Study of the Chemical Composition and Nutritional Value of Buttermilk Iraqi Cow's Milk

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Abstract:
Butter milk was made by fermenting cow's milk with four types of commercial starters, which are YO-MIX S31, YO-MIX 495, YO-MIX 505, and YO-MIX 511 and Churning it by churn electrophoresis and studying the chemical composition using an anlyzer Eko milk device, carbohydrates and organic acids with HPLC technology, active compounds with GC-MS technology, and measuring pH and titratable acidity. The results showed that the type of starter affected the composition of fat, protein, pH, and titratable acidity. It also affected the concentration of carbohydrates, organic acids and active compounds, as the pH ranged from 4.29-4.53 and the titratable acidity was 0.67-0.84%. It also showed the highest value for protein, fat and ash 2.57, 1.20 and 0.61% respectively and The highest concentrations of lactose, glucose and galactose were 1.96, 0.14 and 0.10% respectively and the highest values of the most concentrated organic acids were succinic 142.20% and lactic 121.80% for the starter YO-MIX S31 and many of the active compounds that reached the highest peak area of 26-Nor- 5-cholesten-3.beta.- ol-25-one 31.61131% and cholesterol 14.88812% in YO-MIX S31 and Propanoic acid, anhydride 13.80384% in YO-MIX 505.

Keywords: chemical composition, Buttermilk , cow milk , Nutritional value , Iraqi Cow's Milk.

Introduction:

Buttermilk is the aqueous phase stream produced during butter production It contains water-soluble milk components which are caseins, whey proteins, lactose, and biologically active substances originating from the milk fat granule membrane MFGM (1) . Buttermilk is made from Churning fermented cream or yogurt by separating the water phase which is the buttermilk from the oil phase which is the butter as a result of repeated collisions that break MFGM and merge the fat globules and collect them to form butter and separate it from the milk fat. (2) .Buttermilk contains protein 3.04 g / 100 ml, fat 1.17 g / 100 ml, lactose 36,147 µg / g and pH 4.43 (3).The milk used to make buttermilk is fermented after adding lactic acid bacteria including *Bifidobacterium bifidum* و *Lactococcus Lactis* و *Lactobacillus acidophilus* Which makes it better nutritionally due to the formation of many biologically active compounds (4) . There are four types of buttermilk, which are sweet cream buttermilk the product consists of turning raw milk into cream and stirring it. The second is sour cream buttermilk resulting from stirring fermented cream. The third is cultured buttermilk resulting from adding lactic acid bacteria strains to skimmed milk and commercial buttermilk available in the market. By farmers by adding strains of lactic acid bacteria to free or low-fat cow's milk(5) . Buttermilk has many benefits, including its role in lowering blood pressure by inhibiting the activity of the enzyme that converts incotensin because it contains biologically active peptides resulting from the digestion of proteins by lactic acid bacteria (6).Lactic acid bacteria reduce the negative risks of

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cardiovascular diseases by decreasing the levels of total cholesterol, triglycerides and low-density lipoprotein and increasing the level of high-density lipoprotein (7) .Buttermilk reduces the symptoms of lactose intolerance resulting from the absence or low concentration of the enzyme lactase in the human digestive system because it forms the enzyme lactase needed to convert lactose into easily digestible lactic acid as a result of the metabolic activity of lactic acid bacteria. It also plays a role in removing the acidity of the stomach that causes indigestion and the release of peptides Active antioxidants that reduce the chances of cancer and increase antimicrobial activity as well as reduce or control weight because it contains less than 50% of calories and fats found in milk (5). The lack of studies and trends for the manufacture and use of this type of dairy products in Iraq and because of its high nutritional and health value, in addition to the fact that the chemical analysis of the Buttermilk produced from fermented milk was not dealt with previously. Therefore, this study was conducted to find out the chemical composition and compounds responsible for giving the flavor and the nutritional and health value of the buttermilk. The factory is made of yogurt and the quality of the starter used to give the preferred product is determined.

Materials & Methods

The research was conducted in the laboratories of the Department of Food Sciences in the College of Agriculture at the University of Basrah and some laboratories of the departments of the College of Agriculture and the institutions and units of the University of Basrah.

