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Rewiew Article

Determination of Hydroxymethyl Furfuraldehyde (HMF) Contents in Traditionally Produced Strained Yoghurts

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Abstract

Food products undergo numerous processing phases from the farm to our table. Throughout and after these processing stages, alterations in physical, chemical, and microbial properties transpire in food products. When sweet foods are improperly stored at elevated temperatures and undergo heat treatment during manufacture, chemical processes produce hydroxymethylfurfural (HMF), a quality benchmark. Denizli Burnt-scented yogurt, Burdur Kökez yogurt, and winter yogurt are traditional strained yogurts in Türkiye. Analyses of pH, titratable acidity, total dry matter, fat, protein, ash, color, and HMF were conducted on concentrated yogurts. The analysis of HMF in samples was conducted utilizing a spectrophotometer. The European Food Safety Authority (EFSA) has indicated a maximum detectable level of 15 mg/kg for HMF with established toxicity in milk and dairy products. The current investigation established that the HMF levels in all experimental yogurt samples were beneath this threshold. The HMF content of yogurt samples was determined as follows: Burdur Kökez strained yogurt (6,74 µmol/L) < winter yogurt (7,51 µmol/L) < Denizli burnt scented yogurt (8,63 µmol/L), respectively. L value as 95,87-96,81, the a value as -2.51-3,20 and the b value as 2,66-3,12 in yogurts.

Keywords: 5-Hydroxymethylfurfural (HMF), Yogurt, Strained Yogurt, Burdur Kökez Yogurt, Denizli Burnt-Scented Yogurt, Winter Yoğurt

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Introduction

5-Hydroxymethylfurfural (HMF) is made when sugars break down at high temperatures. It has been found in milk and other foods that have been heated up. There are two types of HMF: "free" HMF, which comes from the Maillard reaction, and "total" HMF, which can also come from the Lobry de Bruyn-Alberda van Ekenstein transformation. This is likely present in milk when subjected to heat treatment and stored at inappropriate temperatures. One type of chemical that HMF is furfurals, which are used as bridge molecules in the advanced stages of the Maillard process to make pigments called melanoidins (64).

HMF is widely available in different food products that are thermally processed. Its concentration might increase with the enhancement of storage duration. Its concentration might increase with the enhancement of storage duration (4). The quantity of HMF observed in the foods is directly associated with the heat load exerted while processing food products rich in carbohydrates (9) and the storage condition. HMF is found in different food products as prepared from fruit, vegetables, cereals, crops, and confectionery food products, etc.(10).

HMF is $C_6H_6O_3$, molecular weight is 126.11 g/mol, density is 1.2062 g/cm^3, refractive index is 1.5627 (at 18°C), flash point is 79°C, melting point is 31.5°C, boiling point is 110–116°C (at 2.67 Pa) and 114–116°C (at 133.32 Pa (34).

Many different foods are known to contain HMF. These include processed fruits, coffee, honey, cookies, bread, baby food, breakfast cereals, toast, and alcoholic beverages. Table 1 displays the HMF content of certain foods. However, the significance of the HMF content varies based on the specific food and the measurement's purpose. For example, adulteration is associated with the formation of HMF in honey, overprocessing in fruit juice and milk, and ripening in vinegar (41).

HMF monitors the thermal processes applied to cereal items, including bread baking, toasting bread slices, morning cereals, pasta drying, and baby cereal extrusion (9). Vinegar, caramel, and dried fruits possess elevated HMF concentrations, but coffee and bread are the primary sources of dietary HMF consumption (42). The lycopene concentration in processed tomato products directly correlates with the HMF concentration. The food sector uses HMF as a quality marker and a sign of adulteration in particular products, including coffee, juices, milk, sauces, honey, and cereals (15,37). Various industrial applications utilize HMF to produce fuels, pharmaceuticals, solvents, and biopolymers (37).

HMF is present in several food items derived from fruits, vegetables, grains, crops, sweets, liquid smoke, and wood smoke. It serves as a flavoring agent and an indicator of thermal processing and preservation degree. The water activity, amount of cations and amino acids, type of sugar, temperature, and pH of the medium all directly affect how well HMF is synthesized (20).

Honey was found to have 20% of HMF, followed by cereals and derivatives (19%), beverages (13%), fruits and vegetables (8%), and infant food (8%). In contrast, milk and milk products contained 4% of HMF (39).

