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Article

Studying the chemical composition and nutritional value of Iraqi buffalo buttermilk and its use in the manufacture of healthy ice cream

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Abstract

Making buttermilk by fermenting raw buffalo milk with two types of commercial starters, YO-MIX 495 and YO-MIX 505, and stirring it by electrophoresis and estimating the chemical content using an analyzer Eko milk, pH, Titration Acidity percentage, carbohydrates and organic acids using HPLC technology, active compounds with GC-MS technology, antioxidant activity, and making ice cream with three mixtures: 0:1, 1:1 and 1:3 from skimmed milk to raw milk, and physical tests were conducted on it, The results showed that the fermentation time had no effect on the chemical content, but it did affect the pH, surface Titration Acidity, the concentration of carbohydrates, organic acids and active compounds. The highest value of pH was 4.37, and ash was 0.65% for buttermilk fermented with YO-MIX 495 starter, and the highest value for Titration Acidity, protein and fat is 0.84, 2.73 and 1.24%, respectively, with YO-MIX 505 starter. The highest value of carbohydrates is for lactose sugar, 3.30 % with YO-MIX starter 505 and the highest value for organic acids is succinic, 169.82% with starter YO-MIX 505 and lactic, 42.50 % with starter YO-MIX 495 and different ratios of active compounds reached the highest area of the peak % Acetic acid, cesium salt 25.08334% with start YO-MIX 495 and it showed the highest antioxidant activity 75% with the starter YO-MIX 495, and the highest starter diameter against Bacillus cereus was 10 mm for the starter. The mixtures manufactured with YO-MIX starter 495 excelled on the mixtures manufactured with YO-MIX starter 505 in sensory evaluation, and the mixture 1:1 showed the highest value in taste and general acceptance. At the same time, 1:3 was superior in color, texture and oral feeling, while 0:1 obtained the lowest acceptance between the three mixtures.

Keywords: Buttermilk, organic acids, carbohydrates, active compounds, ice cream, starter.

Introduction

Buttermilk is the by-product of the butter production process. It is classified as a functional food because of its water-soluble components, including caseinates, whey proteins, polar lipids and milk fat globule membranes [1]. Milk is the primary material for making buttermilk, and it is one of the most essential foodstuffs that contain many nutritional components, classified as GRAS and generally recognized as safe [2]. Buttermilk is formed as a result of cracking the membrane of a milk fat granule and its connection with another pellet, releasing the oil and forming a network made of the accumulated fat globules, which leads to the phase reversal of the emulsion and the accumulation of butter granules and the separation of the milk mixture from it [3]. There are several types of buttermilk, including cultured buttermilk, which has a sour taste due to lactic acid bacteria added to skimmed milk, sweet buttermilk and whey buttermilk, which differ from each other in chemical composition and functional properties [1]. [3] The chemical composition of buttermilk depends on the amount of water added to the cream. The chemical difference between Sour buttermilk and Sweet cream buttermilk. It is due to the surface Titration Acidity that is higher in sour buttermilk at 1%, while Sweet cream buttermilk is 0.15-0.10% as well as it consists of total solids, lactose, lactic acid, protein and fat 4, 3-4, 1.2, 1.3 and 0.8%, respectively. Buttermilk has multiple health benefits resulting from fermentation, where lactic acid bacteria have an inhibitory effect on the harmful effects of hypothyroidism and reduce levels of total cholesterol, LDL and VLDL triglycerides and increase the level of high-density lipoprotein (HDL) cholesterol, so milk and its products fortified with lactic acid bacteria are used to reduce the risks of heart disease and arterial hypertension [4]. Fermentation reduces the symptoms of lactose intolerance due to the ability of starter bacteria to produce the enzyme lactase that converts lactose into lactic acid and removes the Titration Acidity of the stomach by forming a layer on the stomach lining as well as releasing biologically active peptides with health benefits and weight reduction or control [3]. It is rich in biologically active dipeptides or tri-peptides of proline, lysine and arginine sequences at the C-terminal, which show antihypertensive activity by increasing the inhibitory activity of angiotensin-converting enzyme, the blood pressure regulator resulting from the breakdown of casein due to fermentation [5]. The antioxidant activity of yogurt made from buffalo milk is higher than that of cow's milk because of its content of the antioxidant conjugated linoleic acid (CLA), which interacts with peroxide and hydroperoxide and works to bind iron and prevents oxidation in the membranes of body cells due to the abundance of nutrients in buffalo milk and its ability to Secretion of the enzyme Linoleic acid isomerase with a high efficiency responsible for converting linoleic acid to conjugated linoleic acid. [6] indicated that lactic acid bacteria produce compounds such as organic acids, diacetyl, hydrogen peroxide and bacteriocins during lactic fermentation. This explains the prolongation of the shelf life of perishable foods such as fermented dairy products. When studying the antibacterial activities of pathogenic strains of lactic acid bacteria, it was noted that the highest inhibition diameter of 12 mm was produced by Lactobacillus fermentum, and the lowest inhibition diameter of 8 mm was produced by Leuconostoc mesenteroides subsp against Enterococcus faecalis and E. coli. The increase in consumer demand and desire to consume products that reduce many health problems, including cardiovascular diseases, is one of the most essential reasons that prompted the dairy industry to produce low-fat or fat-free milk, including ice cream, by adjusting the percentage of fat or removing it according to the level required in the product as well. Adding ingredients for dry structure, stabilizers, sugar and homogenization are important in the stability of the product and giving it strength [7]. Ice cream is one of the desired snacks by consumers, as it consists of sugars, proteins, salts, water, ice crystals, air bubbles and fat globules. Replacing fats with buttermilk with vegetable milk to treat or prevent many health problems [8]. This study aimed to estimate the chemical content of buffalo milkshake due to the lack of studies and trends for the manufacture and use of this type of dairy product in Iraq in the food industry and in order to develop a new type of ice cream in which butter milk is replaced with buttermilk with health benefits due to its low-fat content and high value. Food contains health-promoting ingredients.

