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Investigation of the 5-hydroxymethylyfurfural value and antioxidant properties of Kazdağı Fir cone syrup

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ABSTRACT

Background and aims Cone syrup, unlike cone molasses, is a food product prepared without adding beet sugar. The use of high temperatures in the production of syrups and molasses causes the formation of 5-Hydroxymethylfurfural (HMF), which causes serious toxic effects on the human body, from glucose, fructose aldose and ketose monosaccharides. Although pine cone syrup is just becoming widespread in our country, it is a new food that is in high demand during the winter months. Therefore, in the study, HMF levels, sugar content, phenolic content and antioxidant levels in Kastamonu local cone syrup prepared without adding beet sugar were experimentally determined.
Methods The color intensity resulting from the reaction of hydroxymethylfurfural, paratoluidine and barbituric acid was determined by reading the absorbance at a wavelength of 550 nm with a spectrophotometer. HMF measured spectrophotometrically was detected at very low levels in the syrup. Brix value, phenolic content and antioxidant values of fir syrup were also evaluated.
Results Spectrophotometric analysis, the HMF content of traditional fir syrup was found to be very low, 8.1 mg/L. In addition, the total phenolic content value of locally produced fir cone syrup was found to be 6,050 mg GAE/g and the % inhibition value calculated by the DPPH method was 48,553, thus shedding light on its potential nutritional content through antioxidant activity analyses.
Conclusions This study is the first to investigate fir syrup. As a result of the study, antioxidant activity analyzes shed light on its potential nutritional content. More importantly, this study was the first to show that fir syrup has a low glycemic index with a % Brix value (2.370±0.058) and that diabetic patients can safely use it instead of molasses.

Key Words: Antioxidant activity, Total phenolic substance, Trojan Fir syrup, 5-Hydroxymethylfurfural

Kazdağı Göknarı kozalak şurubunun 5-hidroksimetilfurfural değerinin ve antioksidan özelliklerinin incelenmesi

ÖZ

Giriş ve Hedefler Kozalak şurubu, kozalak pekmezinden farklı olarak, pancar şekeri ilave edilmeden hazırlanan gıda ürünüdür. Şurup ve pekmezlerin üretiminde yüksek sıcaklık kullanımı, glikoz, früktoz aldoz ve ketoz yapılı monosakkaritlerden insan vücudu üzerinde ciddi toksik etkilere sebep olan 5-Hidroksimetilfurfural (HMF) oluşturabilmesine neden olmaktadır. Kozalak şurubu ülkemizde yeni yeni yaygınlaşmakla beraber kış aylarında oldukça yüksek oranda talep gören yeni bir gıdadır. Bu nedenle çalışmada, pancar şekeri ilave edilmeden hazırlanan Kastamonu yöresel kozalak şurubunda HMF seviyeleri, içerdiği şeker miktarı, fenolik içerik ve antioksidan seviyeler deneysel olarak tespit edilmiştir.
Yöntemler Hidroksimetilfurfural, paratoluidin ve barbitürik asidin reaksiyonu sonucu ortaya çıkan renk yoğunluğu, 550 nm dalga boyunda absorbansın spektrofotometre ile okunmasıyla belirlenmiştir. Spektrofotometrik olarak ölçülen HMF şurupta çok düşük seviyelerde tespit edilmiştir. Köknar şurubunun, brix değeri, fenolik içeriği ve antioksidan değerleri de değerlendirilmiştir.
Bulgular Spektrofotometrik analiz sonucunda geleneksel köknar şurubunun HMF içeriği 8.1 mg/L gibi çok düşük değerde bulunmuştur. Ayrıca yerel olarak üretilmiş köknar kozalak şurubunun toplam fenolik içerik değeri 6.050 mg GAE/g ve DPPH metodu ile hesaplanan % inhibisyon değeri 48.553 olarak bulunmuş.
Sonuçlar Bu çalışma köknar şurubunun araştırıldığı ilk çalışmadır. Çalışma sonucunda antioksidan aktivite analizleri ile potansiyel besin içeriğine ışık tutmuştur. Daha da önemlisi, bu çalışma ile köknar şurubunun % Brix değeri (2.370±0.058) ile düşük glisemik indekse sahip olduğu ve diyabetik hastaların pekmez yerine güvenle kullanılabileceğini gösteren ilk çalışma olmuştur.

