PRIVATNA USTANOVA ŠKOLSKI CENTAR MAARIF SCHOOLS OF SARAJEVO (ŠKOLE „MAARIF“ SARAJEVO)

MATURSKI RAD IZ HEMIJE NA TEMU:

Quantitative determination of lactose by polarimetric method in samples of commercially available milk

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1. Introduction

This paper describes the method of quantitative determination of lactose concentration in a milk sample.

As a child, I discovered that I was lactose intolerant, and since then I have always paid attention to the composition, and primarily to the data on the lactose content, when buying dairy products.

That's why I was wondering if there was a method to check the lactose content of milk.

Analyzing the literature, I concluded that the polarimetric method could be successful with a high degree of sensitivity with a simple apparatus and ease of performing the experiment.

Lactose belongs to the group of carbohydrates, more precisely to the group of disaccharides, or sugars. In the human body, the enzyme lactase breaks down lactose into monomeric units. The polarimetric determination of the lactose content is based on the optical activity of lactose.

This paper describes an experimental approach to determining the concentration of lactose in milk using a polarimeter. The obtained results were analyzed and shown on the graph, and the resulting concentration of the unknown sample was calculated and compared with the concentration on the packaging.

Hypothesis: It is possible to accurately determine the concentration of lactose in milk using the polarimetry method with simpler equipment and a simple method of sample preparation.

Research question: The angle of rotation of the polarized light is directly related to the concentration of lactose. It can be used to measure the percentage of lactose in milk samples.

2. Chemical context

2.1 Carbohydrates

Carbohydrates are biological molecules made of carbon, hydrogen, and oxygen in a ratio of approximately one carbon atom (C) to one water molecule (H2O). "This composition gives carbohydrates their name: they consist of carbon (carbo-) plus water (-hydrates). Carbohydrate chains come in different lengths, and biologically important carbohydrates belong to three categories: monosaccharides, disaccharides, and polysaccharides“. (Khan academy n.d.)

2.1.1 Monosaccharides

„The simplest form of carbohydrate is a monosaccharide. "Mono" means "one" and "saccharide" means "sugar." Monosaccharides are a polyhydroxy aldehyde or ketone that cannot be further hydrolyzed to a simpler sugar. They can again be classified based on the nature of the carbonyl group“. (Khan acedemy n.d.)

Polyhydroxy aldehydes are called aldoses, and polyhydroxy ketones are called ketoses

Image (1).

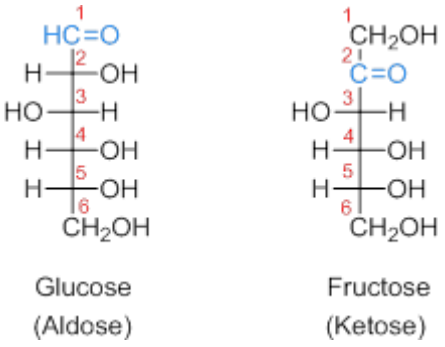


Image (1). Aldoses and ketoses (webstor.srmist.edu)

2.1.2 Glycosides

Glycosides are a cyclic acetal form of sugar, and the bond between the anomeric carbon and the alkoxy oxygen is called a glycosidic bond. They are prepared by an acid-catalyzed reaction of alcohol with pyranose or furanose. Glycosides are named by replacing "e" sugar with "ide". For example, glucose glycoside is a glucoside and if the name pyranose or furanose is used, the acetal is called pyranoside or furanoside. Both α- and β-glycoside as shown in

Figure (2). obtained by the reaction of one anomer with alcohol. (webstor.srmist.edu, Carbohydrates I n.d.)

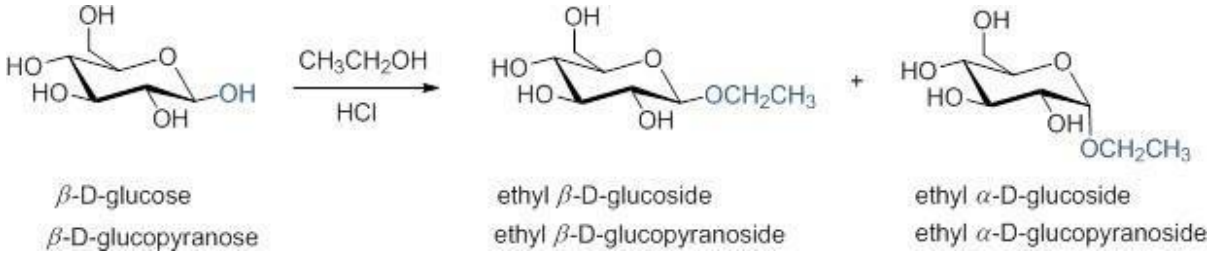


Image 2. α - and β -glycoside reaction with an alcohol (webstor.srmist.edu)

