in the solution. The supernatant was filtered through a 0.45 µm pore size filter (Millex GV, Millipore, Molsheim, France) and directly analyzed by HPLC-DAD [23].

High pressure liquid chromatography (HPLC) separation was performed with a Shimadzu HPLC system (Shimadzu, Tokyo, Japan) consisting of a pump (LC-20AT, Japan), a photodiode array detector (SPD-M20A, Japan), a column oven (CTO-10AS VP, Japan), an auto sampler (SIL-10A, Japan) and data station (LC-20AT, Japan). An ACE 5 C18 column (5 µm, 250 mm x 4.6 mm, Scotland) was used for separation at 24°C temperature. The samples were separated using ultra-pure water:acetonitrile (90:10 v/v) mixture at 1 mL/min flow rate as mobile phase. Detection of HMF, FMC and FA was perforated at a wavelength of 284, 274 and 277 nm, respectively.

Validation and Quantification

The results of correlation coefficient (R^2), limit of detection (LOD) and quantification (LOQ) recovery (%) and repeatability (RSD, %) are shown in Table 1. Standard solutions of furfurals and monosaccharides were prepared with ultra-pure water and diluted at five different concentrations in the range of 0.05-5 mg/kg for HMF and FMC, 0.5-50 mg/kg for FA, and 250-2500 mg/kg for monosaccharides. The curves were linear with R^2 values higher than 0.998. Limit of detection (LOD) and limit of quantification (LOQ) were calculated by taking 3.3 and 10 times the standard deviation, respectively, using the slope of calibration of furfurals and monosaccharide. The LOD and LOQ values were found between 0.01-0.92 and 0.03-0.22 mg/kg for furfurals, respectively and 3.29-8.65 and 9.98-26.2 mg/kg for monosaccharides, respectively. The HPLC procedure for compounds except FA were given good repeatability (Relative Standard Deviation, RSD %=1.32-21.73, *n*=5). Recovery studies were performed in triplicate, by spiking the analyzed samples with different solutions for furfurals (10 and 50 mg/kg) and monosaccharides (1000 and 2500 mg/kg) and obtained recoveries were ranged from 83.54-92.14% for furfurals and 89.62-91.13% for monosaccharides.

Table 1. Performance of the HPLC methods for the determination of furfurals and monosaccharides

Compounds	Coefficient R^2	LOD[a] (mg/kg)	LOQ[b] (mg/kg)	Recovery (%) n=3	Repeatability RSD[c] (%) n=5
2-F	0.999	0.07	0.22	83.54	21.73
2-FMC	0.998	0.31	0.92	87.38	5.03
5-HMF	0.999	0.01	0.03	92.14	2.10
Fructose	0.999	3.29	9.98	91.13	1.32
Glucose	0.999	8.65	26.2	89.62	6.54
Sucrose	0.999	11.00	33.4	92.60	3.53

[a]: Limit of detection; [b]: Limit of quantification; [c]: Relative standard deviation (RSD)

Statistical Analysis

Statistical analysis of the obtained data was conducted using SPSS (version 17.0) package program (SPSS Inc., Chicago, USA). Results were represented as the mean value ± standard deviation. Significant differences between raisin cultivars, and packaged / unpackaged samples were assessed with the Student's *t*-test (P<0.05).

RESULTS and DISCUSSION

Chemical and Physical Characteristics

The chemical and physical characteristics of packaged and unpackaged raisins are given in Table 2. The titratable acidity (expressed as tartaric acid) of the raisins was ranged from 0.78-2.37%. Bhagyashree et al. [24] found that the acidity ranges from 1.21 to 2.29 percent. Normally, fresh grapes contain 0.6-0.9% tartaric acid and after the drying process, raisins contain 2-3.5% of tartaric acid [25]. However, Angulo et al. [26] stated that grapes dried with different drying methods like dry-on-vine or tray-dry contain similar titratable acidity. The titratable acidity of white raisins was detected higher than red raisins ones (P<0.05).

