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DETERMINATION OF TRANS FATTY ACID ISOMERS IN LOCAL RAW MILK BY GAS CHROMATOGRAPHY

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ABOUT ARTICLE

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Abstract: Trans fatty acids (trans isomers) are important lipid components in food products, and their impact on human health and significance from a food safety perspective are substantial. This study aims to identify and quantify the trans fatty acid isomers in a local raw milk sample using gas chromatography (GC-FID).

For analysis, fatty acid methyl esters (FAMEs) were prepared and analyzed using a high-polarity capillary column designed to separate cis and trans isomers. Chromatographic analysis identified two main trans isomers, which were identified based on their retention times: elaidic acid (C18:1 trans-9) - at 46.98 min and 1.547%, and linolelaidic acid (C18:2 trans) - at 48.28 min and 3.137%. The total trans fatty acid content was 4.684%.

The results obtained showed that the trans fatty acids in milk are primarily of natural origin and are associated with the biohydrogenation process that occurs in ruminant animals. The applied GC-FID method is characterized by high accuracy, sensitivity, and reliability in detecting trans isomers in complex food matrices. The research findings have significant scientific and practical importance for monitoring trans fatty acids in dairy products and for assessing food safety.

Introduction. Trans fatty acids (TFAs) are one of the most important lipid components from the standpoint of food safety and human health. Scientific research conducted over the past decades has shown that excessive consumption of trans isomers is closely linked to the development of cardiovascular diseases, metabolic syndrome, and other chronic illnesses [1]. Therefore, strict requirements have been introduced by international organizations to limit and control the amount of trans fats in food products [2].

However, it has been found that not all trans fatty acids have the same biological effects. In particular, there are important differences between industrially produced trans isomers and the trans isomers naturally produced in the bodies of ruminant animals. Natural trans fatty acids, such as trans-vaccenic acid and conjugated linoleic acid (CLA), have been noted to exert positive effects on metabolic processes in some animals [3]. In this regard, when evaluating trans fats, determining their isomer composition, in addition to their total amount, is of particular scientific importance.

Milk and dairy products are considered one of the main sources of naturally occurring trans fatty acids. At the same time, milk fat is a complex lipid system, and the presence of hundreds of different fatty acids in its composition complicates the process of identifying and analyzing them [4]. Furthermore, the amount and composition of trans isomers in milk vary depending on the animal's diet, stage of lactation, and other biological factors.

One of the main challenges in detecting trans isomers is their clear separation from cis isomers and positional isomers. Although these isomers share the same chemical formula, their biological properties differ dramatically, making analytical precision even more critical [5]. Therefore, modern research necessitates the use of highly selective and precise analytical methods.

Gas chromatography, especially in conjunction with a flame ionization detector (GC-FID), is one of the most reliable methods for determining fatty acids, including trans isomers.

This method provides high accuracy and reproducibility by analyzing fatty acids in the form of methyl esters [6]. However, the accuracy of the analysis is directly dependent on the proper execution of the esterification process and the chromatographic separation conditions.

From this perspective, the accurate and reliable determination of trans fatty acids in milk is of significant scientific and practical importance for assessing food safety. The aim of this study is to determine the composition and quantity of trans fatty acids in a local raw milk sample using gas chromatography (GC-FID) and to evaluate them on a scientific basis.

Literature review: In the modern food system, the fatty acid composition is one of the main factors determining not only the technological quality of a product but also its biological safety. In particular, the presence of unsaturated fatty acids in the trans configuration has drawn the attention of the scientific community in recent years, creating the need to assess them separately from the standpoint of food safety [1,7].

Numerous epidemiological and experimental studies have shown that regular consumption of certain trans isomers is associated with impaired lipid metabolism, increased levels of low-density lipoproteins, and an elevated risk of cardiovascular disease [1,5]. Therefore, trans fats are considered an important chemical factor in food products that must be regulated.

At the same time, modern research shows that the biological effects of trans fatty acids depend on their source and isomeric structure. Scientific evidence shows that not all trans isomers have the same adverse effects, and that certain naturally occurring trans fats may play different roles in metabolic processes. For example, fats enriched with trans-vaccenic acid and cis9,trans11-CLA have been observed to improve glucose homeostasis and reduce liver lipid accumulation in mice fed a high-fat diet [3,8].