2.1.3 Mutarotation

Normally D-(+)-glucose has a melting point of 146°C. However, when D-(+)-glucose crystallizes by evaporation of an aqueous solution maintained above 98°C, the second form D-(+)-glucose with a melting point of 150°C can be obtained. When the optical rotations of these two forms were measured, it was found that they differ significantly, but when it is water of any shape is left standing, its rotation changes. A specific rotation of one form decreases and the other increases, until both solutions show the same value. For for example, the specific rotation of a solution of α-D-(+)-glucose (m.p. 146°C) gradually decreases from the initial value of +112.2° to +52.7°, while β-D-(+)-glucose (b.p. 150°C) the specific rotation gradually increases from an initial value of +18.7° to +52.7°. Three forms of glucose reach equilibrium concentrations with a specific rotation of +52.7. This a change ("mutation") in a specific rotation toward equilibrium is called a mutarotation (webstor.srmist.edu, Carbohydrates I n.d.)

2.1.4 Disaccharide

If the glycoside or acetol is formed by reaction of the anomeric carbon of a monosaccharide with OH group of another monosaccharide molecule as is wisible in Image (3), then the glycoside product is a disaccharide.

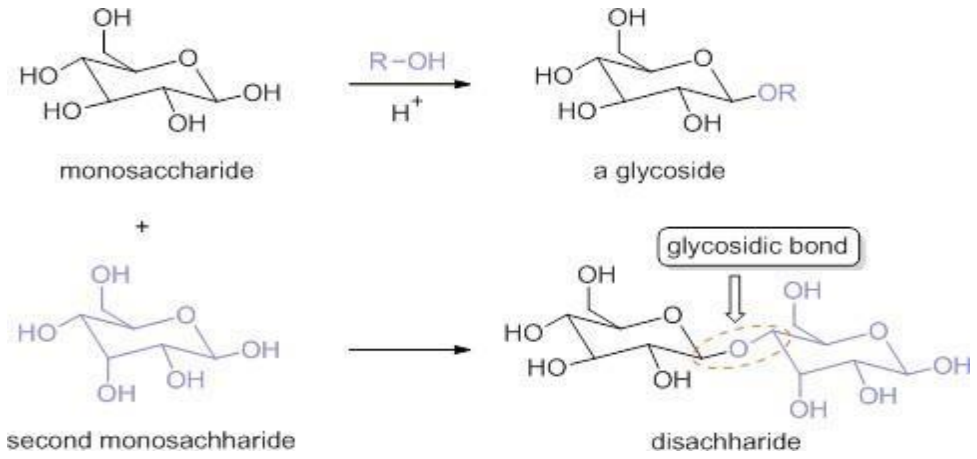


Image 3. Formation of disaccharide (webstor.srmist.edu)

The anomeric carbon can react with any of the hydroxyl groups of another monosaccharide unit to form a disaccharide. Disaccharides can be categorized by the position of the hydroxyl group of another monosaccharide making up the glycoside. (webstor.srmist.edu, Carbohydrates I n.d.)

Disaccharides have three naturally occurring glycosidic linkages:

1-4 link in witch the anomeric carbon is bonded to oxygen on C-4 of second monosaccharide.

1-6 link in witch the anomeric carbon is bonded to oxygen on C-6 of second monosaccharide.

1-2 link: in witch the anomeric carbons of the two monosaccharide unit are bonded through an oxygen.

Prim" superscript indicates that the –OH group is attached to the carbon position of the second monosaccharide unit, α - and β -configuration given by based on the configuration on the anomeric carbon of the first monosaccharide unit. (webstor.srmist.edu, Carbohydrates I n.d.)

1-4' Glycosides : These represent the most common naturally occurring disaccharides.