Milk Collection

Bovine milk was obtained from the Agricultural Research Centre, College of the Agriculture, University of Basrah .

Starter Type

The starters used in fermentation were obtained from the French company Danisco, which is YO-MIX S31 وYO-MIX 495 و YO-MIX 505 و YO-MIX 511 containing bacterial strains *Streptococcus thermophiles* و *Lactobacillus delbrueckii* subsp.*bulgaricus*.

Manufacture of buttermilk

The buttermilk was prepared after pasteurizing the milk at a temperature of 93°C for 10 minutes, cooling it to 42°C, inoculating it with the activated starter , and incubating it at a 42°C for 8-4 hr until the pH decreased to 4.6-4.3. Shake it using homemade electric churning according to the method described (5) .

Determination of pH and titratable acidity and chemical composition

pH has been estimated by using pH-Meter type Sartorius German Origin(8) and titratable acidity has been measured by calibration with standard sodium hydroxide NaOH 0.1 N by using phenolphthalein as mentioned by(9) . protein and fat and ash ratio has been estimated by anlyzer Eko milk device Dutch origin estimated using the method described by(10).

Organic acids and carbohydrates

Carbohydrates and organic acids were estimated according to the method followed (3) were quantified using the high-pressure liquid chromatography HPLC using Dionix reverse phase and column XSELECT ™ CSH ™130 C 18 3.5 µ -4.6 x10 mm , The stationary phase is silica gel at a temperature of 25 °C with a wavelength of lactose, pyruvic acid and uric acid 298 nm and glucose, galactose, lactic acid, alpha-vaketoglutaric, citric and succinic 292, 290, 390, 267, 210 and

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312 nm, respectively, using the solvent acetonitrile.

Active compounds in Buttermilk

An HP-5ms column and helium gas were used as an inert gas with a flow rate of 1 ml/sec. The temperature of the injector and the interconnector was 290 °C. The GC oven program was set to an initial temperature of 40 °C for 5 minutes, then the temperature was raised to 300 °C for 20 minutes, as it increased by 10 m/min. The curves' spectra were matched with the spectral library, and the separated peaks were applied to the spectra database of the NIST 2014 program library, according to the method presented(3).

Statistical analysis

The studied parameters were done by using Complete Randomized Design (CRD) to analyse the inspected parameters, their effects on the different qualities, comparing the significant differences between the average values and choosing the least difference (P<0.05) using a statistical analysis program SPSS (2016) .

Results & Discussion

pH and titratable acidity

Table 1 shows the values of pH and titratable acidity for buttermilk treatments produced by four types of starter YO-MIX S31, YO-MIX 495, YO-MIX 505 and YO-MIX 511. The results of the statistical analysis showed that there were no significant differences in the pH value and significant differences in acidity. The presupposition among the prefixes at the P < 0.05 probability level. The pH values ranged between 4.29-4.53 and the Titratable Acidity was 0.84-0.67%. The results also showed that the process of fermenting milk had an effect on the pH and acidity, while the type of starter showed an effect on the components of milk after fermentation, such as the production of organic acids and coagulation after reaching the electrical neutrality point at pH 4.6, and this varies according to the activity of the starter s used. The reason for the low pH and high acidity is due to the fermentation of lactose sugar and its conversion to lactic acid due to its fermentation by the starter bacteria (11). The results were similar to what was mentioned(5) Which indicated that the acidity in sour sour milk increased to 1%, while in sweet sour milk 0.15-0.10%.

Table 1 values of pH and titratable Acidity for buttermilk

Titratable Acidity %	PH	Starter
0.72^{b}	4.40[a]	**YO-MIX 495**
0.67^{c}	4.53[a]	**YO-MIX S31**
0.80^{a}	4.32[a]	**YO-MIX 505**
0.84^{a}	4.29[a]	**YO-MIX 511**

Different letters indicate the presence of significant differences, and similar letters indicate no significant differences between the treatments at the level of probability (P<0.05). LSD = 0.067 Titratable Acidity LSD =N.S PH