New toxicological studies show that eating foods with high amounts of HMF might be harmful to people's health. It could irritate the eyes, nose, throat, and mucous membranes, as well as neurological disorders, diabetes, and heart diseases. Additionally, HMF can be transformed into several chemicals, including chloromethylfurfural, sulfoxymethylfurfural, and formic acid, whose consumption has detrimental effects, including mutagenicity (9,37,52). The estimated acute oral LD50 of HMF is 2.5–5.0 mg kg−1, with 2.5 mg kg−1 for female and male rats, respectively (8, 16, 37,48).

Assessing HMF, a health hazard, in dairy products is considered a significant quality metric (12). However, it is still unclear what HMF means regarding toxicology, as in vitro studies on its genotoxicity and mutagenicity have produced mixed results. High quantities of HMF are cytotoxic and irritating to the eyes, upper respiratory tract, skin, and mucous membranes; an oral LD50 of 3.1 g/kg body weight has been established in rats (25).

The European Food Safety Authority (EFSA) has indicated that the maximum permissible limit of HMF in milk and dairy products is 15 mg/kg (17, 23). The current investigation observed that the HMF content in yogurt was below this limit (17). Because of this, it is essential to examine the growth of HMF, which harms health above certain levels, in milk and dairy products to protect food safety and quality. Türkiye has conducted numerous research studies to evaluate the quality attributes of shelf-stable yogurts. However, no research has identified the HMF content (38, 64).

Yogurt, an indispensable part of Turkish cuisine culture, has traditional yogurts produced using different production techniques in Anatolia. In the Southeastern and Eastern Anatolian regions (Kars, Van, Şırnak, and Elazığ), "Kurut" is consumed with soup, mantı, and some local dishes. In the Mediterranean (Hatay), Central Anatolia (Sivas), and Eastern Anatolia (Bingöl), **"Kış Yogurt (Winter Yogurt)"** is a traditionally produced type of yogurt. "Silivri yogurt" in the Thrace region, **"Kökez yogurt"** in the Mediterranean region (Burdur), "Tulum yogurt" in Antalya, "Dorak yogurt" in Kayseri and Niğde, "Külek yogurt" in Isparta and Trabzon, Samsun Bafra buffalo (kömüş) yogurt, Kayseri Bünyan pine glass strained yogurt, and **"Burnt-scented (smutty) yogurt"** produced in the Acıpayam, Tavas, Babadağ, and Honaz districts of Denizli are yogurts traditionally made in different geographies in Türkiye (3, 22, 49).

This study aimed to determine the amount of HMF in three traditionally concentrated yogurts: Burdur Kökez strained yogurt, Denizli Burnt Scented yogurt, and winter yogurt. Its purpose was to supplement research on HMF's effects on nutrition and health and to compare the levels of HMF in these three yogurts. The lack of data-based research on concentrated yogurts underscores the significance of this study.

Materials and Methods

Materials

The total number of samples was 23, and they were collected into three main groups: 5 samples of winter yogurt, 8 samples of Denizli burnt-scented strained yogurt, and 10 samples of Burdur Kökez strained yogurt.

Denizli Burnt- Scented Yogurt: It was traditionally made by heating a cauldron over a wood fire, pouring some milk into the cauldron and cooking it, and then the pasteurization process. The milk was heated to approximately 95ºC and kept at these temperatures for 15–20 minutes. The pasteurized milk was taken into another container. The cauldron with the dishwashing milk was heated again over a wood fire, and the milk remaining in the cauldron was ensured to hold the bottom of the cauldron. The milk was poured into the cauldron that held the bottom and boiled to perform the pasteurization process. The pasteurized milk was allowed to cool naturally at room temperature for 4-5 hours to reach 45±2ºC. A tablespoon of the previous day's burnt strained yogurt (yeast/black yeast) was added to the milk that reached the fermentation temperature (45±2ºC) and fermented; the top of the cauldron was covered and left to incubate for 2-3 hours at 45±2ºC. After incubation, the yogurts were transferred to bags made of raw cloth that were cooled in the refrigerator for approximately 15 hours at 4±1ºC and allowed to drain under suspension for 24 hours. Once the straining process is complete, we package the yogurts from the cloth bags (35).

Winter Yogurt: It is one of the most popular varieties of traditional dairy products manufactured inVan. This yoghurt is known as yoghurt cheese or salted yoghurt in the Mediterranean and someregions of Turkey because of its high dry-matter content and long shelf life. In addition, strainedand winter yoghurts have not been manufactured in industrial plants. In most regions of Turkey, sheep milk is combined with cow milk to produce winter yoghurt, which is one of the most well-liked traditional dairy products (33).