The bond is between C-1 of one sugar subunit and C-4 of the other. For example, maltose is a disaccharide with two D-glucose units bearing a 1,4'-glycosidic linkage. The stereochemistry of this bond is α. So the glycosidic bond is called α -1,4'-glycosidic bond as shown in image (4). (webstor.srmist.edu, Carbohydrates I n.d.)

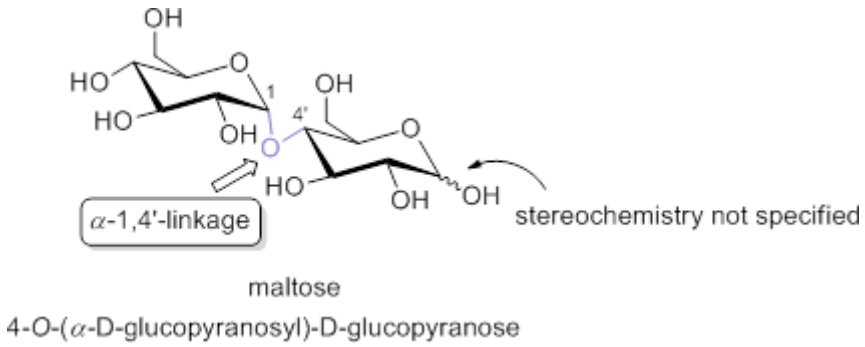


Image 4. α -1,4'-glycosidic linkage (webstor.srmist.edu)

Lactose, a disaccharide present in milk, contains D-galactose (non-reducing) and D-glucose (reducing) monosaccharide units. These units are linked by a β -1,4'-glycosidic bond, as shown in image 5. (webstor.srmist.edu, Carbohydrates I n.d.)

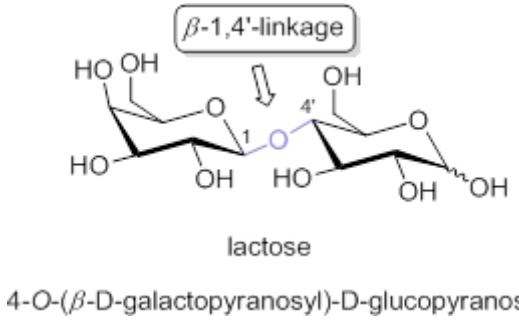


Image 5. β -1,4'-glycosidic linkage (webstor.srmist.edu)

2.1.5 Polysaccharides

„Polysaccharides are carbohydrates that contain many monosaccharide units linked together by glycosidic bonds. All the anomeric carbon atoms of the polysaccharide are included in the acetal forming. Thus, polysaccharides do not react with Tollen's reagent and do not mutarotate.

Polysaccharides that are polymers of one monosaccharide are called homopolysaccharides. If they are made by more than one type of monosaccharide, they are called heteropolysaccharides. For example, glucan is made from glucose and galactan units, which is made of galactose units. There are three important polysaccharides which are starch, glycogen and cellulose“.(C. M. Loudon 2002).

2.2 Sugar in milk

„Lactose, the characteristic carbohydrate in milk, is metabolized by all mammals through enzymatic reactions to form glucose and galactose, which the body needs for energy. Milk sugar, also commonly called lactose, is present in mammalian milk in varying amounts, from about 4.8% in cow's milk to approximately 6.3% in sheep's milk. Human milk contains 7.0% lactose. Also, the lactose content in dairy products varies depending on processing. In concentrated and dried products, for example, it increases in proportion to dry matter, while it is lower in fermented products. Knowing this is an essential part of dairy production, especially since dry lactose powder is often used (eg in the production of cheese, yogurt, chocolate). Sometimes, however, this knowledge is not enough and additional information is needed“. (Anton-Paar n.d.)

Table 1 gives an overview on examples of lactose monohydrate and anhydrous lactose content in cow’s milk as well as in some processed products. (Anton-Paar n.d.)

Table 1. Lactose monohydrate and anhydrous lactose content (Anton-Paar n.d.)

