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RESEARCH ARTICLE OPEN ACCESS

Investigation of the Production and Spectroscopic Determination of Lactulose from Whey, and Exploration of Potential Applications in Cheese

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ABSTRACT
Recently, there has been a growing focus on the practical application of lactulose in both the food and pharmaceutical sectors. Lactulose is a prebiotic that possesses significant functional importance. It is mostly utilized by probiotic bacteria, such as bifidobacteria, which are advantageous for metabolism, since it crosses from the small intestine to the large intestine without any interruption, and promotes the growth of these bacteria. Lactulose can be derived from whey, a significant reservoir of lactose. Whey is a highly significant by-product of milk technology. During the process of cheese production, approximately 85% of every 100 kilograms of milk is transformed into whey. From an economic standpoint, whey is a very valuable by-product that should be efficiently utilized to avoid wastage.
This study, conducted in collaboration with Muratbey Dairy Co., involved the production of lactulose using chemical isomerization synthesis using whey. The amount of lactose and lactulose produced was determined using spectrophotometry. Muratbey Dairy Co. has included lactulose into their spreadable cheese products.
Keywords: Cheese, Lactulose, Spectroscopy, Whey.

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I. Introduction

Lactulose (4-O-β-D-galactopyranosyl-D-fructose) is a disaccharide derivative of galactosine, generated by the binding of fructose β (1-4) to glycoside. It does not occur naturally in the environment. Lactulose possesses a low caloric content of 2.0 kcal/g, exhibits favorable water solubility, and exerts an influence on the texture and stabilization of food.

Lactulose is commonly employed in the pharmaceutical industry for its gentle laxative properties. Nevertheless, its significance has increased in the past several years as a functional element in health foods (Sanlıdere and Uran, 2017). Lactulose is a highly effective prebiotic. Probiotic bacteria, including bifidobacteria, largely utilize it to enhance metabolism and stimulate their proliferation in the small intestinal mucosa without causing any alterations. The efficacy of probiotic bacteria in combating pathogenic bacteria is greatly enhanced by the presence of lactulose. (Akalın, 2002; Kavas and Kavas, 2011). Prebiotic utilization has expanded the potential applications of health food products, making up about 70% of the whole lactulose market. This market is predicted to reach nearly 50,000 tons per year in recent times.

Lactulose is utilized in the global food business due to its high sweetness content, which ranges from 60% to 80% of sucrose (Nooshkam and Madadlou, 2016a). Aside from its functional features, it also holds technological significance and is widely utilized. The composition of gut bacteria in newborns who consume baby meals supplemented with lactulose is expected to resemble that of infants who are exclusively breastfed. In Japan and Europe, lactulose is the favored choice for feeding infants, particularly due to its ability to give functional qualities (Özden, 2005). Lactulose, a dietary ingredient, is marketed in countries like Italy, Japan, and the Netherlands as a beverage additive, a form of kid feed, or a pure prebiotic. Due to its somewhat higher sweetness compared to lactose, it is utilized in diabetic products, the production of lactating-effective syrups, and the medical treatment of hepatic encephalopathy, a condition characterized by

discomfort and constipation. (Nahla and Musa, 2015). By acidifying the intestinal contents, it aids in the elimination of harmful chemicals from the gut, hence lowering the absorption of ammonia and promoting its excretion from the colon (Akın and Erden 2002). Lactulose, because to its impact, is employed in both the food and medicinal sectors.

Various techniques exist for the synthesis of lactulose. The primary methods for obtaining lactulose are through chemical isomerization using acid or base, or through enzymatic synthesis (Sitanggang et al., 2015). The industrial synthesis of lactulose is accomplished using a specific chemical reaction known as the Lobry de Bruyn-Alberda van Ekenstein (LA) reaction. This reaction occurs in an alkaline environment under specific conditions of pH, pressure, and temperature. The catalysts employed include sodium, potassium, and calcium hydroxides, tertiary amines, magnesium oxide, and sodium and potassium carbonates (Nooshkam and Madadlou, 2016b). Furthermore, ammonium carbonate is suggested as an alternate eco-friendly chemical, in addition to the aforementioned catalysts (Seo et al., 2016). Research on alternate catalysts has been growing in recent years. The catalyst selected must possess the following characteristics: affordability, ease of removal from the environment, eco-friendly nature, safety, and non-toxicity (Panesar and Kumari, 2011).

The chemical process of isomerization is the predominant approach employed in the industry for the production of lactulose. Nevertheless, this approach has certain drawbacks. Some of these include the release of coloured by-products and a limited and ineffective response. The chemical isomerisation process involves neutralisation, catalytic separation, and deionisation phases (Sitanggang et al., 2016).