Total chemical composition of buttermilk

Table 2 indicates the composition of proteins, fats and ash for the fermented milk treatments. The results showed that there was no significant difference in the percentage of protein and moisture and the presence of significant differences for fat and ash at the probability level P> 0.05, as the percentage of

proteins ranged between 2.33-2.57% and the percentage of fat between 1.11-1.20 , as for ash, 0.35-0.61%. The different types of starters used had an effect on the components of the milk yield, as the starter YO-MIX S31 gave the lowest percentage of protein and the lowest percentage of fat and ash. Ash percentage to 0.35% compared to other primers. The results agreed with what he said(12) It was noted that the percentage of protein in the milk fat was reduced by 2.75% compared to raw milk by 3.15%, and that approximately 20% of the proteins of the milk fat were found in the membrane of the MFGM granule, which the process of mixing causes its breakage, which affects the percentage of proteins as well as the hydrolysis of proteins due to the activity of bacteria lactic acid; The results also converged with what was found (13) who noticed that the percentage of fat in the stirred milk is 0.6% due to the repeated physical collisions resulting from the shaking, which caused the merging of the fat globules and its transfer to the butter(2) The results agreed (14) Which found that the percentage of ash ranged between 0.70-0.47 due to the composition of the milk used in the industry. As noted(15)The rise in ash led to a decrease in moisture composition and an increase in the percentage. Mentioned(16) By removing fat, the percentage of moisture increases, which leads to an increase in the amount of dissolved salts, and thus an increase in the percentage of ash. While (17) showed that the use of different prefixes containing *Streptococcus thermophiles* and *Lactobacillus delbrueckii* subsp.*bulgaricus* together when fermenting gives different results in the chemical composition of the milk.

Table 2 Chemical composition of buttermilk

LSD	YO-MIX 511	YO-MIX 505	YO-MIX S31	YO-MIX 495	Composition
N.S	2.57^{a}	2.56^{a}	2.33^{a}	2.38^{a}	**Protein%**
0.083	1.20^{a}	1.15^{a}	1.11^{b}	1.12^{ab}	**Fat%**
0.192	0.55^{b}	0.41^{b}	0.35^{b}	0.61^{a}	**Ash%**
N.S	93.58^{a}	93.61^{a}	93.93^{a}	93.34^{a}	**Water%**

Different letters indicate the presence of significant differences, and similar letters indicate no significant differences between the treatments at the level of probability (P<0.05).

Estimation of carbohydrates in buttermilk

The results in Table 3 refer to the percentages of the average concentration of lactose, glucose and galactose in the buttermilk, as it was noted that the fermentation process and the type of starter used had an effect on the concentrations of carbohydrates, which reached the highest value, which is lactose sugar 0.80-1.96% compared to glucose and galactose in the mixed milk treatments, as the glucose was 0.07-0.14% and the galactose was 0.07-0.10%. Lactose is the main carbohydrate source in milk, and its concentration in cow's milk is 4.6 g / 100 ml. When fermentation, it decreases by about a third due to its transformation into lactic acid by lactic acid bacteria (18). The results agreed with what was mentioned (3), which found that lactose was the highest and ranged between 4.77-2.51 g / 100 gm in raw milk due to the difference in the degree of fermentation and lactose 364 µg / g, because lactic acid bacteria do not ferment lactose and it decreased The concentration of glucose to 59.9 µg/gm due to the lack of decomposition of lactose into glucose and galactose and its

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conversion by the action of lactic acid bacteria to organic acids.

Table 3 Carbohydrates in buttermilk

Galactose	Glucose	Lactose	Starter
0.10	0.10	1.96	**YO-MIX 495**
0.08	0.11	0.96	**YO-MIX S31**
0.09	0.14	0.80	**YO-MIX 505**
0.07	0.07	0.86	**YO-MIX 511**