Product	Anhydrous lactose [%]	Lactose monohydrate [%]
Raw milk	4.5	4.7
Lactose-reduced milk	0.27 - 0.84	0.32 – 0.95
Skimmed fresh milk	4.6	4.8
Condensed milk	7.6 – 11.9	8.0 – 12.5
Low-fat milk powder	49	51.5
Whole milk powder	36.1	38
Whey powder 4.4 % H_2O	69	72.8
Farmer's cheese	0.3 – 3.8	0.3 – 4.0

2.2.1 What is Lactose and Lactose Intolerance

Lactose is a sugar found in some dairy products. Lactase is an enzyme in the intestines that is needed to break down lactose. Many adults have trouble digesting lactose-containing foods because their lactase levels decrease after childhood, which is normal. Lactose intolerance is NOT a food allergy. People with intestinal diseases or injuries can also become lactose intolerant, even if they weren't before. The amount of lactose you can eat varies from person to person. Many people with lactose intolerance can eat some foods with lactose by changing

the type, amount and timing of these foods. Other people may need or choose to avoid these foods altogether. (Am Fam Physician 2002)

People with lactose intolerance cannot digest significant amounts of lactose due to a genetically inadequate amount of the lactase enzyme. Treatment primarily consists of avoiding foods containing lactose. Lactase enzyme supplements may be helpful. The degree of lactose malabsorption varies greatly among lactose intolerant patients, but most can consume up to 12 oz of milk per day without symptoms. Patients who cannot tolerate lactose must ensure an adequate intake of calcium. (Am Fam Physician 2002)

2.2.2 Lactose properties

In milk or milk products, lactose exists in two isomeric forms, called α- and β-lactose. The molecular structures of α- and β-lactose differ in the orientation of the hydrogen and hydroxyl groups on carbon atom no. 1 in the remaining glucose. A lactose solution at equilibrium has an optical rotation of 55.7°. The distribution of anhydrous α and β-lactose is about 37.3% anhydrous α-lactose and 62.7% β-lactose. (Anton-Paar n.d.)

As shown from the structure, the bond is a 1,4 glycosidic bond in beta orientation as is wisible in Image 6. (webstor.srmist.edu, Carbohydrates I n.d.)

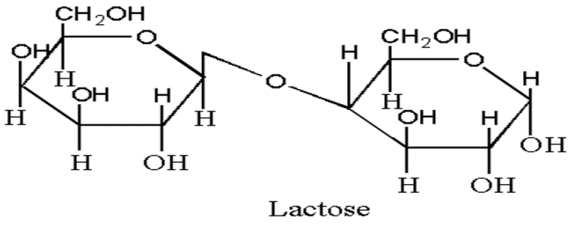


Image 6. 1,4 glycosidic bond in beta orientation (webstor.srmist.edu)

2.2.2.1 Properties of structure

Some of the chemical properties of lactose are:

- The chemical formula of lactose is $C_{12}H_{22}O_{11}$.
- Lactose has a crystalline structure.
- Lactose has free anomeric carbon, therefore, lactose shows reducing properties.
- When lactose is absorbed in the small intestine, the enzyme lactase breaks it into glucose and galactose.

- Lactose is less sweet as compared to sucrose.
- Lactose has 4kcal/g energy.
- The molar mass of lactose is 342.3g/mol.
- It is water as well as alcohol soluble.
- Its melting point is 202.80 C. (Themasterchemistry n.d.)

2.2.3 Lactose in powder

Most common way of obtaining lactose in solid form is in proces of crystallization from solution. When crystallization is carried out at temperatures below 93.5 °C, only α-lactose monohydrate is obtained. α-lactose has the specific characteristic that in the crystalline state each lactose molecule is connected to one molecule of water. And so α-lactose crystallizes as monohydrate. (Anton-Paar n.d.)

2.2.4 Lactose in liquids

α-lactose and β-lactose are present in the liquids, which are constantly changing into each other. This phenomenon is called mutarotation. The rate of mutarotation is determined by factors such as temperature, concentration and pH (acidity) of the solution. Lactose solutions tend to a state of equilibrium between the α and β forms. At room temperature, equilibrium results in a ratio of about 40% α-lactose to 60% β-lactose. Therefore, the measured optical rotation and the resulting specific rotation (SR) change over time until a steady state of equilibrium is reached. (Anton-Paar n.d.)

