International Journal of Food Science and Nutrition
ISSN: 2455-4898; Impact Factor: RJIF 5.14
Received: 27-09-2020; Accepted: 12-10-2020; Published: 29-10-2020
www.foodsciencejournal.com
Volume 5; Issue 6; 2020; Page No. 30-35

Determination of carbohydrate amounts of various cheese types presented to sale in the market

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Abstract
The aim of this study is to determine the amounts of lactose, glucose, galactose, starch and total carbohydrates in cheese types presented to sale in the market. In the study, from different points of sale in Istanbul province 15 different types of cheese were investigated. Carbohydrate amounts of the cheeses examined were determined in two ways as theoretically (by difference method) and analytically (by HPLC). The amount of analytically determined carbohydrate of white cheese types was lower than the theoretically calculated amount ($P<0.05$). However, there was no statistically significant difference between the theoretical and analytically calculated carbohydrate amounts of kashar and other cheese types ($P>0.05$). In addition, it was determined that the amount of carbohydrates of ripened cheeses was lower than carbohydrate amounts in fresh cheeses. Consequently, analytical calculation of carbohydrate amount gives more accurate results. This should be taken into account when evaluating the macronutrient content of cheese.

Keywords: cheese, carbohydrate, lactose, galactose, glucose, HPLC

1. Introduction
Cheese has an important place in our daily diet because it contains high levels of protein and essential amino acids necessary for body and is rich in vitamins B2, B6 and B12 and D and minerals such as Ca, K and Cl [1-3].
Lactose is a naturally occurring disaccharide in milk, which is formed by the combination of glucose and galactose [4]. Lactase is an enzyme responsible for the task of breaking down lactose into glucose and galactose in the small intestine [5]. The absence or decreased production of lactase enzyme causes the lactose to not be metabolized. Ethnicity affects the frequency of lactose intolerance. It affects about 5-17% of the population in Northern Europe, North America and Australia-Asia, more than 50% of the population in South America, Africa and Asia, and about 90% of the population of Far East countries such as China and Japan [6]. The lactose content in dairy products can be reduced by both lactic acid fermentation and lactase breakdown of lactose [7]. However, some lactose may still remain in the structure of the product. Therefore, determining the amounts of lactose, glucose and galactose becomes important when determining the carbohydrate content of cheese. In addition, since starch can be added to provide consistency to cheese, the amount of starch should be determined [8].
Revealing the carbohydrate amounts of foods is important for determining the nutritional value. But carbohydrate amounts are usually calculated by the difference method. Analytical calculation can also be used as an alternative method for calculating the amount of carbohydrates. In the analytical calculation, the amounts of all the sugar components that can be found in the nutrient content are determined and collected. In this way, the amount of carbohydrates contained in the food can be accurately determined. In Turkey, there is no study in which the carbohydrate amount of cheese is calculated analytically.
In this context, the aim of the study is to determine the lactose, glucose, galactose, starch and total carbohydrate amounts found in the cheese types sold in the market. This study is planned considering the carbohydrate content of cheese, which has an important place in nutrition and preparation of nutrition programs, changes with the production method of cheese and the methods used in production.

2. Materials and methods
2.1. Materials
In this study, after determined in accordance with previous studies the most consumed variety of cheese in Turkey, 15 different types of cheese were bought from different sales points in Istanbul randomly selected. A total of 22 cheeses were analyzed by taking 1 of each fresh white cheese, cottage white cheese, ripened Ezine cow cheese, Kars old, Thracian and fresh kashar cheeses, Erzincan tulum cheese, curd, string, cecil cheese, braided cheese, labne, halloumi and village cheese, and 8 of the whole fat white cheese ripened at different durations.
Each of the cheese was bought as 500 g and brought to Istanbul Sabahattin Zaim University Halal Food Ar-Ge Center of Excellence. Two samples were taken from each cheese and the study conducted controlled.

2.2. Methods
Carbohydrate amounts of cheeses were determined in two ways: theoretical and analytical.

2.3. Determination of carbohydrate amount by difference method
Carbohydrate amounts were determined by determining the moisture, ash, protein and fat amounts of cheese and subtracting them from 100.

