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Comparative Determination of Lactose Content in Lactose-Free Milk Using Chemical and Electronic Methods

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التقدير المقارن لمحتوى اللاكتوز في الحليب الخالي من اللاكتوز باستخدام الطرق الكيميائية والإلكترونية

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Abstract

Lactose-free milk products are widely consumed by individuals with lactose intolerance; however, regulatory standards vary globally, and residual lactose may persist after industrial processing. This study aimed to determine lactose concentrations in 20 commercial lactose-free milk samples—14 flavored and 6 unflavored—collected from local markets in Tripoli and Misurata, Libya. Two colorimetric methods based on UV–Vis spectrophotometry were employed: (Method I) using zinc sulfate and barium hydroxide for protein precipitation with Tollen's reagent at 520 nm, and (Method II) using 24% trichloroacetic acid (TCA) with modified Tollen's reagent at 482 nm. In addition, lactose was measured electronically using a TOUCH Master PRO (ultrasonic milk analyzer). The findings revealed significant variability in lactose content, ranging from 0.22% to 2.24 % by Method I and 0.0249% to 0.123% by Method II, while electronic determination yielded overestimated values of 2.40% to 9.45% in flavored samples and 6.34% to 6.62% in unflavored samples. Several samples exceeded international regulatory limits of 0.01% (EU) and 0.1% (FDA) for lactose-free labeling. Method II demonstrated higher consistency and sensitivity for detecting trace lactose levels, while Method I was more susceptible to matrix interference. The electronic method proved unsuitable for flavored samples due to interference from other reducing sugars. The study highlights the need for stricter quality control and harmonized labeling standards to ensure consumer safety.

Keywords: Lactose-free milk, Lactose determination, UV–Vis spectrophotometry, Colorimetric method, TCA precipitation, Electronic determination, Quality control.

الملخص

تُستهلك منتجات الحليب الخالي من اللاكتوز على نطاق واسع من قِبل الأشخاص الذين يعانون عدم تحمّل اللاكتوز. ومع ذلك، تختلف المعايير التنظيمية المعتمدة عالميًا، وقد تبقى كميات متبقية من اللاكتوز بعد عمليات المعالجة الصناعية. هدفت هذه الدراسة إلى تحديد تراكيز اللاكتوز في 20 عينة تجارية من الحليب الخالي من اللاكتوز، شملت 14 عينة من الحليب المنكّه و6 عينات من الحليب غير المنكّه، جُمعت من الأسواق المحلية في مدينتي طرابلس ومصراتة، ليبيا.

استُخدمت طريقتان لونيتان تعتمدان على القياس الطيفي للأشعة فوق البنفسجية–المرئية (UV–Vis). اعتمدت الطريقة الأولى على استخدام كبريتات الزنك وهيدروكسيد الباريوم لترسيب البروتينات، مع كاشف تولنز عند طول موجي قدره 520 نانومتر. أما الطريقة الثانية، فاعتمدت على استخدام حمض ثلاثي كلورو الأسيتيك (TCA) بتركيز 24%، مع كاشف تولنز المعدّل عند طول موجي قدره 482 نانومتر. بالإضافة إلى ذلك، قيس محتوى اللاكتوز إلكترونيًا باستخدام جهاز **TOUCH Master PRO**، وهو محلل حليب يعمل بالموجات فوق الصوتية.

أظهرت النتائج تباينًا ملحوظًا في محتوى اللاكتوز؛ إذ تراوحت التراكيز بين 0.22% و2.24% باستخدام الطريقة الأولى، وبين 0.0249% و0.123% باستخدام الطريقة الثانية. في المقابل، أعطى القياس الإلكتروني قيمًا مرتفعة ومبالغًا فيها، تراوحت بين 2.40% و9.45% في عينات الحليب المنكّه، وبين 6.34% و6.62% في عينات الحليب غير المنكّه. كما تجاوزت عدة عينات الحدود التنظيمية الدولية المعتمدة لوصف المنتج بأنه خالٍ من اللاكتوز، وهي 0.01% وفقًا لمعايير الاتحاد الأوروبي و0.1% وفقًا لمعايير إدارة الغذاء والدواء الأمريكية.