Anahtar Kelimeler: Antioksidan aktivite, toplam fenolik madde, Kazdağı köknar şurubu, 5-Hikroksimetilfurfural

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1. Introduction

Trojan fir syrup, primarily produced in Kastamonu, has gained recent popularity due to its perceived benefits for conditions like asthma and bronchitis. The production process involves boiling mature fir cones without the addition of beet sugar, similar to the method used for pine cone molasses. This syrup is derived from *Abies* trees of the *nordmanniana* species Trojan Fir (*Abies nordmanniana* subsp. *equi-trojani* [Asch. & Sint. ex Boiss] Coode & Cullen). These cones are rich in active biological compounds, including resin acids, monosaccharides, alcohols, lignans, glycerol, gallic acid, sucrose, catechin isomers, and gallocatechin isomers. Additionally, fir syrup has been studied for its potential in preventing asthma and bronchitis (Ermiş 1997; Yücel 2014) contributing to its growing popularity for these health benefits. According to our research, this study is the first attempt to evaluate the phenolic substances and antioxidant levels in fir syrup. Due to the increasing use of these products, this and similar studies will be valuable in informing the public about pine cone syrup and revealing its advantages and disadvantages in terms of public health.

The preparation of cone syrup involves a heat treatment process, akin to other molasses. It's worth noting that the exposure of foods to high temperatures can lead to the formation of HMF. The generation of HMF occurs due to inappropriate temperature storage of sugary foods and the heat treatment applied during their production (Rosatella et al., 2011; van Putten et al., 2013). As a result, HMF is considered a quality indicator in various food products. HMF is considered inert in standard genotoxicity tests (Abraham et al., 2011), yet it can undergo metabolism into a chemically reactive intermediate known as 5-sulfooxymethylfurfural (SMF). Notably, SMF has demonstrated mutagenic and carcinogenic properties (Bakhiya et al., 2009).

In rat studies, the LD_{50} value for 5-HMF was determined to be 3.1 g/kg, underscoring the potential health risks associated with high consumption (Ulbricht et al., 1984). HMF can be encountered in a range of foods, including fruit juices, milk, honey, molasses, cereal products, and jam, due to production and storage processes (Hepsağ and Hayoğlu, 2017). Given that varying consumption rates of these foods can lead to significant 5-HMF intake, it's crucial to ascertain its levels across all food items.

Precise control over production processes is facilitated by quantifying HMF amounts (Batu et al., 2014). To this end, limits have been imposed on the presence of 5-HMF. As outlined in the Turkish Food Codex Honey Communiqué, it is permissible for honey to contain up to 40 mg/kg of 5-HMF. Similarly, under the Turkish Food Codex Grape Molasses Communiqué, liquid molasses is allowed to contain up to 75 mg/kg, while solid molasses can contain up to 100 mg/kg of 5-HMF. However, despite these regulatory standards, research concerning the levels of 5-HMF in sugar-free syrups remains scarce.

Consequently, our study was designed to quantify the levels of 5-HMF in cone syrups devoid of added carbohydrates or clay. By addressing this knowledge gap, we aim to provide a more comprehensive understanding of the composition of such syrups.

2. Materials and Methods

2.1 Preparation of fir syrup

Many products are sold as fir syrup in local markets. However, some of them are actually products containing molasses. The remaining ones fail to meet the standards in terms of color and quality, as they use other pine products. For this reason, our study was carried out using a licensed product that produces original fir syrup from the region and is sold commercially. To carry out our study, fir syrup produced by traditional methods from freshly collected cones by the villagers in Hanönü District of Kastamonu was used. For this, 500 g of mature fir cones of the *A. nordmanniana* subsp. *equi-trojani* species were soaked in 1 liter of water. Subsequently, the boiling process was initiated, maintaining a rolling boil for a duration of 4 hours. The traditional method of fir cone syrup production involved this boiling process, conducted at temperatures ranging from 110°C to 120°C. Notably, no additional sugar or clay was introduced during this process. All reagents and solvents used in the experimental study were purchased from Aldrich, Sigma or Merck Chemical brands and used in analytical purity.