3. Methodology

Lactose detection is very important because of lactose intolerance disease. Small amounts are present in many foods – not just milk and milk products – and therefore traces of lactose can be present in foods under the following headings: milk solids, whey, curd, skimmed milk powder and skimmed milk, which means lactose is present and must be indicated on the food label. Intolerance to lactose is a significant factor in the choice of diet for people sensitive or intolerant to this sugar, so its content in foods must be monitored in order to avoid disorders

and diseases. Therefore, it is important to accurately and precisely quantify the lactose in these products. The chosen method should be economical, fast and sensitive. (EFSA NDA Panel 2010)

3.1. Determination of lactose content

Knowing the exact amount of ingredients in a product improves the quality and safety of food for both consumers and producers. As the content and ingredients must be clearly stated on the product label, it is important for dairy product manufacturers to accurately determine the lactose content of the raw materials they use. In the EU, food manufacturers are required by law to declare all ingredients on the product label, clearly listed in descending order depending on quantity. This includes lactose whenever it is knowingly used in food processing and production. The same applies if lactose is used exclusively as a carrier, for example for flavors or spice mixes. As a consequence, the determination of lactose content is very important in the control of incoming goods, during the production process and the final product in order to ensure the quality and safety of food and to comply with legal requirements. (Anton-Paar n.d.)

3.1.1 Determination of Lactose Content of Milk by Polarimetry

Polarimetric determination of lactose content is based on the optical activity of lactose. Fats and proteins from milk and milk powder, basically one component that causes a white or cloudy color, must be removed before starting the measurement. Volume of precipitated proteins and fats is considered a correction factor in some methods.

Determination of lactose content of milk by polarimetry measuring the specific rotation by [α] of lactose in solution at equilibrium is +52.3° . The specific rotation is defined as the optical rotation of a solution containing 1 g/ml in a 100 mm polarimeter tube, it is affected by temperature (20°C reference temperature) and wavelength (usually the sodium D line , 589 nm) is used:

$$[\alpha]_D^{20} = \frac{\alpha}{c \cdot d}$$

where α is the measured optical rotation; d the light path in dm and c the concentration as

g/ml. (Topac n.d.)

One of the simplest methods for determining the concentration of lactose in milk is polarimetry. Normal milk contains 45-50 g of lactose per liter. For the method to be successful, all optically active compounds other than lactose (eg proteins) must be removed. The solution must be transparent enough for polarized light to pass through easily. Setting the polarimeter at the correct angle for the optical rotation value is difficult and must be done carefully to avoid erroneous values. (Topac n.d.)

3.1.2 Principle and polarimeter

A polarimeter is used to determine optically active substances in solutions. A polarimeter consists of a pair of polarizers placed in line, one of which can be rotated relative to the other, and the angle of rotation can be measured on a scale. When both polarizers cross, meaning their polarization planes are perpendicular to each other, no light passes through the instrument. As soon as an observation tube filled with an optically active liquid, such as a sugar solution, is inserted between the polarizers, the light transmission returns to a certain level. By rotating one of the polarizers relative to the other, light extinction can be achieved again. The angle of this rotation is a measure of the optical activity of the solution in the observation tube. To facilitate handling, the optical system is placed at an angle of 20º.. (FoodChemistry Lab. Milk n.d.)

A detailed picture with an explanation of all parts is given below according to Instruction manual of Polarimeter model PL 1. Image 7. (FoodChemistry Lab. Milk n.d.)

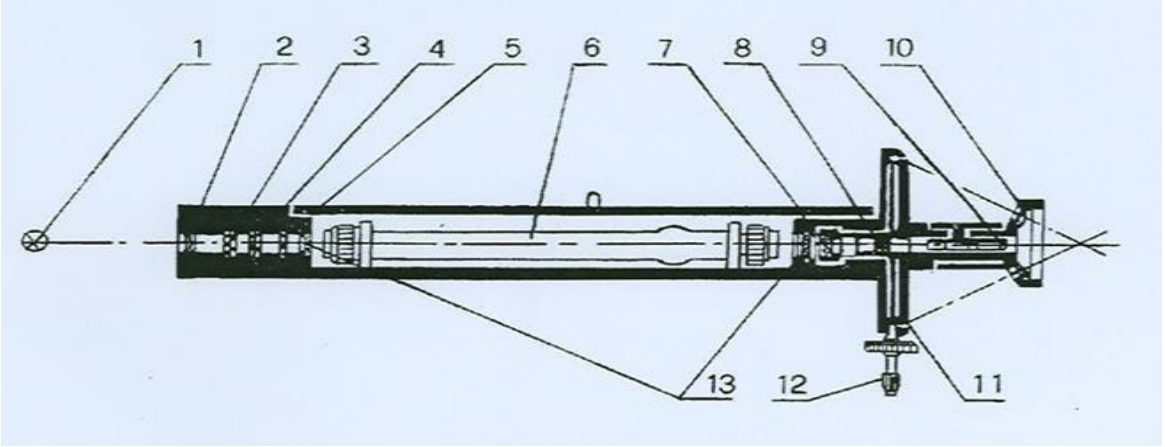


Image 7. Polarimeter model PL 1 (FoodChemistry Lab. Milk n.d.)