First, the milk is filtered with a cloth to separate foreign substances and is cooked thoroughly. This is followed by cooling it to fermentation temperature, fermenting, and leaving it to incubate. The fresh yogurt obtained is placed in clean bags and hung in a high place in the shade. The water from the yogurt is drained for at least 2 and at most 5 hours. The next day, the drained yogurt is cooked by adding clean, cold water equal to the lost water. Cooking continues for 1 to 1.5 hours. The cooked yogurt is poured into clean, flat, tin containers, cooled, and placed in tins, jars, or glazed pots in a way that leaves no air. Pour plain butter, tallow, or olive oil on top to store and consume the yogurt until winter (36).

Burdur Kökez Strained Yogurt: Yogurt production pasteurizes milk in copper boilers at 95°C for 20–30 minutes over a wood fire. After pasteurization, it is transferred to 20-kg containers and left to cool for approximately 5–6 hours. The temperature cools to 40°C (milking temperature) in summer and 45°C in winter. Two tablespoons of strained yogurt are added to the Kökez yogurt from the day before. This is then left to incubate at 40°C for 5 hours. The incubation period is terminated when the yogurt releases its slightly greenish water. After that, the yogurts are taken to a storage room set at 4°C and stored overnight. The following day, the yogurt is transferred onto filter cloths made of cotton, hung on a high hanger in a room at 4°C, and drained after overnight refrigeration. Drainage is achieved at 4°C, and the volume of whey separated is measured periodically. After 24 hours, the cloths are removed from the hanger and stacked on each other to continue draining the yogurt. It is a local type used in Turkish cuisine. It differs from the milk of grazing animals on the plateaus of Kökez in the center of Burdur due to its distinctive production method and aroma, which vary from that of strained yogurt. The most crucial feature of Kökez yogurt is its taste and texture. Kökez yogurt is one of the most popular varieties of traditionally concentrated yoğurt produced in Turkey's Mediterranean region. The product has a cream or white color, a soft and smooth body, and is highly spreadable with little syneresis. The introductory flavors are described as sweet, sour, and astringent (33, 49).

Methods

Gross Composition

The total solids, fat, protein, pH, and lactic acid contents of milk were determined according to the Association of Official Analytical Chemists methods. The pH was determined using a pH meter (Hanna Instrument, Germany). Protein was determined based on total nitrogen content using the Kjeldahl method and multiplied by a factor of 6.38. Fat was determined using the Gerber method. The total solids content was determined using the gravimetric method and then oven-dried in a laboratory at 105^{0}C for 24 h (5, 24).

Determination of Color Values

Color was measured using a Minolta Chroma Meter CR-400 (Minolta, Osaka, Japan). The *L** (luminosity), *a** (red/green color component), and *b** (yellow/blue color component) color measurements were determined according to the CIELab color space system (69).

Hydroxymethylfurfuraldehyde (HMF)

The total HMF amount of yogurt samples will be determined by the spectrophotometric method described by Keeney and Bassette (27). After shaking and homogenizing the yogurt samples, we took 10 mL and added 5 mL of oxalic acid (0.3N). The prepared samples were mixed with a vortex and kept in a water bath (WNB 14, Memmert, Schwabach, Germany) for one hour. After the samples cooled to room temperature, we added 5 mL of a 40% trichloroacetic acid (TCA) solution. Whatman No. 42 filter paper was then used to filter the mixture. After taking 4 mL of the filtrate and adding 1 mL of 0.05 M thiobarbituric acid (TBA) solution, the samples were kept in a 40 °C water bath for 30 minutes. Then, their absorbance at 443 nm (A443) was measured using a spectrophotometer (Varian, Cary 50 Bio UV-Visible Spectrophotometer, Palo Alto, CA). HMF values were calculated as µmol/L.

Statistical Analyses

Statistical analyses will be performed using the SPSS 17 package program. Data are presented with standard deviation values in the tables created as a result of statistical analysis.

Results and Discussion

Gross Composition

The pH values of Winter yogurt vary between 3,78, titratable acidity at 1,80% LA, dry matter at 23,40%, protein content at 9,45%, and fat content at 9,25%. The pH, titration acidity, dry matter, fat, protein, lactose, and ash values of the Denizli Burnt Scented Yogurt samples were 3,28, 1,94%, 22,92%, 8,4%, 12,00%, and 0,7, respectively. For Burdur Kökez strained yogurts, the values for pH are 3,74, for acidity is 1,51%, for total solids is 24,52%, for fat is 8,25%, protein content was 7,42, and ash content was found to be 0,76% (Table.2).

The dry matter content of all yogurt was found to be between 22,92% and 24,52%. These values agree with previous studies (2, 29, 57). It was found to be lower than the findings of some researchers (13, 62), higher than others (26, 29), and lower than those found in Tulum yogurt (66) and Winter yogurt (53). As the researchers stated, the difference in dry matter values is due to the composition of the milk used in production, the production technique, and the pressing method during the straining stage (31, 57).