2.3.1.1. Determination of Moisture Amount
Samples were dried in the oven under a certain temperature and brought to constant weight. The amount of moisture was calculated from the resulting weight loss [9-12].

2.3.1.2. Determination of Ash Amount
The organic matter in the sample was burned in the ash oven at certain temperatures (550 ± 25ºC or 900 ± 10 ° C) and the residue obtained was weighed and the amount of ash was calculated [13].

2.3.1.3. Determination of Protein Amount
The amount of protein was determined according to the Kjehldahl method [14].

2.3.1.4. Determination of Fat Amount
The amount of fat was determined with the soxhlet device [15, 16].

2.4. Determination of carbohydrate amount by analytical method
The sugar components of the samples were determined by HPLC RI detector and the amount of carbohydrate was calculated analytically by summing the obtained amounts [17, 18].

2.4.1 Determination of Starch Amount
50 mg samples were dispersed in 6mL 2M KOH and shaken at room temperature for 30 minutes. To this suspension, 3 ml of 0.4M sodium acetate buffer (pH = 4.75) and 60 µl of amyloglucosidase were added and incubated at 60 ° C for 45 minutes in a controlled shaking water bath. Starch was measured as glucose with Peridochrom Glucose GOD-PAP. The result is multiplied by 0.9 to find the amount of starch [19]. Conversion factor from glucose to starch: 0.9.

2.4.2 Statistical Analyzes
Whether the data fit the normal distribution was determined by the Kolmogorov-Smirnov test. Quantitative data are given as mean ± standard deviation. Wilcoxon test was used to determine whether there is a difference between the amount of analytical carbohydrates and the amount of theoretically calculated carbohydrates. Whether there is a difference between the nutrient contents according to the maturation status was determined by Student t test and Mann Whitney U test. In the evaluation of statistical analysis, the level of significance was accepted as $p < 0.05$.

3. Results & Discussion
In this research, protein, fat, ash, moisture and theoretical and analytical carbohydrate amounts of 22 samples taken from 15 different cheese types taken from different sales points of Istanbul province were determined.

The amounts of protein, fat, moisture and ash in 100 g of cheese are given in Table 1. Accordingly, it was determined that the cheese with the highest amount of protein is the fresh kashar cheese and the lowest is the labne cheese. In addition, it was observed that the cheese with the highest fat content is cottage white cheese and the cheese with the lowest fat content is Erzincan tulum cheese.

Table 1: Protein, fat, moisture and ash amount in 100 g of cheese

Cheese Types	Protein Amount (g)	Fat Amount (g)	Moisture Amount (g)	Ash Amount (g)
Kashar Cheeses				
Fresh kashar cheese	27.0	26.0	41.9	4.0
Kars old kashar cheese	26.0	30.0	37.0	4.5
Thracian kashar cheese	22.8	25.6	46.5	3.1
White Cheeses				
Cottage White cheese	24.0	32.0	38.0	3.2
Whole fat ripened white cheese 4 (120 days)	21.3	22.6	49.8	4.4
Whole fat ripened white cheese 2 (90 days)	20.5	22.2	50.4	4.2
Whole fat ripened white cheese 7 (240 days)	20.3	25.2	47.8	4.8
Whole fat ripened white cheese 5 (180 days)	19.6	25.3	48.2	4.2
Whole fat ripened white cheese 8 (270 days)	19.6	27.3	46.3	3.8
Whole fat ripened white cheese 3 (90 days)	19.0	22.6	51.3	3.5
Whole fat ripened white cheese 6 (180 days)	18.7	28.6	47.9	3.1
Whole fat ripened white cheese 1(90 days)	18.6	20.0	51.1	4.5
Whole fat fresh white cheese	17.6	25.0	50.8	3.4
Other Cheeses				
Erzincan Tulum cheese	26.8	14.3	52.9	4.1
Whole fat ripened Ezine cow cheese	26.7	25.6	43.1	3.2
Braided cheese	25.7	24.0	37.8	10.2
String cheese	22.5	21.3	53.4	1.7
Village cheese	22.4	24.5	43.1	6.2
Cecil cheese	22.1	19.9	50.7	5.2
Halloumi	20.4	23.2	51.5	1.2
Curd	15.6	15.0	64.0	1.4
Labne	4.2	19.7	71.2	2.4

Theoretical carbohydrate amounts of cheese were calculated by the difference method. Analytical carbohydrate amounts of cheeses were obtained by detecting and collecting glucose, galactose, lactose, starch and other sugar components with HPLC-RI detector. In the study, the starch, fructose, sucrose, and maltose amounts of cheeses could not be determined because they were below detection limits.