Organic acids in buttermilk

Table 4 shows The percentages of the concentrations of organic acids in the buttermilk treatments, which are pyruvic, citric, succinic, uric, alpha-ketoglutaric and lactic, as it was noticed that lactic and succinic acids were the most abundant in the milk samples, and the percentage of lactic acid in the milkshake produced using YO-MIX 505 and YO-MIX 511 It is 99.81 and 100.02%, respectively, higher than succinic using the same primers 77.58 and 80.13%, respectively. The percentage of succinic acid in the milk produced using YO-MIX 495 was 124.13% and YO-MIX S31 142.20% higher than lactic acid 85.89 and 128.75%. The reason for this is due to the increase in the fermentation time required for the low pH and coagulation to occur. While the concentration of citric decreased and its highest value was 1.82% in only two treatments, while for pyruvic acid the highest value was 4.79%, uric 1.97% and alpha-ketoglutaric 0.52%. (19) explained that the reason for the decrease in the concentration of citric acid with an increase in the fermentation time is the role of lactic acid bacteria in the metabolism of citrates, meaning that they are able to ferment citrates. And fats and lactose by lactic acid bacteria, as it was found that the concentrations of pyruvic, succinic, lactic and uric acid were 16.2, 136.4, 4575 and 17.68 µg/g, respectively. While (3) mentioned that the fermentation process led to the production of lactic acid in abundance in the milk, while the citric concentration decreased, causing a discrepancy in the ability of lactic acid bacteria to represent citrate and produce acetic acid by metabolizing citrate, while pyruvic acid is An intermediate product in the metabolism of glucose and citrate, and the concentration of succinic is higher with the increase in the fermentation time. The level of citrate representation in lactic acid bacteria is very low as it is consumed at pH 6.9 and absorbed at pH 4.5 due to increased cell permeability at low pH (20). It was mentioned (21) that the variation in the concentrations of organic acids produced was affected by the type of different microorganisms strains used in fermentation, which affected the sensory characteristics of the product.

Table 4 Organic acids in buttermilk

Lactic acid	Alpha keto glutaric	Uric acid	Succinic acid	Citric acid	Pyrovic acid	Starter
85.89	0.26	1.44	124.13	1.82	3.29	**YO-MIX 495**

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128.75	0.42	1.80	142.20	-	3.45	**YO-MIX S31**
99.81	0.52	1.97	77.58	-	4.28	**YO-MIX 505**
100.02	0.39	1.54	80.13	0.26	4.79	**YO-MIX 511**

Estimation of the active compounds in buttermilk

Table 5 and Figure 1 show the active compounds in the YO-MIX 505 buttermilk treatments, and Table 6 and Figure 2 the active compounds in YO-MIX 495 buttermilk. YO-MIX S31, as it was observed that the concentrations of the compounds varied between the samples due to the difference in the activity of lactic acid bacteria in the starters used in fermentation, which affected the sensory and functional properties of the blended milk, as the highest peak area reached 13.80384 % in YO-MIX 505 and 31.61131% for the boat 26 -Nor-5-cholesten-3.beta.-ol-25-one in YO-MIX S31 and propanoic acid, anhydride 9.126932 % in YO-MIX 495 and 14.88812 % cholesterol in YO-MIX 511 with health benefits, (22) indicated that the discrepancy in the concentrations of these compounds is due to the difference in the metabolism of microorganisms and many effective compounds in the milkshake have important health benefits of which are; the anti-fungal effect of the propionic acid and the positive effect of 1,2-Benezenedicarboxylic acid on Alzheimer's, arthritis and cancer (23). Acetic acid is also an antimicrobial due to the effect of this acid on the bacterial cell membrane (24).

Table 5 active compounds in buttermilk YO-MIX 505

Name	Formula	RT	Area %
p-Dioxane-2,3-diol	C4H8O4	5.066	2.721173
(Z)-Difluorodiazene	F2N2	5.964	2.380616
Dimethyl ether	C2H6O	8.547	11.93187
1,2-Cyclopentanedione	C5H6O2	9.008	5.743593
L-Lactic acid	C3H6O3	9.238	3.230663
Octane, 3,5-dimethyl-	C10H22	10.209	3.64879
Propanoic acid, anhydride	C6H10O3	10.314	3.783391
Propanoic acid, anhydride	C6H10O3	12.087	13.80384
Cyclohexanol, 5-methyl-2-(1-methylethyl)-, (1.alpha.,2.beta.,5.alpha.)-(.+/-.)-	C10H20O	13.328	4.371875
Hexadecanoic acid, methyl ester	C17H34O2	22.486	1.352323
Phthalic acid, di(2-propylpentyl) ester	C24H38O4	27.957	2.066553
Ethylamine, N,N-dinonyl-2-(2-thiophenyl)-	C24H45NS	31.725	3.868528
1,3-Benzodioxole-5-methanamine, N-(4-ethoxyphenyl)-	C16H17NO3	33.097	2.419354