The water activity (a_w) of the samples varied within the range of 0.54-0.74 with the mean of 0.64. There was insignificant difference between raisin types, and packaged and unpackaged samples. A_w, important factor for drying process, is an indicator for food as rehydrated fruit [27]. Generally, the rate of forming furfurals increases from the dry state, starting at a critical a_w of 0.2-0.3 for most foods, to a maximum at a_w of 0.5-0.8 and then decreases at higher a_w [28].

Fructose and glucose were dominant monosaccharides with the means of 309.46±46.54 and 313.97±47.2 g/kg, respectively in raisin samples. Although, both of fructose and glucose levels were varied between the raisin samples, there were no significant differences between the white and red ones (*P*>0.05; Table 2).

*CIE L**, *a** and *b** color values of white raisins were significantly different from those of red raisins (P<0.05; Table 2). The *L** values of both of raisins were less than 50, which indicated that the raisins were dark in color. During drying process, the colors of white and red grapes had been probably turned to dark color. Positive *a** and *b** values indicated that red and yellow coloration were dominate all of the samples. According the Chayjan et al. [29], higher value of *L** and lower value for *a/b* ratio are desired for raisins.

Table 2. The physicochemical characterizations of white and red raisins

Cultivars	Acidity (%)	a_w	Fructose (g/kg)	Glucose (g/kg)	*L**	*a**	*b**
White Raisin (*n*=39)	1.85±0.4[a]	0.63±0.03[a]	311.95±52.69[a]	314.19±52.29[a]	29.08±1.43[a]	5.21±3.64[a]	10.38±3.64[a]
Red Raisin (*n*=23)	1.07±0.25[b]	0.65±0.05[a]	304.77±32.72[a]	313.55±37.09[a]	17.92±2.56[b]	2.59±1.03[b]	3.45±1.03[b]
Package							
Packaged (*n*=19)	1.55±0.44[a]	0.62±0.04[a]	315.91±33.69[a]	307.26±44.41[a]	21.55±6.04[a]	3.43±1.49[a]	6.88±3.61[a]
Unpackaged (*n*=43)	1.59±0.54[a]	0.64±0.03[a]	309.46±46.54[a]	315.38±48.15[a]	25.98±9.06[a]	4.48±3.49[a]	8.21±4.63[a]

The data were explained as mean±standard deviation, [a-b] Means with the same letters in a column within each category are statistically significant at P<0.05.

Furfural Contents

The HMF, FA and FMC concentrations in raisins were found as means of 11.23, 24.39 and 25.22 mg/kg, respectively. The HMF values ranged from a low of 1.15 to a high of 44.43 mg/kg for white raisins and from a low 2.32 to a high of 75.85 mg/kg for red raisins (Figure 1). Differences in HMF levels of white and red raisins were not significant (*P*>0.05). Similarly, FA and FMC values of white raisins were found lower than red raisins but not statistically different between raisins (*P*>0.05; Figure 1). The results were in agreement with the findings of Caglarirmak [9], who found that the mean HMF value of sultanas (pretreatment before sun drying) was 5.5 mg/kg. Whereas, the obtained HMF concentrations of raisins (not pretreated before sun drying) was much higher than our results for white and red raisins. Karadeniz et al. [30] reported that sun dried, dipped and golden raisins had HMF an average values of 53.4, 85.5 and 20.6 mg/kg, but fresh grapes did not contain HMF.

The mean HMF, FA and FMC levels were determined as 3.59, 17.36 and 44.04 mg/kg for packaged raisins and 12.84, 25.87 and 92.22 mg/kg for unpackaged raisins, respectively (Figure 2). The HMF and FMC values of the statistical analysis show that there was a difference between the packaged and unpackaged raisins (*P*<0.05). This situation can be explained with effects of storage conditions such as temperature, long storage time, oxygen, packaging situation etc. In addition, packaging situations of foods are important for Maillard reaction. Waletzko and Labuza [31] reported that adequate packaging significantly decreased the rate of the Maillard reaction wherefore the packs keep of oxygen-free atmosphere. Presence of oxygen in environment surrounding a food can effect on Maillard reaction rate because of linking water system of food (water activity and water content) [32]. It is found that Maillard reaction rate decreased in model system without oxygen and so that longer shelf life of foods could be obtained with oxygen-free atmosphere [31].