2.2 Analysis of 5-HMF in fir syrup

The presence of HMF was determined quantitatively by the colorimetric method, one of the harmonized methods of the International Honey Commission (Bogdanov et al., 2002). This method was based on the reaction between hydroxymethylfurfural, paratoluidine, and barbituric acid and absorbance readings taken using a spectrophotometer. Fir syrup sample (20 g) was made up to 100 milliliters with distilled water and then filtered using a filter paper. From this filtrate, 2 milliliters were transferred to separate test tubes: one as a control and the other as the sample. To both test tubes, 5 milliliters of p-toluidine solution were added. Following this, 1 milliliter of distilled water and 1 milliliter of barbituric acid were included in each test tube. The contents were mixed using a vortex mixer. Then, absorbance measurements of the samples in the test tubes were carried out at a wavelength of 550 nanometers using a Shimadzu UV-1900 spectrophotometer with the help of a pair of quartz cuvettes. The calculated value for HMF (mg/L) was determined using the formula HMF (mg/L) = 162 × A, where 162 represents the correction factor and A stands for the absorbance value measured at 550 nm (Binici et al., 2023).

2.3 Analysis of total phenolic contents

For the determination of total phenolic substances, the spectrophotometric method applied by Elmas et al. (2019) was used with minor modifications (Elmas et al., 2019). For this, the fir syrup concentrations to be analyzed were used at 50% dilution rates. First of all, the test tubes were prepared by mixing 2.5 mL of 10% Folin Ciocalteu reactant and 2.5 mL of 7.5% $NaHCO_3$ on fresh rhubarb solution. The blank solution was prepared by mixing this solution without fir syrup in the same proportions. After the samples were incubated in a dark environment for 45 minutes, measurements were made on a spectrophotometer at 760 nm. The same procedure was repeated for the gallic acid solution. The absorbance obtained was

compared to a standard curve (Gallic acid, 0-10 mg) and the results were expressed as milligrams of gallic acid equivalents per gram of dry matter (mg GAE/g dry matter).

2.4 Analysis of antioxidant activity

To evaluate antioxidant activity, the 2,2-diphenyl-1-picrylhydrazyl (DPPH) method applied by Baysan et al. (2019) was used (Baysan et al., 2019). For this, fir syrup samples prepared at 50% dilution were mixed with 3 mL of methanolic solution containing DPPH radicals and brought to a total volume of 6 mL by the addition of methanol. The mixture was shaken vigorously and left in the dark for 30 minutes. The absorbances of the sample-containing solution were then measured against a blank sample without sample at 517 nm. The measurements were conducted spectrophotometrically using the Soif Optical Instruments V-5000H spectrophotometer (Turkey). Radical scavenging activity was calculated from the following formula:

Inhibition (%) = $[(A_0 - A_1) / A_0] \times 100$,

Here, A_0 is the absorbance of the control solution and A_1 is the absorbance of the sample solution (Huang et al., 2005). The antioxidant activity was quantified in micromoles per gram of dry matter and expressed as trolox equivalent (mg trolox/g dry matter). All analyses were conducted in triplicate.

2.5 Analysis of moisture content

Determinations of moisture content and amount of dissolved dry matter in water were evaluated by both oven and refractometer methods. The moisture content of fir syrup was determined using an infrared moisture analyzer (Daihan, MA10, Korea) set at 105°C. This method exhibited a strong correlation (0.99) with the oven method. In addition, the amount of solid matter in fir syrup was measured with an Abbe 2 model (0-95 % brix, temperature range: 0-70 °C) analog refractometer device. The fir cone syrup sample used for dry matter analysis was taken as 3.370±0.394 g.