1. Light source (sodium light)
2. Collector lens
3. Colour filter
4. Polarizer
5. Half-wave plate
6. Test tube
7. Polarization analyzer
8. Object lens
9. Eye lens
10. Magnifying glass
11. Dial vernier
12. Dial rotary hand-wheel
13. Protective plate

3.1.3 Equipment and Chemicals

Analyzing the literature according to the available information, we decided to use chemicals and equipment as in the established literature.

- Polarimeter - model PL 1
- volumetric flasks – 100 ml
- pipettes – 5 ml
- beakers – 100ml
- funnels
- filter paper
- potassium ferrocyanide $K_4[Fe(CN)_6]*3H_2O$ solution (c = 0,150 g/ml)
- zinc sulphate $ZnSO_4*7\ H_2O$ solution (c = 0,3 g/ml^{-1})

3.1.4 Procedure of sample preparation

We had three parallel samples for analysis. Through literature analysis, for optimal results of the experiment, it was necessary to prepare the sample gradually. The procedure itself consisted of several steps listed below.

1. It was weighed accurately 50 g of milk into a 100 ml volumetric flask,
2. then was added 5 ml of $K_4[Fe(CN)_6].3H_2O$ and shake well,
3. then 5 ml of $ZnSO_4.7\ H_2O$ solution was added and shake well,
4. water was added up to the 100 ml mark,
5. then mixed again and allow the flask to stand at room temperature for 20 min,
6. then the content and filter was mixed through funnel with a fluted filter paper and collect the clear filtrate containing the lactose to be estimated,
7. then the measurement was started of the optical activity of this solution (specific rotation – α) and calculate the lactose concentration of this solution.

3.1.5 Measuring the sample by polarimeter

A picture with explanation and detailed instructions for the handling and measurement procedure with the given polarimeter is given below according to Instruction manual Polarimeter model PL 1 (FoodChemistry Lab. Milk n.d). The procedure itself consisted of two main steps given below and described in image 8.

1. Open the sample compartment
2. Filling the tube:
    - unscrew the cap near to the annular enlargement of the observation tube,
    - take away the inner cap, the glass window and the gasket,
    - fill the tube with the sample to be measured, keeping the tube well upright and maintaining it by the metal ring, to avoid any warming –up of the glass tube and the sample,

- -fill the tube until the meniscus formed by the liquid of the sample stands on the top of the glass tube,
    - slide the glass window over the top of the glass tube pushing the liquid forming the meniscus away,
- -re-install the rubber gasket in the inner cap and push them together over the glass window
    - screw the outer cap on,
    - turn the complete observation tube into horizontal position.
    - capture the eventual air bubbles into the annular enlargement before introducing the tube into the polarimeter's sample compartment.

- Rotate the scale control wheel until a uniformly illuminated field is obtained described in image 9.