أظهرت الطريقة الثانية درجة أعلى من الاتساق والحساسية في الكشف عن التراكيز الضئيلة من اللاكتوز، في حين كانت الطريقة الأولى أكثر تأثرًا بالتداخلات الناتجة عن مكونات العينة. كما تبيّن أن الطريقة الإلكترونية غير مناسبة لتحليل عينات الحليب المنكّه بسبب تداخل السكريات المختزلة الأخرى. وتؤكد الدراسة ضرورة تطبيق رقابة أكثر صرامة على جودة هذه المنتجات وتوحيد معايير وضع البيانات على الملصقات الغذائية، بما يضمن سلامة المستهلكين.

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الكلمات المفتاحية: الحليب الخالي من اللاكتوز، تقدير اللاكتوز، القياس الطيفي بالأشعة فوق البنفسجية–المرئية، الطريقة اللونية، الترسيب بحمض ثلاثي كلورو الأسيتيك، التقدير الإلكتروني، ضبط الجودة.

1.Introduction :

Lactose constitutes the main disaccharide in mammalian milk, typically accounting for 4.5–5.0% of the total composition in fresh bovine milk. In the small intestine, β-galactosidase catalyzes the hydrolysis of lactose into glucose and galactose through specific cleavage of the β-1,4 glycosidic bond, facilitating nutrient absorption. It is estimated that 65–70% of adults worldwide experience a decline in lactase activity following childhood, a condition known as lactose malabsorption that often manifests as gastrointestinal discomfort after dairy intake [1][2][3][4]. The widespread occurrence of lactose malabsorption has significantly fueled the expansion of the lactose-free dairy sector. Commercial lactose-free dairy is generally manufactured by incorporating exogenous lactase to catalyze the breakdown of lactose into its monosaccharide components, thus lowering the residual lactose content to less than 0.1%. Nevertheless, trace amounts of lactose, typically between 0.01% and 1.5%, may persist in the final product owing to incomplete enzymatic conversion or unintentional contamination during production [5][6][7][8]. Regulatory authorities, particularly the U.S. Food and Drug Administration (FDA), have established 0.1% w/v as the maximum permissible lactose level for products marketed as “Lactose-Free,” a benchmark that has been widely adopted globally. Achieving accurate quantification at this concentration poses substantial analytical difficulties due to the intricate composition of milk and the potential interference from other reducing sugars such as glucose and galactose [6][9]. Analytical approaches for residual lactose determination can be classified into four main categories. The enzymatic method with TCA precipitation is adapted from AOAC Official Method 984.15. Prior to enzymatic hydrolysis, trichloroacetic acid (TCA) is employed for protein precipitation to eliminate matrix