Trans fatty acids are typically classified into two main groups based on their origin: natural (ruminant) and industrial trans isomers. This classification reflects not only their formation mechanisms but also the differences in their biological effects [1,5]. Therefore, evaluating trans fats as a single group is not scientifically justified.

Natural trans fatty acids are formed as a result of the biohydrogenation process that occurs in the rumen of ruminant animals. In this process, microorganisms partially convert the polyunsaturated fatty acids in the feed, producing isomers such as trans-vaccenic acid (C18:1 trans-11) and conjugated linoleic acid (CLA) [3,8]. These compounds have been noted to exert positive effects on lipid metabolism and metabolic processes in some cases.

Industrial trans fatty acids are primarily produced by the partial hydrogenation of vegetable oils. During this process, cis-configured double bonds are converted to trans, resulting in isomers such as elaidic acid (C18:1 trans-9). These isomers are associated with more adverse biological effects and can lead to alterations in the lipid profile and an increased risk of cardiovascular disease[1,7].

Milk and dairy products are considered one of the natural sources of trans fatty acids, and the trans isomers in them are primarily formed as a result of the biohydrogenation process that occurs in the bodies of ruminant animals[1,5]. During this process, the rumen microflora partially converts the unsaturated fatty acids in the feed, producing various trans isomers.

The main trans isomers identified in milk fat include trans-vaccenic acid (C18:1 trans-11) and conjugated linoleic acid (CLA). These compounds are natural products of ruminant lipid metabolism, and their levels vary depending on the animal's diet and physiological state [4,9].

The complex lipid system of milk fat is explained by the presence of hundreds of different fatty acids in its composition. This diversity also leads to a wide spectrum of trans isomers and complicates the process of detecting them[10,11]. At the same time, trans isomers in milk fat are typically present in low amounts, and the majority of them are naturally occurring.

Studies show that the trans fatty acids in milk are not only limited to biological processes but are also closely linked to nutritional factors. For example, adding large amounts of polyunsaturated fatty acids to feed can increase the levels of trans isomers, particularly vaccenic acid and CLA[9,12].

Moreover, it has been noted that trans isomers in dairy products, unlike industrial trans fats, typically have different biological characteristics, and their metabolic effects are not the same [5,8]. Therefore, when evaluating trans fatty acids in milk, it is important to consider their source and isomer composition.

As a result, milk is a natural and important source of trans fatty acids, and its complex and variable composition further underscores the need to accurately identify and assess these compounds.

The determination of trans fatty acids and the accurate assessment of their isomeric composition is considered one of the most complex challenges in analytical chemistry. The main reason is that trans and cis isomers have the same chemical formula and differ only in their geometric structure, which makes it difficult to separate them using conventional analytical methods [1,5].

Furthermore, the problem of positional isomers is also of significant importance. For example, although elaidic (trans-9) and vaccenic (trans-11) acids in the C18:1 series have the same molecular formula, their double bond positions give them different physical and biological properties[5,7]. Therefore, their precise identification requires a highly selective analytical approach.

Another factor that intensifies analytical challenges is that milk is a complex matrix. Milk fat contains hundreds of fatty acids, including components with very similar retention times[10,11]. This situation can lead to overlapping chromatographic peaks and complicate the interpretation of the results.

Moreover, the fact that the amounts of trans isomers are usually very low also complicates the detection process. To detect them at low concentrations, methods with high sensitivity are required; otherwise, some isomers may go undetected or be misidentified [6,13].

Moreover, problems can arise during the analysis process itself. In particular, during the esterification stage, isomerization or the loss of certain fatty acids may occur, which negatively affects the accuracy of the results [14,15].

As a result, to ensure high accuracy and selectivity in the detection of trans fatty acids, it is necessary not only to use modern analytical methods but also to optimize the sample preparation stage. This is crucial for reliably detecting trans isomers and assessing their true concentrations.

Among modern analytical methods for detecting fatty acids, especially trans isomers, gas chromatography techniques hold a leading position. These methods offer high accuracy, sensitivity, and selectivity, enabling the separation and quantification of individual fatty acids in complex food matrices [6,10].