Theoretical and analytical carbohydrate amounts of cheddar and other cheese types are shown in Table 2. In the analysis, it was determined that the cheese with the highest carbohydrate content was curd cheese and village cheese according to the theoretical calculation analytical calculation, respectively.

Table 2: Theoretical and Analytical Carbohydrate Amounts of Cashar and Other Cheeses

Cheese Types	Glucose Amount (g)	Galactose Amount (g)	Lactose Amount (g)	Analytical Carbohydrate Amount (g)	Theoretical Carbohydrate Amount (g)	p
Kashar cheeses						
Kars old kashar cheese	0.0	1.8	0.4	2.2	2.5	0.109
Thracian kashar cheese	0.15	1.65	0.0	1.8	2.0	
Fresh kashar chees	0.0	1.0	0.0	1.0	1.1	
Other cheeses						
Curd	0.03	0.54	2.7	3.27	4.0	0.079
Vilage cheese	0.0	3.76	0.0	3.76	3.8	
Halloumi	0.0	2.2	1.3	3.5	3.6	
Labne	0.4	0.4	1.6	2.4	2.5	
Braided cheese	0.0	2.2	0.0	2.2	2.3	
Cecil cheese	0.1	1.8	0.1	2.0	2.1	
Erzincan Tulum cheese	0.0	2.0	0.0	2.0	1.9	
Whole fat ripened Ezine cow cheese	0.0	1.4	0.0	1.4	1.4	
String cheese	0.02	0.95	0.17	1.1	1.1	

Theoretical and analytical carbohydrate amounts of white cheeses are given in Table3. In the analysis, it was determined that the white cheese with the highest carbohydrate content was 90 days ripened white cheese according to the theoretical calculation and full-fat fresh white cheese according to the analytical calculation.

Table 3: Theoretical and Analytical Carbohydrate Amounts of White Cheeses

White cheeses	Glucose amount (g)	Galactose amount (g)	Lactose amount (g)	Analytical carbohydrate amount (g)	Theoretical carbohydrate amount (g)	p
Whole fat ripened white cheese 1 (90 days)	0.0	1.8	0.0	1.8	5.8	0.005
Whole fat ripened white cheese 3 (90 days)	0.0	1.9	0.0	1.9	3.6	
Whole fat fresh white cheese	0.0	3.0	0.0	3.0	3.2	
Whole fat ripened white cheese 8 (270 days)	0.0	1.8	0.0	1.8	3.0	
Cottage white cheese	0.3	1.2	1.2	2.7	2.8	
Whole fat ripened white cheese 5 (180 days)	0.0	1.4	0.0	1.4	2.8	
Whole fat ripened white cheese 2 (90 days)	0.2	1.3	0.0	1.5	2.7	
Whole fat ripened white cheese 4 (120 days)	0.0	0.6	0.0	0.6	1.9	
Whole fat ripened white cheese 7 (240 days)	0.0	1.3	0.0	1.3	1.9	
Whole fat ripened white cheese 6 (180 days)	0.0	0.7	0.0	0,7	1,7	

This study was planned considering that the carbohydrate content of cheese, which has an important place in nutrition and preparation of nutrition programs, changes with the production method of cheese and the technological methods used in production.

In a study conducted by the Kafkas University it is noted that 193 varieties of cheese produced in Turkey [20]. However, it is reported that a large amount of production is made to meet local needs. Additionally, these local cheeses are not well known by other regions. The most important varieties of cheese produced in Turkey are white cheese, kashar cheese and tulum cheese. This is followed by mihalic, string, herbed, circassian, halloumi, meadow, curd and civil cheeses [21].