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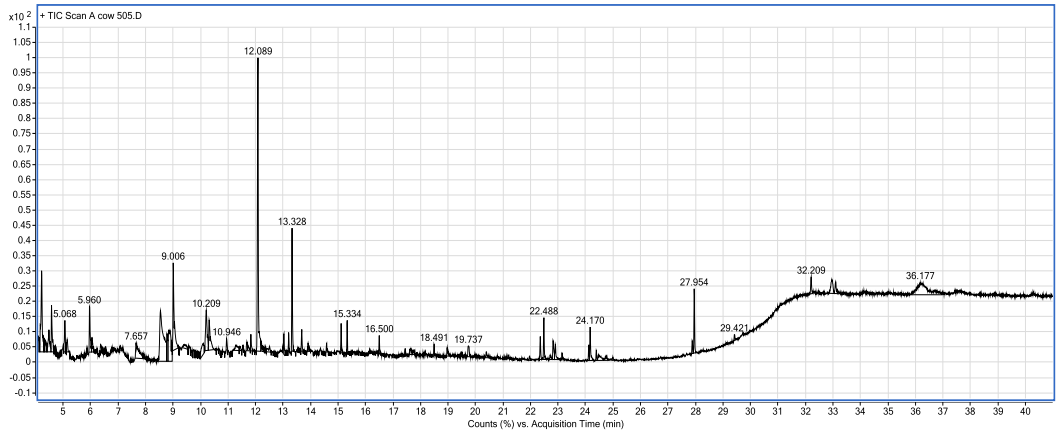

Figure 1 Chromatogram of active compounds in buttermilk YO-MIX 505

Table 6 active compounds in buttermilk YO-MIX 495

Name	Formula	RT	Area %
1,3,5,7-Tetroxane	C4H8O4	4.497	2.793455
Propane, 2-fluoro-2-methyl-	C4H9F	4.664	3.803601
(Z)-Difluorodiazene	F2N2	5.665	2.018788
Methylal	C3H8O2	6.301	2.741629
Ethene, 1,1-dichloro-	C2H2Cl2	6.749	2.332148
Benzene, 1,3-dimethyl-	C8H10	7.005	2.273381
1,2-Cyclopentanedione	C5H6O2	8.91	5.687978
Benzoic acid	C18H24O6	9.55	0.107907
1-Pentanone, 1-phenyl-	C11H14O	10.103	2.418217
Octane, 3,5-dimethyl-	C10H22	10.212	4.532739
Propanoic acid, anhydride	C6H10O3	10.256	3.745026
Propanoic acid, anhydride	C6H10O3	12.049	9.126932
Cyclohexanol, 5-methyl-2-(1-methylethyl)-, (1.alpha.,2.beta.,5.alpha.)-(.+/-.)-	C10H20O	13.326	4.504448
Butanoic acid, 2-methyl-	C5H10O2	15.515	0.156031
Tolycaine	C15H22N2O3	22.354	1.170444
Hexadecanoic acid, methyl ester	C17H34O2	22.485	3.282396
Phthalic acid, 6-ethyl-3-octyl butyl ester	C22H34O4	22.904	0.353258
9-Octadecenoic acid (Z)-, methyl ester	C19H36O2	24.17	1.869351
Phthalic acid, di(2-propylpentyl) ester	C24H38O4	27.956	2.355774
1,4-Benzenedicarboxylic acid, bis(2-ethylhexyl) ester	C24H38O4	29.419	0.139745
1,2-Benzenedicarboxylic acid, 4-methyl-, dimethyl ester	C11H12O4	31.648	2.208316
Cholesterol	C27H46O	32.206	7.050611
4H-Pyrazolo[4,5-E]1,3-oxazine, 4,4-bis(trifluoromethyl)-3-methyl-1-phenyl-6-(1-piperidyl)-	C19H18F6N4O	32.957	6.962987

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Acetic acid, trifluoro-, 1,1-dimethylethyl ester	C6H9F3O2	34.127	1.074556
Succinic acid, di(2-(pentafluorophenoxy)ethyl) ester	C20H12F10O6	40.242	1.218453