interference and clarify the sample matrix. This pretreatment step enhances the specificity of β-galactosidase activity and improves the accuracy of subsequent spectrophotometric glucose determination at 520 nm. The method demonstrates high selectivity with a limit of detection of 0.13 mg/100 g and is widely adopted for quality control in lactose-free dairy products [8][10][11]. The modified Tollen's reagent method is based on the reduction of the silver diammine complex $[Ag(NH_3)_2]^+$ by the free aldehyde group of lactose under alkaline conditions, producing a visible colorimetric response measurable at 482 nm. The modification involves adjusting reagent concentration and pH to minimize interference from other reducing sugars while maintaining sensitivity for lactose detection. Although less specific than enzymatic assays, this approach offers advantages in terms of simplicity, cost-effectiveness, and suitability for routine screening applications [12][13][14]. The electronic biosensor method employs portable amperometric devices with immobilized β-galactosidase for rapid on-site lactose analysis. The Touch Master Pro analyzer (Carlsberg Research Laboratory, 2021) represents a commercial application of the AOAC First Action 2020 LactoSens® amperometric method. The instrument quantifies lactose within 90 seconds by measuring the electrical current generated from enzymatic oxidation, with a working range of 0.01–2.00% and precision of ±5%. This technology provides strong correlation with reference enzymatic assays while minimizing matrix-related interferences [15][16][17][18]. Chromatographic techniques, particularly high-performance anion exchange chromatography with pulsed amperometric detection (HPAEC-PAD), deliver the highest analytical sensitivity with detection limits below 10 mg/L. Additionally, high-performance liquid chromatography (HPLC) with post-column fluorescence derivatization enables simultaneous determination of lactose and related oligosaccharides such as allolactose and lactulose [19][20]. Several parameters influence the efficiency of lactose hydrolysis, including enzyme concentration, temperature, pH, and incubation time. The Michaelis constant (Km) for lactase generally ranges from 20–40 mM between 5–37°C, reflecting enzyme-substrate affinity. Optimal catalytic activity occurs at 37–45°C, while temperatures exceeding 60°C result in complete enzyme denaturation. The presence of calcium ions at 5–10 mM enhances enzyme stability and extends its half-life beyond 29 hours at 12°C. Conversely, lipids and proteins in the milk matrix can cause matrix interference, affecting the accuracy of spectrophotometric measurements [21][22][23][24]. In view of these considerations, the current study aims to compare three analytical methods—enzymatic assay with TCA pretreatment, modified Tollen's reagent method, and Touch Master Pro biosensor—for the quantification of residual lactose in commercial lactose-free milk products, and to evaluate their compliance with FDA regulatory standards.

2. Materials and Methods

2.1 Chemicals and Reagents:

Table 1: Chemicals and Reagents Used in the Experimental Work

Chemical Formula	Chemical Name
$Na_2S_2O_5$	Sodium Metabisulfite
$ZnSO_4$	Zinc Sulfate
C_6H_6O	Phenol
H_2O	Distilled Water
$C_6H_3N_3O_7$	Picric Acid
$C_{12}H_{22}O_{11}$	Lactose

Prepared in Laboratory	Tollen's Reagent
$Ba(OH)_2$	Barium Hydroxide
—	Milk Samples
NaOH	Sodium Hydroxide
CCl_3COOH	Trichloroacetic Acid (TCA)
$NaHSO_3$	Sodium Bisulfite

2.2 Instrumentation:

Table2: Instruments Used in the Experimental Work

Type	Model
UV–Vis spectrophotometer	(SPECORD 210/PLUS, Analytik Jena, Germany)
quartz cuvettes	1cm
Centrifuge	(Model E800, West Germany).
Analytical balance	(AE ADAM AAA250L)
Boiling water bath	(BUCHI 461, Switzerland)
TOUCH Master PRO (ultrasonic milk analyzer)	(Model PRO TOUCH 31338

2.3 Analytical Methods:

Twenty lactose-free milk samples (14 flavored, 6 unflavored) were purchased as available from local markets in Tripoli and Misurata, Libya.

Method I: Lactose was determined colorimetrically following the procedure described by Teles et al. (1978). Sample dilution, deproteinization, and color development were carried out exactly as specified in the original method without modification. Proteins were precipitated with 5% $ZnSO_4$ and 4.5% $Ba(OH)_2$, followed by color development with Tollen's reagent and absorbance measurement at 520 nm [25] .

Lactose concentration was calculated as:

$$\text{Lactose concentration} = \frac{A\ sample}{\text{A standared}} \times 100\ mg/ml$$

And Lactose % was calculated as:

$$\text{Lactose \%} = \frac{\text{Lactose concentration mg/ml}}{10}$$

Method II: Lactose was determined colorimetrically following the modified procedure described by Sen et al. (2023). Milk samples were deproteinized using 24% trichloroacetic acid (TCA), and the filtrate was reacted with the working reagent prepared from 1% phenol, 5% sodium hydroxide, 1% picric acid, and 1% sodium disulfite according to the original method of Teles et al. Absorbance was measured at 482 nm against a reagent blank, and lactose concentration was calculated using a standard lactose calibration curve[26].