It is known that the amount of fat in cheese varies according to the production method, starter culture used in production, lipolysis and ripening duration [22, 23]. Lipolysis occurs by breaking the ester bonds between triglycerides and glycerol and fatty acids with lipolytic enzymes. This biochemical event is mainly caused by the activities of lipase and esterase enzymes [24].

In this study, it was determined that the amount of fat in cheeses ranged between 14.3 and 32.0 g and the average fat amount of cheeses was 23.6 ± 4.3 g. When the obtained data are compared with TURKOMP, USDA and FINELI, the results are similar. In addition, it has been determined that the fat content of full-fat fresh feta cheese, fresh kashar cheese and Kars old kashar cheese (respectively; 25.0; 26.0; 30.0 g) are similar to the fat content data obtained from TURKOMP (respectively 23.6; 26.1; 29.4 g) [25]. When the fat amount of whole fat white cheese is compared with the data in USDA, it was seen that the amount of fat obtained in the study (25.0 g) is higher than the amount of fat in USDA (17.9 g). When whole fat ripened white cheese is analyzed, it was observed that the fat amount of white cheese ripened for 180 days and above was higher than the data in USDA, while the fat amounts of white cheese ripened for 90 days and 120 days were similar to the data in USDA [26]. In addition, the determined fat amount (23.2 g) of halloumi cheese was found to be compatible with the fat amount data (22.1 g) obtained from FINELI [27]. Fat amounts of labne, curd and string cheese (19.7; 15.0; 21.3 g, respectively) were similar to data obtained from USDA (20.0; 15.3; 21.4 g, respectively) [26]. In addition, when the determined fat amounts were compared with the available label information, the results were found to be similar.

When the fat amount of cheese was examined according to the maturation status, it was determined that the fat amounts of the analyzed ripened cheese were higher than the fresh cheeses. Similarly to this study, in a study, it has been determined that the amount of fat increased of kashar cheeses during ripening [28].

When the protein amounts of the samples in the study were examined, it was seen that the cheese with the highest protein amount was 27.0 g with fresh kashar cheese, the cheese with the lowest protein amount was 4.22 g with

labne cheese and the average protein amount in the cheese examined was 21.0 ± 4.9 g. In addition, when the data obtained were compared with TURKOMP, FINELI and USDA, protein amounts were found to be similar. When the cheese types were examined, it was observed that the average protein amounts of the whole fat fresh white cheese, fresh kashar cheese and Kars old kashar cheese (17.6; 27.0; 26.0 g, respectively) were similar to the protein amount data in TURKOMP (16.0; 26.0; 27.0 g respectively) [25]. In addition, it has been observed that the amount of protein (17.6 g) of whole fat fresh white cheese is lower than the amount of protein (25.0 g) reported in USDA. In addition, protein amounts of whole fat ripened white cheese were found to be lower than the data in USDA [26]. When the halloumi cheese is examined, the average protein amount in the study was found to be 20.4 g, while the amount determined in FINELI was found to be 19.5 g and the results were similar [27]. When protein amounts of labne, curd and tongue cheese were examined, it was found that the data obtained in the study (4.2; 15.6; 22.5g, respectively) were similar to the data obtained from USDA (respectively; 3.3; 14.1; 21.4 g) [26].

It is known that the protein amount of cheeses is changed by proteolysis which continues during ripening [29]. In a study on Cheddar cheeses, it was determined that there was an increase in the amount of protein during the ripening period of cheeses. Likewise, in a study, it was seen that protein amount of kashar cheese increased during ripening [28, 30]. In this study, when the protein amounts of the samples were compared according to the ripening condition, it was determined that the protein amount of the ripened cheeses (21.6 ± 2.9 g) was higher than the protein amount in the fresh cheeses (19.8 ± 7.4 g).