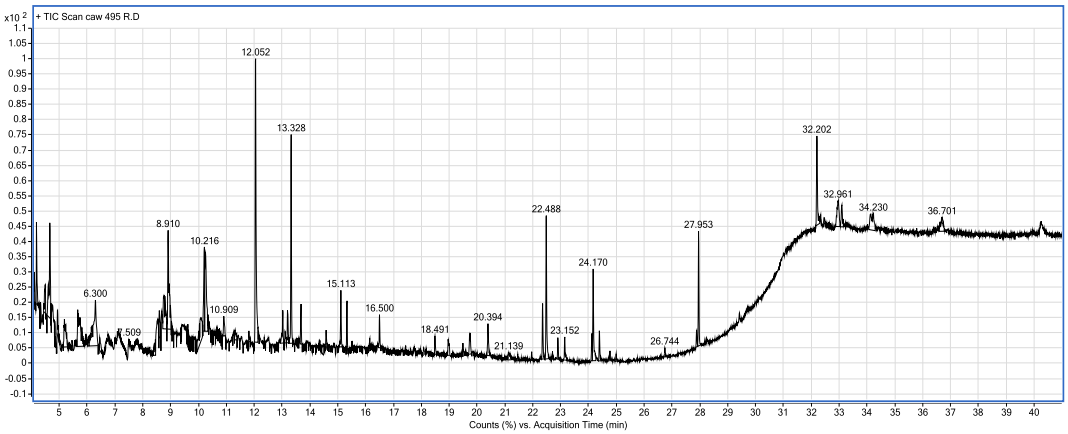

Figure 2 Chromatogram of active compounds in buttermilk YO-MIX 495

Table 7 active compounds in buttermilk YO-MIX 511

Name	Formula	RT	Area %
Ethylbenzene	C8H10	7.106	2.14734
Methylenecyclopropanecarboxylic acid	C5H6O2	7.592	2.435763
Dimethyl ether	C2H6O	8.515	8.274632
Acetic acid, cesium salt	C2H3CsO2	8.677	0.155451
4-Pentynoic acid	C5H6O2	8.858	0.555327
1,2-Cyclopentanedione	C5H6O2	8.982	5.791949
Benzene, 1,2,3-trimethyl-	C9H12	10.085	0.751786
Octane, 3,5-dimethyl-	C10H22	10.21	2.410599
Propanoic acid, anhydride	C6H10O3	10.302	3.201362
Propanoic acid, anhydride	C6H10O3	12.087	11.35347
Cyclohexanol, 5-methyl-2-(1-methylethyl)-, (1.alpha.,2.beta.,5.alpha.)-(.+/-.)-	C10H20O	13.327	2.519784
Hexadecanoic acid, methyl ester	C17H34O2	22.483	2.140227
n-Hexadecanoic acid	C16H32O2	22.821	0.868401
9-Octadecenoic acid (Z)-, methyl ester	C19H36O2	24.167	1.264376
Phthalic acid, di(2-propylpentyl) ester	C24H38O4	27.954	1.246352
1-Pentene, 4,4-dimethyl-1,3-diphenyl-1	C22H30OSi	31.81	9.460473
Adipic acid, 2-isopropylphenyl propyl ester	C18H26O4	32.195	0.167739
Cholesterol	C27H46O	32.205	14.88812

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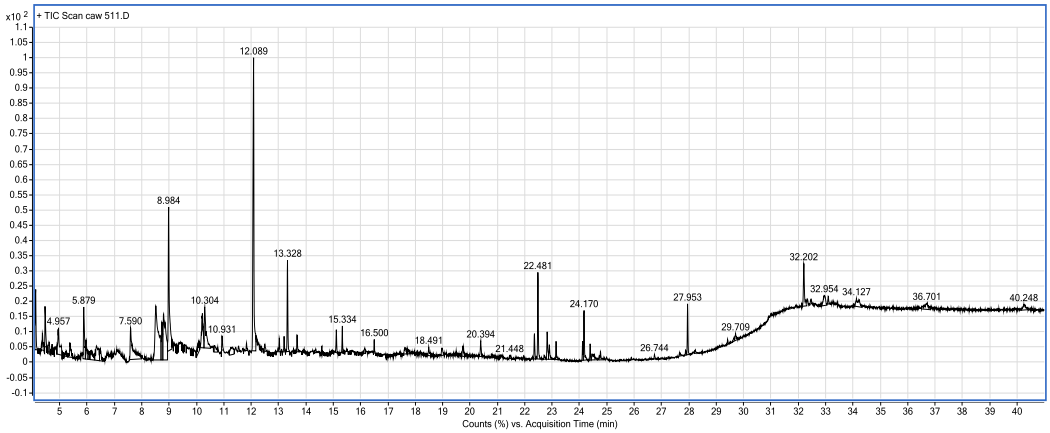