Materials and methods

The research was conducted in the Department of Food Sciences and some laboratories of the departments of the College of Agriculture, institutions and units affiliated with the University of Basrah. Raw buffalo milk was prepared from a breeder in the Karma area in Basrah province.The starter YO-MIX 495 and YO-MIX 505 containing Streptococcus thermophiles and Lactobacillus delbrueckii subsp.Bulgaricus strains were obtained from the French company Danisco.

Buttermilk industry

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, incubating it at a temperature of 42° C for 8-4 hours, and separating the fat, from the buttermilk using homemade electric percussion according to a method he followed [3].

Estimation of pH, Titration Acidity and chemical content

The milk and ice cream pH was estimated using a German-origin Sartorius pH meter [10] and pH by scaling with 0.1N of NaOH with phenolphthalein index [11]. The protein, fat and ash percentages in buttermilk were estimated using an analyzer Eko milk of Dutch origin [9].

Determination of carbohydrates and organic acids using HPLC technology

Carbohydrates and organic acids were estimated according to the method followed by [12] using high-performance liquid chromatography (HPLC) using Dionix reversible phase and XSELECT™ CSH™130 C 18 column 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 298 nm, glucose, galactose, lactic acid, alpha-vaketoglutaric, citric and succinic 292, 290, 390, 267, 210 and 312 nm, respectively, using the solvent acetonitrile.

Determination of Active Compounds in Buttermilk Using GC-MS

Use a column of type HP-5ms and helium gas as inert gas at a 1 ml/sec flow rate. The temperature of the injector and the interconnector is 290°C. The GC oven program was set at a temperature of 40°C for 5 minutes and raised to 300°C for 20 minutes as it increased by 10°C/minute. The spectra of the curves were matched with the spectral library, and the separated peaks were applied to the spectra database of the NIST 2014 program library, as mentioned in method [12].

Determination of antioxidant activity by DPPH method

The antioxidant activity was estimated by measuring the ability of skimmed milk to reduce DPPH radicals and comparing it with the synthetic antioxidant BHT by the method mentioned [14].

Manufacture of milk ice cream

1000 gm was prepared in three mixtures of 1:1, 1:1 and 1:3 from curdled milk to buttermilk with modifications to the method mentioned [15] a using the TREBS Dutch-made milk ice cream maker.

Ice cream physical tests

The spreadability and melting rate of the ice cream samples were estimated as in the method mentioned by [16].

sensory evaluation

The ice cream was evaluated by 10 specialists from the Department of Food Sciences, College of Agriculture, University of Basra

statistical analysis

Statistical analysis of the results was conducted using the Completely Randomized Design (CRD) in data analysis to study the effect of different treatments, and the significant differences between the means were compared with the least significant difference (LSD) test at the 0.05 probability level using the statistical analysis program SPSS ver.23.

Results and discussion

The results showed that the fermentation process did not affect milk components. However, it affected the pH and Titration Acidity and produced organic acids and active compounds with health benefits [17].