When the moisture content of the cheese was examined, it was determined that the lowest moisture content was 37.0 g with Kars old cheese, the highest humidity was 71.2 g with labne cheese, and the average moisture content of the analyzed cheeses was 48.9 ± 7.9 g. When the obtained data were compared with the food composition databases, the results were similar. Accordingly, the moisture content of whole fat fresh white cheese, fresh kashar and Kars old kashar cheeses (50.8; 41.9; 37.0 g, respectively) in the study are similar to the data in TÜRKOMP (48.9; 40.7; 37.0 g, respectively) [25]. When the moisture content of cheeses was examined according to the ripening condition, it was found that the moisture content of fresh cheeses (51.0 ± 12.0 g) was higher than the moisture content of ripened cheeses (47.6 ± 4.3 g). When the ash content of the cheese was examined, it was found that the lowest ash amount was 1.2 g with halloumi cheese, the highest ash amount was 10.2 g with braided cheese and the average ash amount of the analyzed cheeses was 3.9 ± 1.9 g. When the obtained data were compared with the food composition databases, the results were similar. Accordingly, the average ash amounts of the whole fat fresh feta cheese, fresh cheddar and Kars old cheddar cheese (3.4; 4.0; 4.5 g, respectively) found in the study are similar to the ash content data in TÜRKOMP (3,3; 3,7; 4,4 g, respectively) [25].

When the ash amounts of cheeses were examined according to the ripening condition, it was found that the ash amount of fresh cheeses (3.9 ± 2.8 g) was similar to the ash amount of ripened cheeses (3.9 ± 1.1 g).

When the theoretical carbohydrate amounts of the samples in the study were examined, it was determined that the cheese with the highest carbohydrate amount was 5.8 g, whole fat ripened white cheese, and the cheese with the lowest carbohydrate amount was fresh kashar and string cheese with 1.1 g. In addition, the average amount of carbohydrates in the cheese examined was found to be 2.6 ± 1.1 g. When the carbohydrate amounts of whole fat white cheese, fresh kashar cheese and Kars old kashar cheese calculated by the difference method were examined, the determined carbohydrate amount (3.2 g) of the whole fat white cheese is lower than the carbohydrate amount (8.2 g) in TURKOMP. The determined carbohydrate amounts (1.1 g, 2.5 g, respectively) of fresh kashar cheese and Kars old kashar cheese are compatible with the data in TURKOMP (2.6 g, 3.1 g, respectively) [25]. In addition, the amount of carbohydrates (3.6 g) determined by the difference method of Halloumi cheese was found to be similar to the data (2.5 g) examined in FINELI [27]. While the amount of carbohydrates (4.0; 2.5, 3.2 g, respectively) determined by the difference method of curd, labne and whole fat fresh white cheese were coherent with the data in USDA (3.6; 3.3, 3, 3, respectively), the amount of carbohydrate (1.1 g) determined by the difference method in the study of string cheese was lower than the amount of carbohydrate (3.6 g) in USDA. When whole fat ripened cheeses were examined, it was found that the theoretically calculated carbohydrate amount of white cheeses ripened for 90 days and 270 days was higher than the data in USDA, while the theoretical carbohydrate amounts of 120, 180 and 240 days ripened cheeses were similar to the data in USDA [26].

When the theoretical carbohydrate amounts of the samples were compared according to the ripening condition, it was determined that the amount of carbohydrates (2,8 ± 1,2 g) of the ripened cheeses was higher than the carbohydrate amount (2,4 ± 1,0 g) in the fresh cheeses. However, during the ripening period of cheeses, lactic acid bacteria convert lactose to glucose and galactose, use them as an energy source, and as the cheese ripenes, the amount of carbohydrates in its content decreases [7, 31]. Thus, the amount of carbohydrates of ripened cheeses is expected to be lower than the amount of carbohydrates in fresh cheeses.

Analytical carbohydrate amounts of cheeses were obtained by determining and addition amounts of glucose, galactose, lactose, starch and other sugar components.

In a study examining the glucose and galactose amounts during storage of fresh cream cheeses, it was observed that the amount of glucose decreased significantly during storage and the amount of galactose increased significantly ($P<0,05$) [32].