Lactulose can be derived from whey, which serves as a significant lactose source. Whey is a significant by-product of milk technology. The content and qualities of cheese vary based on the quality of milk and the specific variety of cheese being made. The residual liquid resulting from the milk-to-cheese manufacturing is commonly referred to as whey or lactoserum. During the process of cheese production, approximately 85 kg of milk is transformed into cheese liquid for every 100 kg. There are situations where it is preferable to expeditiously eliminate cheese liquid. The economic value of whey as a by-product is significant, and it should be assessed to prevent wastage (Kurt and Gülümser S, 2011; Kurt, 1994). The dry matter content of whey is approximately half that of milk. The whey composition consists of around 6.96% milk solids. The composition of this material is as follows: 0.36% fat, 0.84% protein, 5.76% lactose, and a maximum of 0.2% lactic acid with salts (Pala, 1997). The properties and makeup of whey differ based on the manufacturing process and the milk quality employed in cheese production (Kaptan, 1986; Karagözlü and Bayarer, 2004; Konar, 1981; Metin, 1983; Yerlikaya et al., 2010). Individuals are increasingly embracing nutritious food choices as a response to the challenges posed by global transformations, recognizing that a good diet is fundamental to sustaining life. This enhances the significance of inventive, practical, and healthful items.

Although numerous articles have been published on the quantification of lactose or lactulose in dairy products using various analytical techniques such as high performance liquid chromatography with refractive index detection (HPLC-RID) (Chavez-Servín, Castellote, & Lopez-Sabater, 2004; ISO/IDF, 2007; Neves, Carvalho, Aguiar, & Silva, 2017), gas chromatography with flame ionisation detection (Montilla, Moreno, & Olano, 2005), capillary zone electrophoresis with indirect detection in ultraviolet region (CZE-UV) (Neves and de Oliveira, 2020; Soga & Serwe, 2000), capillary microchips (Duarte-Junior et al., 2019), electrochemical biosensors and membranes (Tsaturyan et. al., 2023; Conzuelo, Amella, Ampuzano, Uiz, & Eviejo, 2010), and enzymatic spectrophotometric methods (ISO/IDF, 2004; Marconi et al., 2004), only a limited number of studies have applied spectrophotometry to determine both carbohydrates in dairy matrices (Adhikari, Sahai, & Mathur, 1991; Zhang, Wang, Yang, & Jiang, 2010),

This study, conducted in collaboration with Muratbey Dairy Co., involved the production of lactulose using chemical isomerization synthesis using whey. The amount of lactose and lactulose produced was measured using spectrophotometry. Muratbey Dairy Co. has included lactulose into their spreadable cheese product. A group of 10 informed students from the Department of Food Engineering at the University of Uşak, along with food engineers from Muratbey, conducted a sensory investigation of the cheese. The sensory analysis was conducted following the amended "Consumer Test Evaluation Form" outlined in Altuğ and Elmacı's (2011) study.

II. Experimental Procedure

The chemicals used in the study were used without any purification. Lactose, lactulose standards, boric acid, triethylamine, $CuSO_4.5H_2O$, molybdic acid, sodium tungstate, benzoic acid, tartaric acid and resorcinol were obtained from Sigma-Aldrich (St. Louis MO, USA).

Absorbance measurements were performed with a Biochrom Libra S80PC UV/VIS Spectrometer, using 1 cm cuvette light path. Ultrapure water device (Millipore Direct-Q 3UV; 0.22 µm), precision balance (Mettler Toledo), magnetic stirrer (Falc), centrifuge (TD5 LAB312), pH meter and electrode (Mettler Toledo, Hanna HI 1332 Ag/AgCl combined glass electrode, Hanna Ins), vortex (IKA Genius 3), water bath (Selecta H-D, Bandelin) were used in experiments as apparatus.

III. Results and Discussion

3.1 Spectrophotometric Determination of Lactose

In order to make a phosphomolybdic acid solution, 3.5 g of molybdic acid, 0.5 g of sodium tungstate, and 20 mL of 10% sodium hydroxide are brought to a boil. The boiling process continues for twenty to forty minutes. The volume should be brought up to 50 mL by adding distilled water and then adding 12.5 mL of phosphoric acid that is 85%.1.5 mL of 1% lactose solution is completed with 50 mL of 0.2% benzoic acid for preparation of lactose standard solution.

For preparation of copper reagent, 40 grams of sodium carbonate (Na_2CO_3) in 400 mL of water were dissolved. Then, 7.5 grams of tartaric acid and 4.5 grams of $CuSO_4.5H_2O$ were added. The final volume equals to one liter.