The most widely used variant of gas chromatography is the GC-FID, which is equipped with a flame ionization detector. This method is based on the analysis of fatty acids in the form of methyl esters (FAME), and because the detector signal is proportional to the number of carbon atoms, it is very convenient for quantitative analysis [6,15]. At the same time, cis and trans isomers can be effectively separated using high-polarity capillary columns.

Another important variant of gas chromatography is gas chromatography–mass spectrometry (GC-MS). This method not only separates components but also allows their identification at the molecular level. GC-MS provides particularly high accuracy in detecting components in complex mixtures or at low concentrations[13].

One of the key steps in fatty acid analysis is the methylation (derivatization) process. This process converts fatty acids into a volatile form suitable for chromatography. In practice, various methylation methods are used, including basic (KOH/methanol), acidic (BF_3/methanol), and direct methylation methods, each with its own advantages and disadvantages [14,15].

Furthermore, modern research is also proposing approaches aimed at developing faster and more accurate methods for determining fatty acid composition. However, at present, GC-FID and GC-MS methods are regarded as the primary and most reliable techniques for determining fatty acid composition in food products [10,16].

Thus, modern analytical methods ensure high accuracy and reliability in detecting fatty acids, especially trans isomers, but their effectiveness is directly dependent on a properly chosen methodology and optimal laboratory conditions.

Although existing analytical methods are highly efficient, their associated limitations require a cautious approach when detecting trans fatty acids. This underscores the need to develop more advanced methods and optimize existing techniques.

Therefore, conducting research aimed at the accurate and reliable detection of trans fatty acids is of significant scientific and practical importance. In particular, the gas chromatography (GC-FID) method serves as an important tool for individually detecting trans isomers and quantifying them to ensure food safety and assess product quality.

This study is specifically aimed at filling this scientific gap, serving to enrich existing knowledge by identifying trans fatty acids in local raw milk samples and evaluating them on a scientific basis.

Materials and methods. Analytically pure reagents were used to convert fatty acids into methyl esters during the analysis. A potassium hydroxide (KOH) solution in methanol was used for the methylation process. For extraction, a 2:1 (v/v) mixture of chloroform and methanol (Folch solution) was used.

Fatty acid methyl esters (FAME) standard mixtures (Sigma-Aldrich, USA) were used for gas chromatography analysis. The standards contained major fatty acids, including cis and trans isomers.

Methyl tricosanoate (C23:0) was used as the internal standard. All chemicals were of analytical grade (≥99% purity) and were used without further purification.

A raw milk sample obtained from the local area was used as the study object. The sample was collected from the Kitob district and stored at +4 °C until analysis.

Total lipids were extracted from the milk using the Folch method (chloroform: methanol, 2:1). The isolated lipid fraction was dried and then subjected to methylation.

Fatty acid methyl esters (FAME) were prepared as follows:

- Approximately 50 mg of the lipid sample was taken in a test tube;
- 2 mL of methylation reagent was added;
- the mixture was incubated at 60 °C for 20 minutes;

- then 2 mL of isobutanol was added, and the phases were separated;
- The upper phase was used for chromatographic analysis.

The resulting FAME solution was directly introduced into the GC-FID system.

The fatty acid composition was determined by gas chromatography. Analyses were performed on a Shimadzu GC-2010 gas chromatograph equipped with a flame ionization detector (FID).

A high-polarity capillary column was used for separation:

- Column type: SP-2560
- Dimensions: 100 m × 0.25 mm × 0.20 μm

Helium (He) was used as the carrier gas, with a flow rate of 1.0 mL/min.

The chromatographic conditions were as follows:

- Injection volume: 1 μL
- Split ratio: 1:50
- Injector temperature: 250 °C
- Detector temperature: 260 °C

Oven temperature program:

- Initial: 140 °C (5 min)
- Gradient: 4 °C/min
- Final temperature: 240 °C (15 min)

Fatty acid identification was carried out by comparing retention time values with those of standard FAME mixtures.

The method's reliability and accuracy were evaluated using a series of validation parameters. The high accuracy and reproducibility of the GC-FID method for fatty acid determination have been confirmed by previous studies (Validation of the determination of fatty acids in milk by gas chromatography).