Electronic Method: Lactose content in milk samples was determined using a TOUCH Master PRO analyzer. UHT milk was selected as the sample type on the digital display, and samples were placed in the designated sample cells and loaded into the instrument according to the manufacturer's instructions. Lactose percentage was automatically calculated and displayed on the screen. The instrument was calibrated after each measurement.

3. Results and Discussion

3.1 Lactose Concentrations by Method I (520 nm):

Table 3: Lactose Concentrations Determined by Method I

Lactose (mg/100 mL)	Lactose (%)	Absorbance	Flavor	Company	Sample No
133.81	1.34	0.186	Chocolate	Scotti	1
153.24	1.53	0.213	Chocolate	Scotti	2
50.36	0.50	0.070	Hazelnut	Nilky	3
51.80	0.52	0.072	Hazelnut	Nilky	4
73.38	0.73	0.102	Coconut	Nilky	5
168.35	1.68	0.234	Coconut	Scotti	6
23.02	0.23	0.032	Almond	Nilky	7
21.58	0.22	0.030	Almond	Nilky	8
187.05	1.87	0.260	Almond	Scotti	9
64.03	0.64	0.089	Oats	Nilky	10
46.76	0.47	0.065	Oats	Scotti	11
150.36	1.5	0.209	Almond	Nilky	12
223.88	2.24	0.311	Coconut	Scotti	13
48.92	0.49	0.068	Coconut	Nilky	14
128.06	1.28	0.178	Unflavored	Juhayna	15
173.38	1.73	0.241	Unflavored	Juhayna	16
182.01	1.82	0.253	Unflavored	Juhayna	17
161.15	1.61	0.224	Unflavored	Sterilgarda	18
109.35	1.09	0.152	Unflavored	Sterilgarda	19
130.94	1.31	0.182	Unflavored	Sterilgarda	20

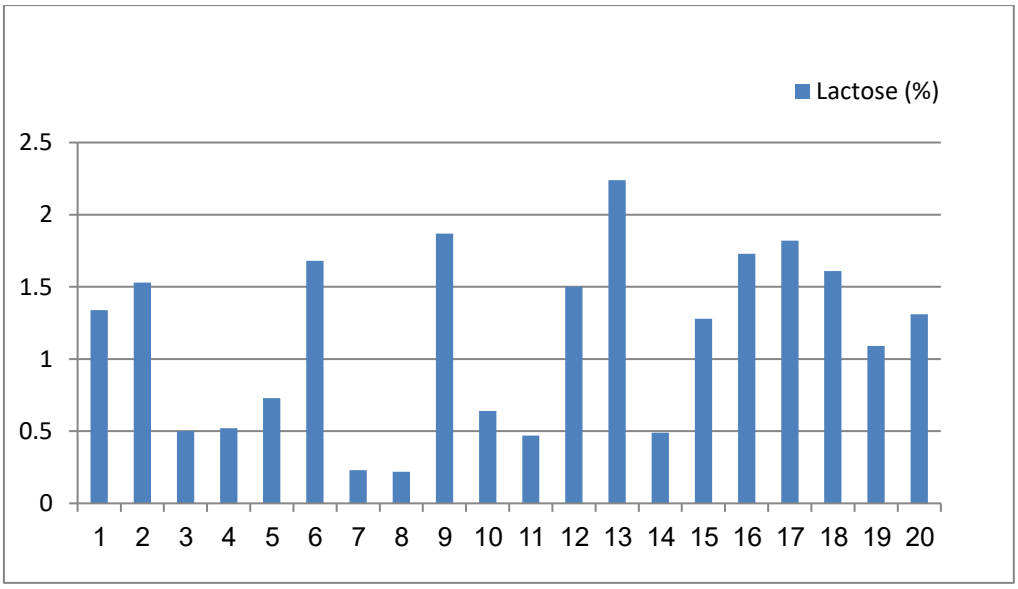

Figure 1: Lactose % Determined by Method I**.**

Lactose concentrations ranged from (0.22% to 2.24%). The highest level was in Scotti coconut-flavored milk, while the lowest was in Nilky almond-flavored milk. Unflavored samples ranged between (1.09 % and 1.82 %), which exceed both the EU limit of 0.01% and the FDA limit of 0.1% for lactose-free labeling.