pH, surface Titration Acidity and chemical content

Table 1 shows the pH values, Titration Acidity, protein, fat and ash content for the buttermilk treatments. The statistical analysis results showed no significant differences at the probability level of <0.05 for the pH values, which reached the highest value of 4.37 for fermented buttermilk with the starter YO-MIX 495. Significant differences in the values of the Titration Acidity reached the highest value of 0.84% with the starter YO-MIX 505. There were no significant differences in protein content. The fat and ash were at a probability level of $P<0.05$, where the highest percentage of protein was 2.73%, fat 1.24% with YO-MIX 505 starter, and ash 0.65% with YO-MIX starter 495. The reason is due to the ability of the starter bacteria to convert the sugar lactose into lactic acid and other organic acids, which lowers the pKa value inside the cell and makes its membrane more permeable to substances such as lactate and acetate, which leads to a decrease in the pH value and an increase in the total Titration Acidity [18]. The results also showed differences in Titration Acidity, pH and chemical content between the types of starters. It was noticed [19] that there was a difference in the chemical content of buttermilk when using different primers. When adding the primers 11, 21, 25, 29 and 41 to ferment goat milk, using starter 21, it gave the highest water content of 91.21%, which negatively affected the ash content of 0.70 % and fat 1.03% due to the low activity of this starter that affected the total solids. As for starter 11, it gave the lowest water content of 90.48%, the highest ash content of 0.76%, fat 1.92%, and the highest lactic acid and protein production. The fat percentage in starter 41 was the highest at 2.37%, and the lowest percentage produced by starter 21 because it produces the lipolytic enzyme in a higher percentage. The results agreed with what was mentioned by [20], which found that the pH value of the curd ranged between 4.80-4.83 and the Titration Acidity was 0.612-0.615%. It did not agree with what was found by [3]. which found that the percentage of protein is 1.3% and fat is 0.8%. With what was mentioned by [21], the percentage of protein and fat in buttermilk is 4.28 and 0.59%. It was shown [1] that MFGM proteins represent about 19% of the milk proteins that have biologically active properties, and the mixing process caused their breakdown, which affected the proportion of proteins [22]. As well as the hydrolysis of proteins into smaller peptides due to fermentation [23], part of the protein is transferred to butter after curing. A low percentage of fat in the shaken milk indicated that there was no centrifugal step to remove the fat when it was shaken, as well as the presence of parts of MFGM, small fat globules and free fats that cannot be extracted by centrifugation. When the fat is removed, the moisture content increases, which leads to an increase in the amount of dissolved salts and, thus, an increase in the percentage of ash [24].

Starter	PH	Titration Acidity %	Protein%	Fat%	Ash%
YO-MIX 495	4.37^{a}	0.78 ^{b}	2.71^{a}	1.22 ^{a}	0.65 ^{a}
YO-MIX 505	4.20^{a}	0.84^{a}	2.73^{a}	1.24^{a}	0.53^{a}

Table 1. Values of pH, Titration Acidity and chemical content in buttermilk.

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

Carbohydrates in buttermilk

The results in Table 2 indicate the percentages of the average concentration of lactose, glucose and galactose in the buttermilk made from buffalo milk. It was noticed that the concentration of lactose sugar was the highest compared to glucose and galactose in the buttermilk treatments, as it reached for the YO-MIX 495 and 505 YO-MIX starters 3.02 and 3.30%, and the glucose with 1.11 and 0.14%, respectively, and the galactose 0.09% for both starter. This is because the starter bacteria fermented the lactose and used only part of the glucose during fermentation to reach the required pH, and the lactose was not fermented [17]. The results converged with what was mentioned. [3] who found that the percentage of lactose in buttermilk is 3-4%. [12] found that the lactose content ranged between 2.51-4.77 g/100 g, lactose 364 mg/g and glucose 59.9 mg/g. As the lactic acid bacteria do not ferment lactose, the glucose concentration decreases when the fermentation time is increased.

Starter	Lactose%	Glucose%	Galactose%
YO-MIX 495	3.02	1.11	0.09
YO-MIX 505	3.30	0.14	0.09

Table 2. Carbohydrate Concentration in Buttermilk.

Organic acids in buttermilk

The results in Table 3 indicate the percentages of the concentrations of organic acids in the buttermilk treatments, which are pyruvic, citric, succinic, uric, alpha-ketoglutaric and lactic. It was noted that lactic and succinic acids were the most abundant in the buttermilk samples. It was found that the highest percentage of lactic acid was 42.50% when fermenting with starter YO-MIX 495 and succinic 169.82% with starter YO-MIX 505. The results also showed that the percentage of pyruvic acid was between 4.11 and 3.50% for both starters. [12] indicated that pyruvic acid is an intermediate product in the metabolism of glucose and citrate, and it occurs as a result of the action of lactic acid bacteria and that the succinic concentration is higher with the increase in the fermentation time. Succinic is a by-product of the fermentation process, produced when glucose, produced from the decomposition of lactose, is converted into two molecules of phosphoenolpyruvate, then to oxaloacetic acid, and turns into malate after reacting with two hydrogen molecules, then to fumarate and then succinate. to pyruvate and then to lactic as a result of fermentation [25]. The results converged with the mentioned [26] in that the concentration of lactic acid is 5.6% less than succinic 11.26%. The concentration of citric acid decreased compared to other acids, and its value was 0.82% with the starter YO-MIX 495. No concentration of this acid was observed. When using YO-MIX 505 in fermentation, [12] found that citric acid ranged between 52 and 1712 µg/g, which indicates a decrease in its concentration. As [13] justified, the low concentration of citric acid is the role of lactic acid bacteria in citrate metabolism and their ability to ferment citrate. As for lactic acid, it dominates the other acids, and its participation in fermentation is linked to its ability to benefit from the other components of milk, such as proteins, fats and lactose, as a result of the activity of lactic acid bacteria. A small percentage of uric acid was observed, ranging between 1.89-1.93% and alpha-ketoglutaric acid 2.65-0.90%. The organic acids add a positive hydrogen ion, which lowers the pH inside the cytoplasm and thus prevents molecular interactions in the bacterial cell [27]. The starter activity of acids depends on the value of the acid dissociation constant PKA, as the high value has a lower starter activity than the low value. [28] The variation in the concentration of the resulting organic acids was affected by the type of starters used in fermentation, affecting the final product's characteristics [29].