Using a gentle shaking, 250 µL of the sample, 500 µL of sodium tungstate, and 500 µL of 0.7 N H_2SO_4 solution were added. After waiting for five minutes, the volume up to 25 mL was completed by adding distilled water and filtered.
Sample, standard and blank tubes are prepared according to the Table 1.

Table 1. Sample preparation amounts for lactose analysis

Solutions / mL	Sample	Standard	Blank
Filtrate	0.5	-	-
Lactose standard	-	1	-
Distilled water	0.5	-	1
Copper reagent	1	1	1
Phosphomolybdic acid	2	2	2

During the process, after adding the copper reagent, the tubes are placed in a boiling water bath for 8 min and then cooled. Also, the tubes are maintained at ambient temperature for a duration of 10 minutes after phosphomolybdic acid addition. During this time, the tubes are shaken until the gas outlet process is finished.
The absorbance of the sample and standard are measured at a wavelength of 630 nm. The quantity of lactose in the sample is determined using the following formula;
Lactose % = [Absorbance of sample / Absorbance of standard] x 100
Determination of lactose from whey was carried out from samples obtained from 3 different cheese processes. All the measurements were performed in triplicate and the results obtained are 76.90%, 64.72% and 70.19%, respectively.

3.2 Spectrophotometric Determination of Lactulose

Spectrophotometric method using Seliwanoff reagent are performed according to Amine et. al. by slightly changes. Seliwanoff's reagent was generated in our laboratory, which is an aqueous solution of HC1 with a concentration of 4 mol/L and contains 0.1% resorcinol. This solution has demonstrated long-term stability for several months.

The reaction takes place in a solution of hydrochloric acid that is heated to its boiling point and contains resorcinol. The analysis of lactulose relies on the premise that this component is the sole origin of fructose in whey. Hence, an increase in the color intensity during the Seliwanoff's reaction is directly proportional to the concentration of lactulose. 2 mL of Seliwanoff's reagent and 0.5 mL of either a whey sample or a standard lactulose were added sequentially to each of the glass-capped test tubes. The test tubes were subjected to a water bath at a temperature of 70°C and 90°C ± 0.1°C for a duration of 10 minutes, and subsequently cooled in tap water. Afterwards, the solution obtained was passed through a disposable filter with a porosity of 0.22 µm. The absorbance of the filtered solution was then measured at a wavelength of 482 nm relative to distilled water, after a brief period of time.

For the calibration graph, standard lactulose solution was prepared and the necessary dilutions were made from this solution and the calibration graph was drawn by reading the absorbance values at 482 nm. The calibration graph is given in Fig 1.

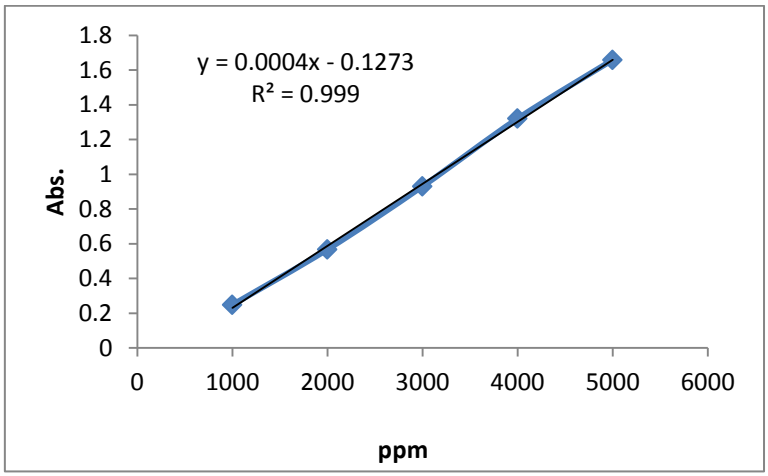

Fig 1. Calibration graph plotted at 482 nm using lactulose standard

The calibration curve was linear in the range of 1.10^3–5.10^3 µg/mL with a correlation coefficient of 0.999.

Subsequently, to assess the impact of various catalysts, NaOH and H_3BO_3 were employed individually and in combination, and their influence on the quantity of lactulose was analyzed. The influence of time, considered as an additional variable, on the quantity of lactulose was evaluated as well.

A volume of 50 mL of whey was taken and 0.35 gram of sodium hydroxide (NaOH), 6.31 grams of H_3BO_3 and combined with 6.31 grams of H_3BO_3 and 0.35 grams of NaOH were added, separately. The pH was raised to 11 by adding HCl. The solution was subjected to a temperature of 70°C for a duration of 70 minutes. Subsequently, 2.5 mL of these solutions were taken out and combined with 2 mL of Seliwanoff reagent and 0.5 mL of water. The resulting mixture was then analyzed for absorbance values at a wavelength of 482 nm.