3.2 Lactose Concentrations by Method II (482 nm) :

Table 4: Lactose Concentrations Determined by Method II

Lactose (%)	Absorbance	Flavor	Company	Sample No
0.123	0.1303	Chocolate	Scotti	1
0.1062	0.1143	Chocolate	Scotti	2
0.1028	0.1102	Hazelnut	Nilky	3
0.1023	0.1096	Hazelnut	Nilky	4
0.103	0.1105	Coconut	Nilky	5
0.105	0.1127	Coconut	Scotti	6
0.0779	0.0849	Almond	Nilky	7
0.301	0.642	Almond	Nilky	8
0.098	0.1048	Almond	Scotti	9
0.1001	0.1069	Oats	Nilky	10
0.0964	0.1032	Oats	Scotti	11
0.098	0.1047	Almond	Nilky	12
0.1052	0.1129	Coconut	Scotti	13
0.0887	0.0965	Coconut	Nilky	14
0.0261	0.0589	Unflavored	Juhayna	15
0.0249	0.0628	Unflavored	Juhayna	16
0.0305	0.0692	Unflavored	Juhayna	17
0.0287	0.0673	Unflavored	Sterilgarda	18
0.0258	0.0627	Unflavored	Sterilgarda	19
0.0284	0.0641	Unflavored	Sterilgarda	20

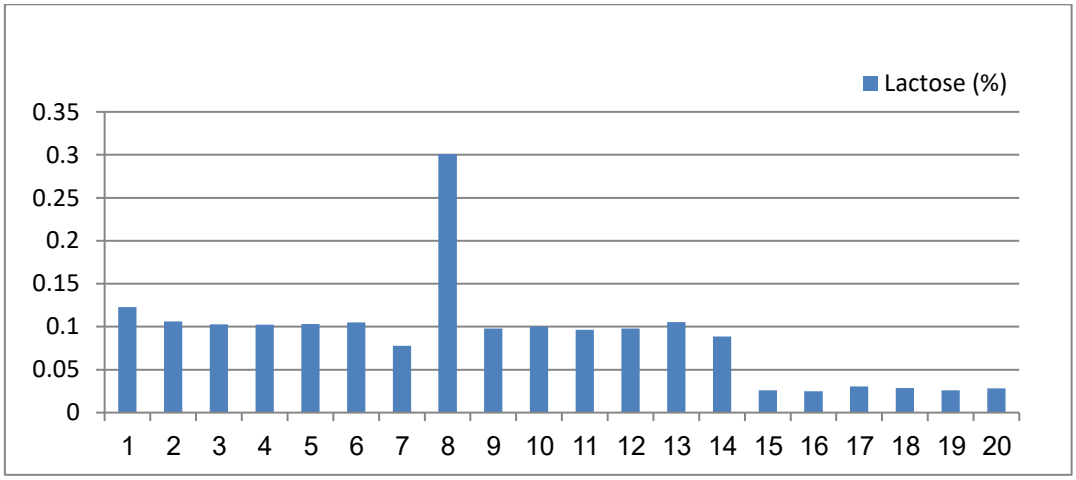

Figure 2: Lactose % Determined by Method II**.**

Lactose% ranged from (0.0249% to 0.301%). Sample(8) exhibited a notably higher value (0.301%) compared to other Nilky almond samples (0.0249–0.055%), suggesting possible batch variation or incomplete protein precipitation.