Recovery ranged from 95 to 102%, indicating the method's efficiency. Each sample was analyzed at least $n = 3$ times, and the results were expressed as mean values.

Repeatability (RSD) did not exceed 3%. The limit of detection (LOD) was determined to be 0.01 mg/mL, and the limit of quantification (LOQ) was 0.03 mg/mL.

The method is characterized by high sensitivity and selectivity in detecting fatty acids, especially trans isomers.

Results and discussion. Gas chromatography analysis successfully separated and identified the fatty acid methyl esters (FAMEs) in milk fat. In the chromatogram, the fatty acid peaks were clearly separated based on their retention times (RT), indicating that the high-polarity capillary column (SP-2560) used was effective in resolving cis and trans isomers.

According to the analysis results, the milk sample contained, in addition to the major fatty acids, isomers in the trans configuration. In particular, the trans isomers of the C18 group appeared as distinct peaks. The trans isomers were identified by comparing their retention times with those of standard FAME mixtures.

The main identified trans isomers were as follows:

- trans-9-octadecenoic acid (elaidic acid) – RT: 46.98 min
- trans-9, trans-12-octadecadienoic acid (linolelaidic acid) – RT: 48.28 min

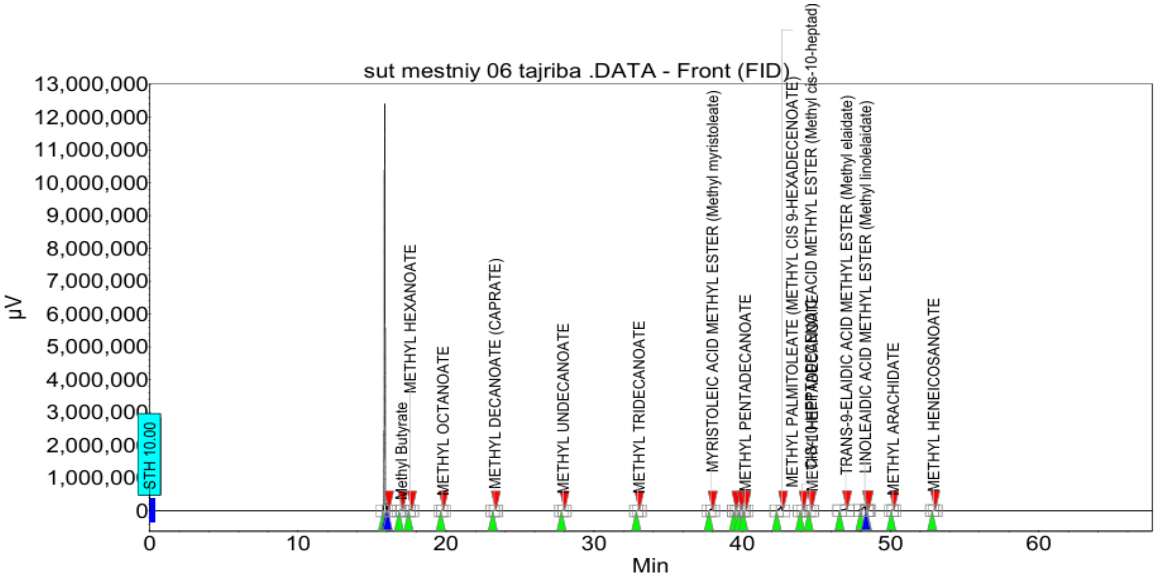

Figure 1. The GC-FID chromatogram of fatty acid methyl esters (FAMEs) in a milk sample.

These retention time values confirm the chromatographic identification of these isomers.

Table 1

Composition of fatty acid methyl esters (FAME) in a milk sample, determined by the GC-FID method.