In a study examining the amount of glucose, galactose and lactose in hard lactose-free cheeses, it was determined that the average amount of galactose (1.36 ± 0.89 mg / 100 g) was higher than the average lactose (0.45 ± 0.12mg / 100g) and glucose (0.46 ± 0.13 mg / 100 g) amounts [33]. In a study examining the galactose content of Cheddar cheeses that are half ripe and fully ripe, it has been determined that the amount of galactose (<10 mg / 100) of semi-ripened Cheddar cheeses is lower than the amount of galactose (95.5 mg / 100 g) of fully ripened Cheddar cheeses [34].

The main bacteria used as starter culture in cheese production are Lactobacillus helveticus, Lactococus lactis and Streptococcus thermophilus. While Lactococcus lactis is a mesophilic bacteria, Lactobacillus helveticus and Streptococcus thermophilus show thermophilic properties. Researchs show that mesophilic bacteria can convert both

glucose and galactose into lactic acid and use them as an energy source. However, some thermophilic bacteria can only use glucose in lactic acid fermentation [35]. In addition, as the ripening time increases, the amount of lactose decreases or is completely depleted, as these bacteria show more activity.

In this study, when the glucose, galactose and lactose amounts of the samples were examined, it was determined that the amount of galactose was higher in fresh cheeses compared to fresh cheeses, while the amount of glucose and lactose was lower in ripened cheeses than fresh cheeses. In addition, when the glucose, galactose and lactose amounts of the cheese examined were compared, it was found that the amount of galactose was higher in fresh or ripened cheeses compared to the lactose and glucose amount. In this study, the higher amount of galactose may be due to the more frequent use of thermophilic bacteria in cheese production.

When the analytical carbohydrate amounts of the samples were examined, it was determined that the cheese with the highest carbohydrate amount was 3.76 g with cottage cheese and the cheese with the lowest carbohydrate amount was 0.6 g with full-fat ripe cheese (120 days). In addition, the average amount of carbohydrates in the cheese examined was found to be 2.0 ± 0.9 g. In addition, when the glucose, galactose and lactose amount of halloumi cheese was examined, it was seen that the amounts obtained in the study (0.4; 0.4; 1.6g) were higher than the data in FINELI (0.0; 0.0; 1.2 g) [27].

When the analytical carbohydrate amounts of the samples were compared according to the ripening status, it was determined that the amount of carbohydrate (1.8 ± 0.9 g) of ripened cheeses was lower than the carbohydrate amount (2.2 ± 0.8 g) in fresh cheeses. Knowing that the amount of carbohydrates decreases during the ripening of the cheese supports the results obtained from the study. Besides, there was no statistically significant difference between the theoretically and analytically calculated carbohydrate amounts of cheeses. However, theoretically calculated carbohydrate amounts were generally higher than analytically calculated carbohydrate amounts.

This study has some limitations. First, the nutritional elements of many cheese varieties are not included in the food composition databases. Therefore, it was not possible to compare the nutrient content of most cheese types examined in the study with the same kind of cheese. In addition, the limited number of cheese samples in the study and the presence of approximately 200 kinds of cheese in our country are seen as the limitations of this study. In addition, despite the presence of approximately 200 kinds of cheese in our country, to use the limited number of cheese samples, are seen as the limitations of this study.

Using the HPLC device to analyze the carbohydrate amount analytically is one of the recommended methods. In addition, it is known that the RI detector can detect even small amounts of sugar components. In this study, using HPLC-RI detector is the strength of the study. In addition, theoretical and analytical measurement of the amount of carbohydrates in cheeses helped determine the difference between the two methods. This situation reflects the strength of the study.

4. Conclusions

Statistically significant difference was found between the difference method and analytical calculation in evaluating the amount of carbohydrates in white cheeses. When the cheeses were examined, it was seen that the amount of carbohydrates calculated by the difference method was higher according to analytical method. There is no study on determination of carbohydrate amounts by analytical methods in Turkey. Only the results obtained by the difference method are included in the food composition database. The results from the two analyzes show that after validation studies, the determination analytically of carbohydrate amounts in cheeses and the inclusion of results in the food composition database provide a more accurate assessment.

5. Acknowledgments

The work was supported by Marmara University Scientific Research Projects Committee (Project Number: SAG-C-YLP-200318-0102).

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