The corrected range of Lactose (%) was from (0.0249% to 0.123%). Unflavored samples from Juhayna and Sterilgarda showed the most consistent values, averaging (0.028%), These values fall within the (FDA) acceptable limit but exceed the stricter (EU) standard.

3.3 Electronic Determination Using TOUCH Master PRO

Table 5: Electronic Lactose Determination – Flavored Samples

Lactose (%)	Flavor	Company	Sample No
7.43	Chocolate	Scotti	1
9.45	Chocolate	Scotti	2
2.92	Hazelnut	Nilky	3
2.96	Hazelnut	Nilky	4
2.45	Coconut	Nilky	5
7.47	Coconut	Scotti	6
2.63	Almond	Nilky	7
2.56	Almond	Nilky	8
7.68	Almond	Scotti	9
3.94	Oats	Nilky	10
3.92	Oats	Scotti	11
6.51	Almond	Nilky	12
7.85	Coconut	Scotti	13
2.40	Coconut	Nilky	14

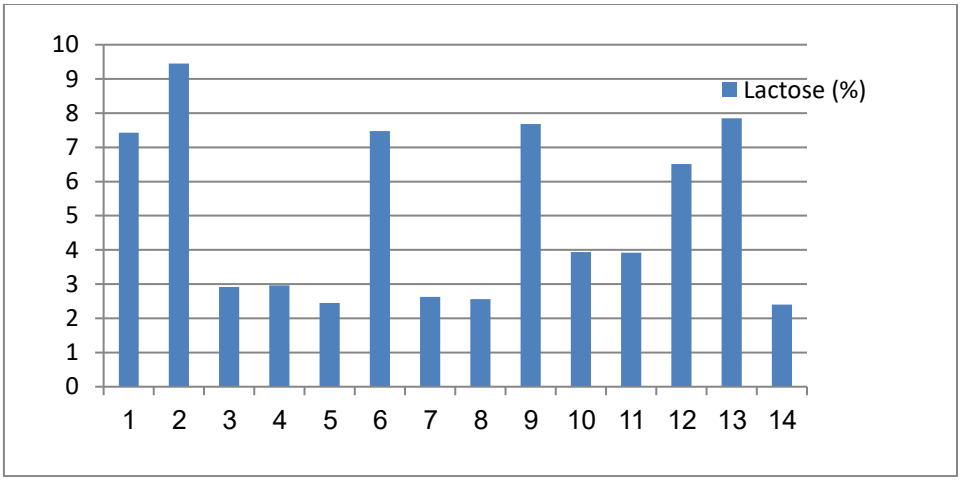

Figure 3: Lactose % Determined by TOUCH Master PRO (Flavored Samples)

Table 6: Electronic Lactose Determination – Unflavored Samples

Lactose (%)	**Flavor**	**Company**	**Sample No**
6.43	Unflavored	Juhayna	15
6.34	Unflavored	Juhayna	16
6.38	Unflavored	Juhayna	17
6.62	Unflavored	Sterilgarda	18
6.60	Unflavored	Sterilgarda	19
6.6	Unflavored	Sterilgarda	20