Figure 3 Chromatogram of active compounds in buttermilk YO-MIX 511

Table 8 active compounds in buttermilk YO-MIX S31

Name	Formula	RT	Area %
Acetic acid, methoxy-, methyl ester	C4H8O3	4.308	2.755036
(Z)-Difluorodiazene	F2N2	6.071	2.13384
Benzene, 1,3-dimethyl-	C8H10	7.128	2.234477
Methyltartronic acid	C4H6O5	8.588	1.838334
1,2-Cyclopentanedione	C5H6O2	9.041	5.487094
Octane, 3,5-dimethyl-	C10H22	10.207	3.067507
Propanoic acid, anhydride	C6H10O3	10.329	2.332921
Propanoic acid, anhydride	C6H10O3	12.092	10.77059
Cyclohexanol, 5-methyl-2-(1-methylethyl)-, (1.alpha.,2.beta.,5.alpha.)-(.+/-.)-	C10H20O	13.328	3.185141
Hexadecanoic acid, methyl ester	C17H34O2	22.485	1.518314
Phthalic acid, di(2-propylpentyl) ester	C24H38O4	27.955	1.505232
1-Pentene, 4,4-dimethyl-1,3-diphenyl-1-(trimethylsilyloxy)-	C22H30OS	32.038	10.88327
26-Nor-5-cholesten-3.beta.-ol-25-one	C26H42O2	32.202	31.61131

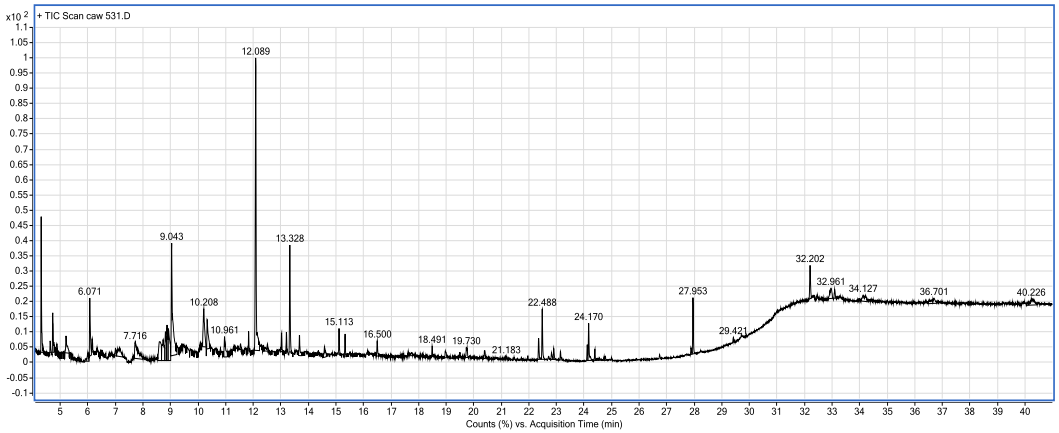

Figure 4 Chromatogram of active compounds in buttermilk YO-MIX S31

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Conclusions

Making buttermilk after churning the yogurt and separating the milk fat from it and studying its chemical composition . It was noted that the pH was low and the acidity was high for the percentage in which coagulation takes place. The proportions of its components such as carbohydrates, proteins, fats and ash were estimated. It was noted that compounds were formed after the fermentation process that affect the quality of this product and these compounds showed A noticeable variation in their concentrations after the decomposition of the basic compounds, including organic acids and active compounds with health-promoting properties resulting from the metabolic activity of lactic acid bacteria, which leads to the possibility of using this product in many healthy food industries due to the local market need for this type of product for its health benefits.

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Conflict of interest

We declare that we have no conflict of interest.

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