3. Results and Discussion

3.1 Assessment of 5-HMF values

HMF, which is not found in the structure of fruits but is formed by transforming from monosaccharides under the influence of heat, is an important quality factor that indicates the heat treatment intensity (temperature and duration) of many products (Türkben and Uylaşer, 2018). Thermal treatment is one of the basic processes in food technologies, especially for substances such as molasses. The primary effect of heat treatment is the formation of HMF during the production of various foods such as fruit juices, milk, honey, molasses, cereal products, jams. The HMF value gives information about whether the boiling process is applied correctly. Therefore, HMF is an important parameter for health and serves as a quality indicator for foods and requires constant monitoring. In our study, we measured the HMF value in Kastamonu local pine cone syrup spectrophotometrically and found that HMF was present at very low levels. In our study, the HMF values obtained for fir syrup prepared without adding sugar were found to be 8.1 mg/L, and this value was significantly lower than the reference data. This situation emphasizes the importance of applying short heat treatment under appropriate conditions. The closest comparable study was conducted on Kastamonu cone molasses, where the range was measured as 4.54-101.58 mg/kg (İncemehmetoğlu, 2021). The main reason for this inequality is the absence of sugar in cone syrup. As it is known, syrup samples are generally prepared without adding extra sugar. A study involving tomato paste samples revealed that an increase in glucose levels led to higher levels of HMF (Kus et al., 2005). Similarly, the lowest level of 5-HMF was detected in pasteurized milk samples, while lactose-free milk samples exhibited a higher concentration of HMF due to the increased reactivity of glucose and galactose (Urgu et al., 2017). The similarity between the amounts of dry matter and water-soluble substances indicates the presence of carbohydrate-derived molecules in the fir itself. As shown in this study, these molecules do not cause an increase in HMF values unless there is an additional carbohydrate input from outside. One of the most critical factors influencing the HMF value is the combination of temperature and time during the applied heat treatment (Eskin, 1990; Rosatella et al., 2011; van Putten et al., 2013). In our study, our sample was prepared by boiling at a low temperature for as little as 4 hours, resulting in very low HMF values. This underscores the importance of maintaining low temperature and limited time for optimal food quality.

Another factor affecting the HMF rate is the storage process and temperature. In certain cases, this can cause rates to increase nearly twofold (Labuza and Saltmarch, 1981; Bharate and Bharate, 2014). In our analyzed sample, it was refrigerated without undergoing any storage process. As a result, there was no interference in the HMF values due to improper storage conditions. The HMF values of the fir syrup we obtained were compared with the Turkish Food Codex reference data. According to the 2007 Agricultural Food Codex, the acceptable ranges are a maximum of 75 mg/L for liquid grape molasses and 100 mg/L for solid molasses (Anonim, 2007). Remarkably, the HMF values obtained for the syrup prepared without the addition of sugar were notably low (8.1 mg/L), demonstrating a stark contrast to the reference data.

3.2 Assessment of antioxidant activity and some physicochemical parameters

Previous studies have shown that pine cone extracts have a reactive oxygen scavenging effect (Kwak et al., 2006). It has been reported that pine cones belonging to the Pinus koraiensis species contain bioactive substances such as flavonoids, polysaccharides and phenolic compounds with antitumor and antioxidant activities (Yi et al., 2017). Meng (2010) examined the bioactivities of polysaccharides obtained from the cones of Pinus koraiensis and stated that they exhibited high antioxidant activity with an IC_{50} value of 28.0 mg/mL (Meng, 2010).

In this study, samples obtained by diluting 500 g of pine cone syrup by 50% were examined by the DPPH radical quenching method. The absorbance values obtained for the samples with and without pine cone addition are given in Table 1 and the % inhibition values were calculated. The results were given as the average of 4 different samples taken from the sample. Additionally, the antioxidant capacity value against trolox, which was used as a standard antioxidant, was given as 0.299

mg trolox/mL. Antioxidant activity and total phenolic values of pine cone syrup with 5% moisture content are summarized in Table 1. To compare total phenolic contents, the results were calculated as gallic acid equivalent (mg/g) and found to be 6.368 mg GAE/g dry weight.