The fat content of all yogurts ranged from 8,25% to 9,25%. Similar to our findings, other researchers have reported values ranging between 5,53 and 10,82% in strained yogurts (7, 19, 31, 36). Furthermore, similar fat values were also found by Uysal et al. (62) and Sömer and Kılıç (51).

The results of this work indicated that strained yogurts are an essential source of animal protein in the diet (13, 51). According to the results obtained, the average acidity values are consistent with the studies of Kırdar and Gün (31), Tekinşen et al. (57), and Şimşek et al. (55) and are lower than those reported by Atay (7), Atamer et al. (6), Uysal et al. (62), and Çağlar et al. (13). Several factors could cause the acidity levels to differ: the method of milk production, the quality of the raw milk, the amount of starter culture added, the acidity level of the starter culture, the high temperature and long incubation period, and/or insufficient cooling after incubation and during storage (2, 31, 51, 57). This study found that the Kökez yogurts ranged in pH from 3.52 to 3.94. Atamer et al. (6) stated a pH of 3.52; Seçkin and Nergiz (43) reported a pH of 3.67 in strained yogurts. According to Kırdar and Gün (30), the pH is 3.38-3.91; Kırdar and Gün (31) reported 3.69; Şahan and Kaçar (54) found 3.76; and Şimşek et al. (55) reported that it was between 3.65 and 4.22. The pH values obtained from the research are similar to those stated.

Hydroxymethylfurfural (HMF)

HMF is an important quality indicator for milk and dairy products. The later stages of the Maillard reaction, where reducing sugars and amino acid groups react with heat, produce HMF. Researchers have reported that temperature and duration are the most critical parameters affecting the formation of furfural in milk and dairy products. However, the fermentation process and additional ingredients are also effective (18).

The HMF content of yogurt samples was determined as follows: Burdur Kökez strained yogurt (6,74 µmol/L) < winter yogurt (7,51 µmol/L) < Denizli burnt scented yogurt (8,63 µmol/L), respectively (Table 3).

HMF is one of the most common Maillard reaction by-products in over-processed foods. Several food products use the HMF level to indicate the absence of thermal processing. In a study, researchers examined over five hundred food items and found high levels of HMF (1-9.5 g/kg) (45, 46, 47).

Li et al. (38) reported that the HMF content of fermented dairy products increased from 1.14 mg kg^{-1} to 1.85 mg kg^{-1} during the 21-day storage period. The values obtained in the study are high.

Within his research on traditional Kökez yogurts, Saçak (49) found that the HMF value of these yogurts increased from 6.43 µmol/L at the start of the 30-day storage period to 11.38 µmol/L on the last day.

The HMF contents of the control group and the samples with different amounts of pineapple added were determined as follows: control group (17.16 µmol/L) < 5% pineapple-added yogurt sample (33.67 µmol/L) < 7.5% pineapple-added yogurt sample (35.51 µmol/L) < 10% pineapple-added yogurt sample (39.04 µmol/L), respectively (58).

The HMF content was highest in goat's yogurt (21.59 µmol/L), followed by sheep's yogurt (17.81 µmol/L) and buffalo's yogurt (16.33 µmol/L). It has been reported that heat treatment temperature, heat treatment time, milk composition, and pH affect the HMF content of foods (63).

There aren't many studies that look at the HMF content in yogurt samples. Cui et al. (11) found that the HMF content in yogurt samples was up to 3.43 mg/kg. The European Food Safety Authority (EFSA) has reported the maximum detectable limit of 15 mg/kg in milk and dairy products for HMF with proven toxicity (17). The current study observed that the HMF content in the strained yoğurt samples was below the maximum limit. The research results are similar to the results of Terzioglu et. al. (59). Denizli burnt scented yoghurt had the highest HMF value among concentrated yogurts.

Color Value

Table 3 provides the color values of the yogurt samples. Color is a visual property consisting of the spectral distribution of light that affects a food's acceptability, quality, flavor, and sensory properties. The color of a food product is one of the essential characteristics that influences the consumer's preference for that food and the quality of the final product. Many spoilage reactions in foods also manifest themselves as color changes (44, 68).

The color analysis determined the brightness (L), redness or greenness (a), and yellowness or blueness (b) values of the lemon drink samples. The "L" value represents brightness and varies between 0-100. It takes the value of zero in black when there is no reflection, while it takes the value of 100 in white when there is perfect reflection. The "a" color scale is known as the redness value. Positive A values indicate redness, while negative A values represent green. The "b" color scale is known as the yellowness value. Positive b values represent yellowness, while negative b* values represent blueness. At the zero cut-off point (a=0 and b=0), there is colorlessness, i.e., grayness. (40, 67).