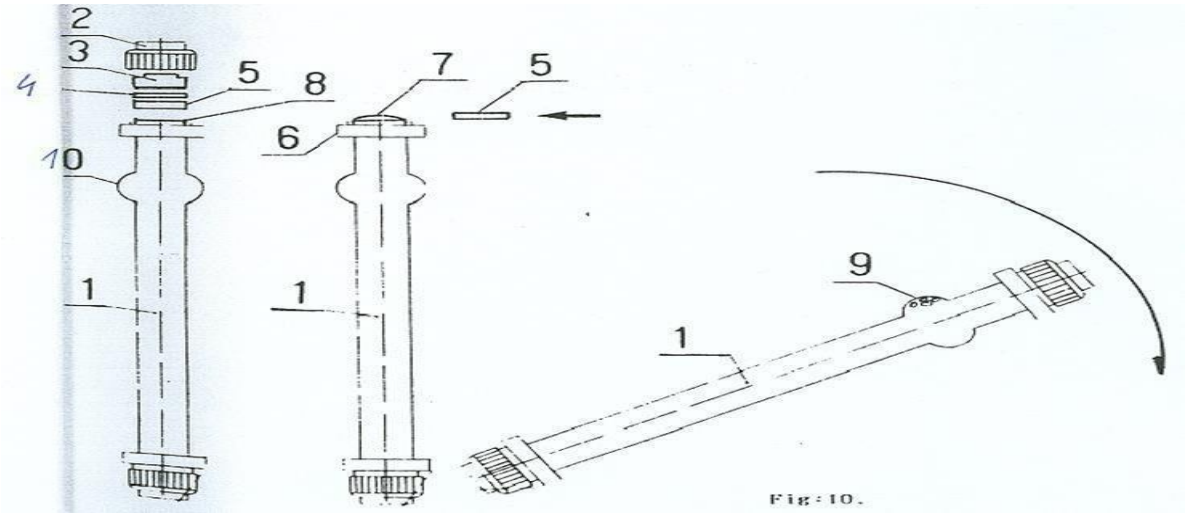


Image 8. Procedure of filling and measuring with polarimeter

1. observation tube
2. cap
3. inner cap
4. gasket
5. glass window
6. annular enlargement
7. meniscus
8. glass tube
9. bubbles
10. annular enlargement

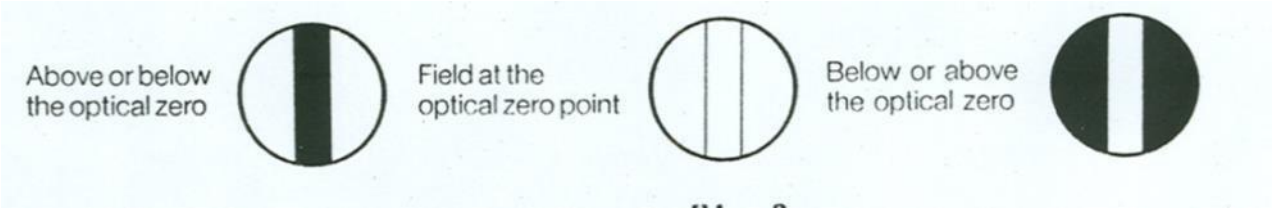


Image 9. Uniformly illuminated field

4. Results and analysis

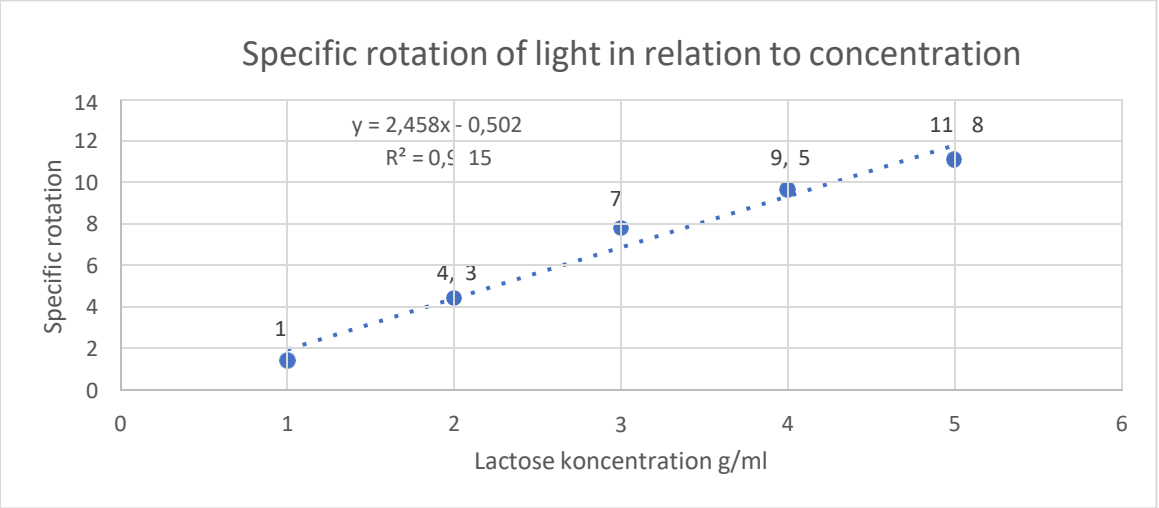
The measurement results are presented in Table 2.