After the data were analyzed, it was found that using H_3BO_3 and NaOH together produced the highest yield.

The analysis in the second step focused on examining the impact of time on the optimal condition. For this purpose, a volume of 50 mL of whey was taken and sodium hydroxide (NaOH) and H_3BO_3 were added. The pH was adjusted to 11 by adding HCl. The solution was subjected to a temperature of 70°C for a duration of 120, 150 and 180 minutes, respectively. Subsequently, 2.5 mL of these solutions were taken out and combined with 2 mL of Seliwanoff reagent and 0.5 mL of water. The resulting mixture was then analyzed for absorbance values at a wavelength of 482 nm.

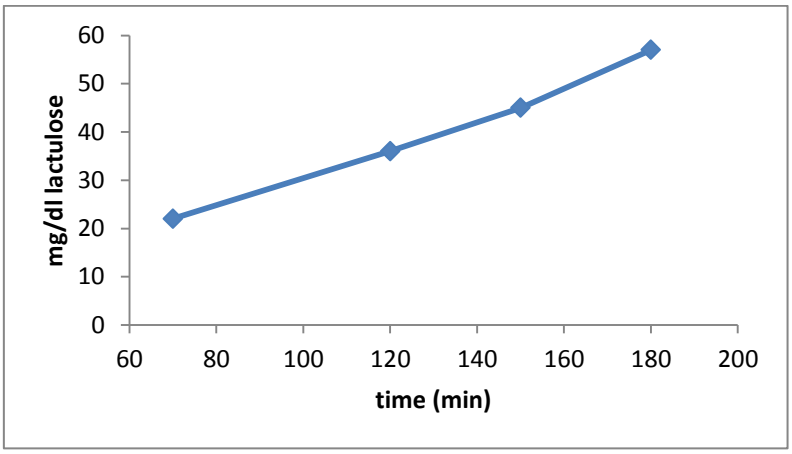

Fig 2. Temporal variation in lactulose concentration

As can be deduced from Fig. 2, lactulose production is increased by time increasing.

3.2 Sensory Analysis

In order to evaluate the usage of lactulose in cheese production, 0-2-5-7% lactulose converted whey was added to the spreadable cheese and sensory analyses were carried out by an experienced panel group of 10 people consisting of graduate students of the Department of Food Engineering, lecturers and food engineers working in the company. The sensory analyses were evaluated on a 1-5 point scale according to the revised version of the "Consumer Test Evaluation Form" described in Altuğ and Elmacı (2011).

The sensory analysis results for the spread cheese samples containing lactulose in groups A, B, C, and D are presented in Table 2. The sensory scores of the cheese samples ranged from 48.67 to 43.83 points.

Table 2. Results of sensory analysis of cheese samples

Number of Panelists 10									
	Samples	**External Appearance**	**Interior Appearance**	**Structure**	**Taste**	**Odor**	**Mouth Sensation**	**Total**	**Average**
1	Spreadable Cheese (A)	50	48	47	49	50	48	292	48.67
2	Spreadable cheese with 2% lactulose (B)	50	48	48	48	44	48	286	47.67
3	Spreadable cheese with 5% lactulose (C)	49	47	44	45	52	45	272	45.33
4	Spreadable cheese with 7% lactulose (D)	48	45	41	46	40	43	263	43.88

IV. Conclusion

The inclusion of lactulose in spreadable cheese was motivated by its low caloric content, excellent water solubility, and its impact on both texture and stabilization. Lactulose supplementation greatly enhances the efficacy of probiotic bacteria in combating pathogenic bacteria, as demonstrated. Thus, it possesses a high functional value as a prebiotic.

The spectrophotometric approach has successfully been used to determine the presence of lactose and lactulose in whey. Upon analyzing the data, it came out that the combination of H_3BO_3 and NaOH resulted in the highest yield of lactulose production.

The addition of lactulose to spreadable cheese received high scores in the sensory analysis. However, the addition of lactulose, at concentrations of 5% and 7%, into whey-based cheese resulted in a noticeable laxative effect as seen by sensory evaluators.

In countries with an advanced dairy industry, the evaluation of milk and its by-products is carried out in a very sensitive manner and every component is evaluated. It is thought that studies should continue in order to obtain lactulose, which has both functional and technological importance, with the most economical and environmentally friendly processes at high yields and to use it in appropriate areas.

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