The changes in color values of yogurt samples are given in Table 3. The L* color value serves as a parameter, revealing the brightness and opacity of the object under measurement. The a* color value is a color parameter that provides information about the redness and greenness of the object whose color is being measured. When the a* value is positive (+), it defines redness; when it is negative (-), it defines greenness. The b* color value serves as a color parameter, revealing the yellowness and blueness of the object under measurement. When the b* value is positive (+), it defines yellowness, and when it is negative (-), it defines blueness (69).

Few reports have been available, if any, on the L, a, and b values of Winter yogurt, Burdur Kökez strained yogurt, and Denizli burnt -scented yogurt. In CIELAB systems, the a- and b- scales indicate pigment content in the sample. Constant stirring may have led to smaller fat globules in the lighter yogurt products. This is because fat globules may reflect light better. The increases in yellowness could be due to the caramelization of the milk carbohydrates by high processing temperatures (50).

Coşkun and Karabukulut determined the L value as 104.48, the a value as -2.55, and the b value as 7.50 in yogurts. Sert ve Ark. (50) determined the L value in yogurts to be 93.9, the a value to be -5.5, and the b value to be 8.7. In yogurts, the L value was defined as 93.9, the a value as -5.5, and the b value as 8.7 (21).

The highest L value was determined for Denizli burnt scented yogurt. The lowest was determined for Burdur Kökez strained yogurt. The differences in whiteness/lightness values of yogurts arise from the fragmentation of fat globule diameters due to continuous mixing during processing (28). The Burdur Kökez strained yogurt sample yielded the highest value, while the Winter yogurt sample showed the lowest. The highest B value was determined for Winter yogurt and the lowest for Burdur Kökez strained yogurt. These differences occurring in the value of samples may be due to the production method; heat treatment at a high temperature causes caramelization of lactose, which is the main carbohydrate in milk, and leads to a Maillard reaction through interactions with proteins. This also affects the yellowness values of the samples. Yazıcı and Akgün (65) reported that increasing the fat proportion of Torba yogurt samples gives rise to the b values, and that storage time affects the b values.

Conclusion

The highest HMF value among concentrated yogurts was detected in Denizli burnt scented yogurt. This was followed by winter yogurt, and Burdur Kökez strained yogurt, respectively. Concentrated yogurts showed lower HMF values compared to other foods. The European Food Safety Authority (EFSA) has established an acceptable daily intake value of 0.5 mg/kg body weight for FF. It considers the maximum use limit of 5-HMF in dairy products to be 15 mg/kg. The current study observed that the HMF content in yogurts was below the maximum limit. It is known today that HMF poses a significant risk to food safety and negatively affects human health. However, HMF formation in foods during production and storage should be measured, and HMF reduction studies should be conducted.

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Table 1. HMF Contents of Some Foods

Besinler	HMF miktarı mg/kg
Coffe	100-1900
Honey	10.4-58.8
Beer	3.0-9.2
Jam	5.5-37.7
Fruit juice	2.0-22.0
Wine(red)	1.0-1.3
Kurabiye	0.5-74.5
Bread(white)	3.4-68.8
Breakfast cereal	6.9-240.5
Baby food (milk based)	0.18-0.25
Baby food(Cereal based)	0.-57.18
Dried Fruit	25-2900
Roasted almonds	9
Caramelized products	110-9500

Tablo 2. Chemical Properties Of Concentrated Yoghurts.

Compozisition	Winter Yogurt	Denizli Burnt Scented Yogurt	Burdur Kökez Strained Yogurt
pH	3,78±0,01	3,28±0,01	3,74±0,01
Acidity (%)	1,80±0,01	1,94±0,15	1,51±0,02
Protein(%)	9,45±2,05	12,00±0,21	7,42±0,12
Fat (%)	9,25±1,61	8,4±0,01	8,25±0,01
Total Solid(%)	23,40±0,51	22,92±1,02	24,52±0,55
Ash(%)	0,97±0,22	0,70±0,31	0,76±0,62

Table 3. Color Value And HMF Of Strained Yoghurt Samples

Compozisition	Winter Yogurt	Denizli Burnt Scented Yogurt	Burdur Kökez Strained Yogurt
L	96,81±0,25	96,48±1,20	95,87±0,75
a*	-2,51±0,04	-2,81±0,01	-3,20±0,51
b*	3,12±0,03	2,98±0,42	2,66±0,32
HMF(µmol/L)	7,51±2,61	8,63±1,45	6,74±0,61