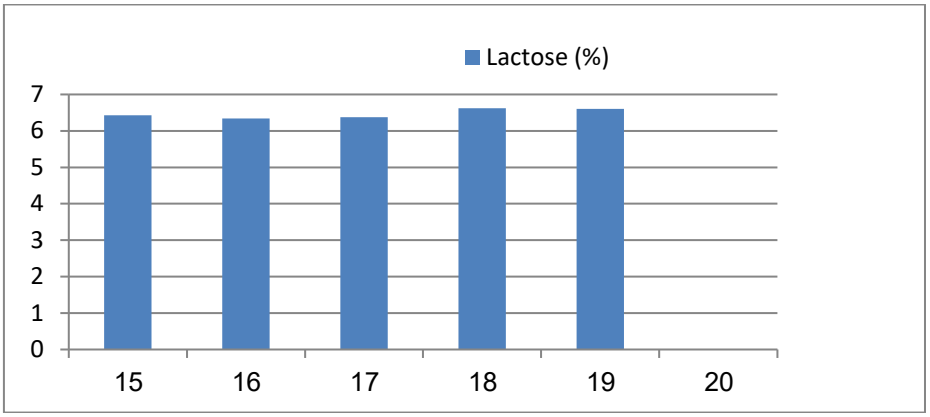

Figure 4: Lactose % Determined by TOUCH Master PRO (UnFlavored Samples)**.**

The electronic analyzer reported excessively high values ranging from (2.40% to 9.45%) in flavored samples and (6.34% to 6.62%) in unflavored samples, exceeding the normal lactose content of whole milk (4.8%) This is physiologically implausible for products labeled as "lactose-free. . The consistently high values across all analyzed samples indicate that the electronic method measures total reducing sugars rather than lactose specifically, as it cannot differentiate lactose from other carbohydrates such as sucrose and maltodextrin present in flavoring agents.

3.4 Discussion of Methodological Discrepancies:

I . Method II is the most accurate, **TCA** achieves more complete protein precipitation compared to Method I where ($ZnSO_4/Ba(OH)_2$), reducing matrix interference and light scattering during spectrophotometric measurement. This resulted in lower variability and higher reproducibility between replicates.

Method I is less precise: Incomplete protein removal led to elevated absorbance readings and overestimation of lactose content.

Electronic method is unsuitable for flavored products: The TOUCH Master PRO ultrasonic analyzer estimates total reducing sugars based on acoustic and refractive properties rather than lactose specifically. It cannot distinguish lactose from other carbohydrates such as sucrose, glucose, or maltodextrin present in flavoring agents. Consequently, the high readings reflect total reducing sugars, not actual lactose content.

II. Reasons for Differences Between Companies and Flavors:

1. Difference in Enzyme Efficiency: Scotti showed higher levels than Nilky and Juhayna, indicating that the enzymatic hydrolysis of lactose was incomplete or that the concentration of β-galactosidase enzyme used was lower.

2. Flavoring additive interference: Flavored samples exhibited higher lactose readings across all methods due to the presence of added reducing sugars in chocolate, coconut, and almond flavorings.

3. Contamination During Manufacturing: Lactose contamination may occur during the packaging or production stages..

4. Regulatory compliance: None of the samples met the EU standard of <0.01%. Only unflavored samples analyzed by Method II complied with the FDA threshold of <0.1%. The "lactose-free" labeling on most products is inaccurate and may pose health risks to severely lactose-intolerant individuals.

III. Compliance with International Standards:

European Standards: Requires a lactose concentration of less than (0.01%), and none of the studied samples met this requirement.
US Standards : The (FDA) requires a concentration of less than (0.1%); only the unflavored samples in Method II met this requirement.
Conclusion: The "lactose-free" labeling on the packaging is inaccurate in most samples, and this poses a risk to consumers with severe lactose intolerance.

4. Conclusion and Recommendations

4.1 Conclusion:

1. Method II using TCA precipitation is the most reliable and reproducible method for quantifying trace lactose in lactose-free milk.
2. Method I can serve as a rapid screening tool but is more susceptible to matrix interference.
3. Electronic ultrasonic analysis is not suitable for lactose quantification in flavored dairy products due to cross-reactivity with other reducing sugars.
4. Most of the studied commercial products do not comply with international lactose-free standards.

4.2 Recommendations:

1. Adopt Method II with TCA precipitation as the standard protocol for routine quality control in dairy laboratories.
2. Use HPLC for confirmatory analysis where ultra-low lactose detection is required.
3. Enforce stricter regulatory oversight on dairy manufacturers to verify “lactose-free” claims on product labels.
4. Harmonize international standards for lactose-free classification to ensure consumer safety safety and prevent misleading labeling.

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Compliance with ethical standards

Disclosure of conflict of interest

The authors declare that they have no conflict of interest.