α (H2O)	α (0,01 g/ml) lactose	α (0,03g /ml) lactose	α (0,05g /ml) lactose	α (0,07g/ ml) lactose	α (0,09g /ml) lactose	α (sampl e 1)	α (sampl e 2)	α (sampl e 3)
-2,86	0,12	1,35	3,90	6,80	9,98	3,75	3,73	3,72
-2,90	0,13	1,30	3,89	6,80	9,97	3,22	3,77	3,78
-3,00	0,16	1,30	3,90	6,70	9,97	3,73	3,70	3,78
-2,92								
Average	0,14	1,33	3,90	6,76	9,97	3,73	3,73	3,76

The table also shows the values of the turning angle for pure water, which had to be measured in order to calibrate the device and reduce possible measurement errors. Specific rotation is defined as the optical rotation of a solution containing 1 g/ml in a 100 mm polar tube as affected by temperature (20°C reference temperature) and wavelength (typically sodium D line, 589 nm).

$$[\alpha]_D^{20} = \frac{\alpha}{c \cdot d}$$

In the table we see the data for the optical activity values of different concentrations of pre-prepared lactose solutions of known concentration. For the sake of accuracy and error zeroing, all the procedures listed for the samples were performed on all concentrations according to the same steps, and three values were read for each of the samples, after which the average value was calculated and used to draw the graph 1.



Graph 1. Average value of optical activity values of known concentrations of lactose

Using the above formula, we calculated the angle of rotation for known concentrations. After that, by knowing the angle of unknown samples (sample 1, sample 2 and sample 3), we can determine the concentration of lactose in milk.

Applying the measurement results and using the formula, we came to the data that the measured lactose concentration is 0.0311g/ml. On the milk packaging that we analyzed, you can find the information that the lactose content in 1 liter of milk is 3,4%, which if compared to our result represents a deviation of 0.003g/ml, in percentagei of 8.82% of the total mass of lactose.

Calculation:

$[\alpha] = \alpha/c*d$

$a= \alpha/ [\alpha]*d$

$a= 3,74/52,3 * 0,01$

$a= 3,74/ 0,523$

$a= 7,151$

a – ange of rotation
[α] = 52,3
d = 10cm
α – value from polarimetre

If we include the obtained results in the equation from garficon, we get that the final measured concentration of lactose is listed below from the equation:

$y = 2,458x – 0,502$

$x= 7,151 + 0,502/2,458$

$x = 3,11\%$

The data from the milk packaging that we analyzed for lactose concentration shows a result of mass fraction of 3,4%. If we compare that with our values of 3,11% obtained by the polarimetric method of analysis we can come to the conclusion that the deviation from the total amount of lactose is 8.52%.

The method is less sensitive than expected, and with a measurement error of more than 8% there is room for improvement.

5. Conclusion

Through this research, I came to the conclusion that it is possible to determine the lactose content in milk by the polarimetric method in a simple way, which confirmed the hypothesis set at the beginning. However, despite its simplicity, the method is not sensitive enough to be used for analytical purposes.

The relatively large deviation of 8.52% of the measured concentration from the concentration of lactose on the packaging leads to the conclusion that the method is less sensitive than other methods of analysis.

However, the polarimetric method of lactose determination can be used when it is necessary to quickly and simply determine the lactose content in the food without the use of expensive and complicated equipment.

One of the disadvantages of the method is that the measurement of the rotation angle should be performed at a certain temperature. in our case at 20 degrees Celsius. Because the co-concentration of lactose changes with the temperature, as well as the angle of rotation of the light itself.

The biggest challenge was to keep the temperature constant at 20 degrees Celsius during the measurements. If a closed system like a calorimeter were used, I think the error would be much smaller.

6. References

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KOMENTAR

Datum predaje rada:_____
Datum izlaganja rada:_____

Ocjena:_____

Članovi komisije: 1._____
2._____
3._____

Tabela konsultacija mentora sa kandidatom

Predmet:
Mentor:
Učenik/kandidat:
Tema maturskog rada:

Broj konsultacija	Datum konsultacija	Prisustvo učenika (prisutan/odsutan)
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Učenik je prisustvovao		
Potpis mentora		