N:	Fatty acid (FAME)	Time (min)	Quantity (μg/ml)	Height (μV)	Area (μV·min)	Area (%)
1	Methyl butyrate	16.93	0.17	19225.8	1493.8	0.216
2	Methyl hexanoate	17.57	13.33	42170.7	2216.3	0.321
3	Methyl octanoate	19.74	4.50	23910.3	1325.7	0.192

4	Methyldecanoate (caprate)	23.25	2.29	12148.7	713.3	0.103
5	Methylundecanoate	27.90	2.58	23448.8	1424.9	0.206
6	Methyltridecanoate	32.95	2.50	25556.3	1563.4	0.226
7	Methyl myristoleate	37.92	10.30	74725.2	6469.6	0.936
8	Methyl pentadecanoate	40.18	2.10	10799.2	672.5	0.097
9	Methylpalmitoleate (cis-9-hexadecenoate)	42.66	25.96	136597.2	22413.2	3.242
10	Methyl heptadecanoate	44.05	5.05	18143.8	1400.9	0.203
11	Cis-10-heptadecenoic acid methyl ester	44.58	2.01	7077.8	491.6	0.071
12	Trans-9-elaidic acid methyl ester	46.98	23.34	54300.7	10694.1	1.547
13	Linolelaidic acid methyl ester	48.28	40.11	125506.8	21686.5	3.137
14	Methyl arachidate	50.16	4.54	17995.1	1203.6	0.174
15	Methyl heneicosanoate	52.91	2.45	7987.2	585.8	0.085
	Total		**141.24**	**13174682.3**	**691334.6**	**100.000**

The quantitative composition of trans fatty acids was evaluated based on peak areas (%). The amounts of the identified trans isomers were as follows:

Table 2

Composition of trans fatty acid methyl esters (FAME) in the milk sample, determined by the GC-FID method

No	Fatty acid (fame)	Notation	Retention time (min)	Area (%)
1	Elaidic acid methyl ester	C18:1 trans-9	46.98	1.547
2	Linolelaidic acid methyl ester	C18:2 trans	48.28	3.137
	Total trans fatty acids (tfa)	—	—	**4.684**

This result indicates that the analyzed milk sample contains trans fatty acids, the majority of which are isomers belonging to the C18 group.

When the obtained results were compared with scientific literature, it was determined that they fall within the range of naturally occurring trans fatty acids found in dairy products. According to studies, trans fats in ruminant products are typically formed as a result of the biohydrogenation process, and their levels vary depending on dietary and physiological factors [1].

Furthermore, the composition of milk fat is significantly dependent on the animal's diet and stage of lactation, which explains the variability of the fatty acid profile [4]. Although one of the main identified isomers—elaidic acid—is characteristic of industrial trans fats, its low levels in milk may also be linked to the natural biohydrogenation process. Furthermore, the presence of elaidic acid is explained as a result of isomeric changes in fatty acids and metabolic processes.

The obtained results indicate that the amount of trans fatty acids in milk is relatively low, and they are primarily isomers of natural origin. The detection of 4.684% total TFA is consistent with the natural lipid profile of milk fat.

The presence of trans isomers is a natural outcome of the biohydrogenation process that occurs in the rumen, in which unsaturated fatty acids are partially converted into trans forms. Therefore, directly comparing the trans fats in dairy products with those from industrial sources is not scientifically justified.

At the same time, the retention times and quantification parameters of the identified isomers confirm that the employed GC-FID method offers high accuracy and selectivity. This indicates that this method can be used as a reliable tool for detecting trans fatty acids in food products.

Conclusion. In this study, fatty acid methyl esters in a local raw milk sample were analyzed by gas chromatography (GC-FID), and trans fatty acid isomers were identified. According to the analysis results, the main trans isomer was elaidic acid (C18:1 trans-9) – 1.547% and linolelaidic acid (C18:2 trans) – 3.137%, with the total trans fatty acid content amounting to 4.684%.

The results obtained confirmed the presence of trans fatty acids in milk and showed that they are primarily formed through the biohydrogenation process that occurs in ruminants. At the same time, the relatively low levels of the identified isomers indicate that the trans fats in dairy products are of natural origin.

The GC-FID method used in the study was characterized by its high sensitivity, selectivity, and reproducibility in detecting fatty acids, particularly trans isomers. This makes it possible to use this method as a reliable analytical tool for assessing fatty acid composition in food products.

Thus, the results of the conducted research are of significant scientific and practical importance for the detection and monitoring of trans fatty acids in dairy products, as well as for assessing food safety.

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