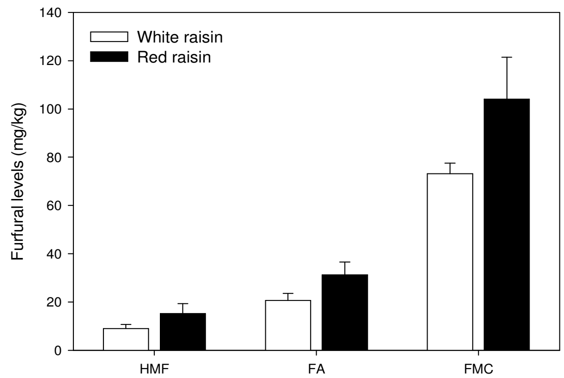

Figure 1. Furfural levels of white and red raisins (mg/kg)

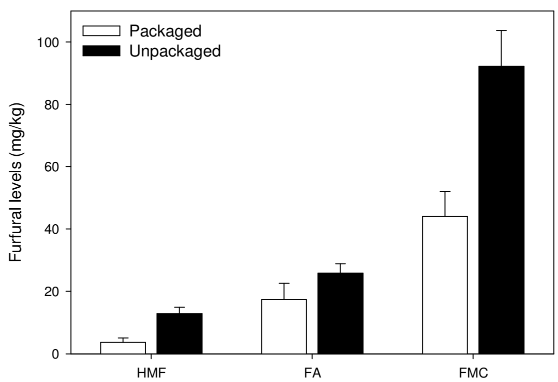

Figure 2. Furfural levels of packaged and unpackaged raisins (mg/kg)

The formation of furfurals is affected by some factors like titratable acidity, a_w, glucose and fructose contents of the samples. The HMF, FA and FMC were not correlated with stated factors according the correlation analysis (P>0.05). Whereas, the correlation results showed that the HMF level in white raisins were increased with the *L** value of the samples become darker (P<0.05), but not in red raisins (P>0.05). Similarly, HMF level was found as negative correlation with *b** values (P<0.05). Lee and Nagy [33] reported that the HMF formation provides the dark brown color of the product. In addition, HMF and FMC levels were increased in association with each other (P<0.01).

There is no any limitations about the HMF, FA and/or FMC levels in raisin. According the Turkish Food Codex, the HMF limit values of liquid and solid grape molasses are 75 and 100 mg/kg, respectively [15], and in this study, HMF levels of raisins were found lower than expressed limits. An Acceptable Daily Intake (ADI) value for furfurals was established as 0.5 mg/kg body weight by European Food Safety Authority (EFSA), but not references are written for HMF or others [34]. In this study, Estimated Daily Intake (EDI) of each furfural have been calculated as <0.05 mg/kg body weight for raisin as snack food. The EDI value of furfurals for unpackaged raisins was determined as nearly 2 times higher than other. Although value of EDI is lower than ADI, furfural levels of unpackaged raisin using as raw material for some grape based products are important. On the other hand, a dietary HMF intake estimated as 1.6 mg/person per day based on modified Theoretical Added Maximum Daily Intake approach in the Scientific Panel about the food additives, flavorings, processing aids and materials in contact with foods [35]. In addition, the major concern for HMF is related to its bioactive derivative 5-sulfoxymethyl-2-furfural (SMF) that has been stated as a mutagenic compound [1]. Sulfotransferases (SULTs) enzyme plays a role in the conversion of HMF to SMF as a catalyst [36]. Therefore, human SULTs are more active than those expressed by rodents, since they are also more extensively present in human tissues than rodents and therefore human are more sensitive to HMF [37].

CONCLUSION

In this study, HMF, FA and FMC contents of white and red raisins were determined. Packaged raisins should be consumed as a snack food because of its low furfural contents. Packaging of raisins rather than the variety has an effect on furfurals. There is a positive correlation between HMF and FMC levels. Packaging of raisins is important for not only the consumption directly as a snack food in daily life but also the use as a raw material in grape based products such as grape molasses, grape juices and bakery products in terms of initial furfural levels.

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