Table 1. DPPH radical scavenging activity and total phenolic substance content of 50% diluted pine cone syrup

	DPPH Method			Folin Ciocalteu Method
	Blank Absorbance (500 nm)	Sample Absorbance* (500 nm)	Inhibition (%)	Total Phenolic Substance (mg GAE/g)
Trojan fir cone syrup	0.795	0.409±0.011	48.553	6.050

*Average absorbance value was calculated (n=4).

Akar (2022) examined the antioxidant activities of extracts prepared at different concentrations from cones collected from Pinus nigra jf. arnold and Thuca occidentalis L. trees growing in the Nevşehir region and calculated their DPPH scavenging abilities. In his study, Akar (2022) reported that the best value in terms of DPPH radical scavenging activity was found in Pinus nigra (IC_{50}: 55.8 µg/ml) and the lowest value in Thuca occidentalis (IC_{50}: 200.4 µg/ml) (Akar, 2022).

Dry matter analysis was carried out through moisture determination in an oven. The cone syrup obtained from fir cones revealed no detectable levels of protein or lipids, with carbohydrates accounting for 97% of the dry matter content. Following the dry matter analysis, the quantification of water-soluble substances was performed using a refractometer. The results are presented in Table 2.

Table 2. Determination of the amount of dry matter dissolved in water using the moisture analysis method and the Brix (%) method at 105°C

	Moisture Analysis (105 ℃ Oven) (Mean±Std.)		Amount of dry matter dissolved in water, Brix (%) (Mean±Std.)
Trojan fir cone syrup sample	Humidity (%)	Dry matter (%)	
	97.731±0.066	2.270±0.066	2.370±0.058

The fact that the amounts of dry matter and water-soluble substances are close to each other shows that fir has carbohydrate derivatives in its content. While the water-insoluble dry matter consists of polysaccharides such as cellulose and starch, the water-soluble dry matter consists of sugars such as fructose and glucose and organic acids such as citric acid, malic acid and tartaric acid. It was stated in Table 2 that the fir syrup prepared without added sugar had a low glycemic index, as a low dry matter ratio calculated by the Brix (%) method. *Abies* species have also been reported to reduce postprandial glycemic response, prevent blood sugar increases through enzymes associated with high glucose levels in the diet, and protect against oxidative damage (Kreft, 2021; Schoss et al., 2022).

4. Conclusion

This study represents the first instance of evaluating the levels of phenolic substances and antioxidants in fir syrups. Given the absence of similar studies before, its findings are essential for contributing to the literature as a reference point. Considering the potential adverse effects on human health, it becomes imperative to focus on the 5-HMF limit values stipulated in food regulations concerning heat-treated products. It is evident that intense heating stands out as the primary contributor to both nutrient degradation and elevated 5-HMF levels. Given that 5-HMF functions as a gauge of product quality, its presence should ideally be minimized in optimal food items. Our analysis leads us to project that to avert the formation of 5-HMF in heat-treated foods like syrups, it is anticipated that the 5-HMF value can be curtailed by preparing them without the addition of extra sugar, employing briefer heating durations instead of prolonged processes, and ensuring swift consumption within a limited shelf life, all while storing them in refrigerated conditions. These collective measures substantially contribute to the preservation of both nutritional value and the overall quality of heat-treated food products.

Author Contributions

Concept: V.K.; Design: V.K., S.B.O.; Control: V.K., T.K.B., S.B.O., S.Ü.; Sources: V.K., and S.B.O., Materials: V.K., and S.B.O.; Data Collection and/or Processing: V.K., S.B.O., B.Ö; Analysis and/or Interpretation: V.K., S.B.O., T.K.B, B.S; Literature Review: V.K., S.B.O., B.Ö.; Manuscript Writing: V.K., S.B.O., T.K.B; Critical Review: V.K., T.K.B; Other: S.B.O, S.Ü., B.Ö.

Conflict Of Interest

The authors declare that there is no real, potential, or perceived conflict of interest for this article.

Ethics Committee Approval

The authors declare that the ethics committee approval is not required for this study.

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