Starter	Pyrovic acid	Citric acid	Succinic acid	Uric acid	Alpha keto glutaric acid	Lactic acid
YO-MIX 495	3.50	0.82	135.02	1.89	2.65	42.50
YO-MIX 505	4.11	-	169.82	1.93	0.90	13.24

Table 3. Organic acids in buttermilk.

Active Compounds in Buttermilk

Tables 4 and 5 indicate the active compounds in Buttermilk treatments, the time of detention and the area of the peak %, where it was observed that the concentrations of the compounds varied due to the difference in the activity of the starter bacteria used in the fermentation. The highest peak area of %Acetic acid, cesium salt reached 25.08334% for the starter YO-MIX 495 and 4-Methyl-2,4-bis(p-hydroxyphenyl)pent-1-ene, 2TMS derivative 20.3756 % for the starter YO-MIX 505. The results also showed the presence of high concentrations of 1,3-Benzodioxole-5-methanamine, N-(4-ethoxyphenyl) and 2,5-Octadiyne, 4,4-diethyl compounds amounting to 19.34917 and 17.98694 %, respectively. As shown in Figures 1 and 2, the chromatograms of separated active compounds, it was observed that many active compounds with health benefits and volatile compounds responsible for flavor and sensory acceptability, as well as the production of saturated fatty acids resulting from lipolysis, [30] the reason for the formation of compounds such as aldehydes and ketones, is that they result from the oxidation of unsaturated fatty acids and esters resulting from the association of free fatty acids and alcohols. The presence of antibacterial benzene compounds and antifungal propionic compounds was also observed in both starters. [31] mentioned that the active compounds in the milkshake have many benefits, including Dodecenoic acid, which is responsible for the treatment of kidney disease; Glucopyranoside, an antibacterial; benzoic acid, an antimicrobial, a starter of lipase and lipoxygenase; propionic acid, an antifungal, and 1,2-Benezenedicarboxylic acid. Anti-Alzheimer's, Cancer, Arthritis, 1-Docosene, Antibacterial, Anti-cancer, Anti-inflammatory, 9-Eicosene, Antioxidant, Hyperglycemic, 3-Chloropropionic Acid, Antidepressant, Nephrotoxic. [32] Also found that lactose is the source of aldehyde, diacetyl and acetoin produced by lactic acid bacteria and that the main volatile compounds responsible for giving flavor are carbonyl and aldehyde compounds at a concentration of 4–23 mg/kg, diacetyl, acetone, acetoin and butanone, despite their production in small quantities. As well as the production of lactic acid by 0.9% and some other acids resulting from the fermentation of 20-40% of lactose responsible for giving the distinctive flavor to dairy productsThe main alcohol produced is ethanol resulting from the breakdown of glucose and amino acids at a concentration of 0.2-9.9 mg/kg and [33] showed that acids have health benefits such as acetic acid, which is considered an antimicrobial for bacteria resistant to different types of antibiotics, including E. coli, Pseudomonas aeruginosa due to its effect on the bacterial cell membrane.[34] explained that the discrepancy in the concentrations of the active compounds is due to the difference in the metabolism of microorganisms.

Name	Formula	RT	%
Silanediol, dimethyl-	C2H8O2Si	4.441	1.554851
4-Methyl-2,4-bis(p-hydroxyphenyl)pent-1-ene, 2TMS derivative	C24H36O2Si2	6.245	0.432769
Trichloromonofluoromethane	CCl3F	6.656	0.979625
Benzene, 1,3-dimethyl-	C8H10	6.996	2.445417
Ethylbenzene	C8H10	7.768	1.261635
Oxime-, methoxy-phenyl-_	C8H9NO2	8.669	0.963845
Proline, 2-methyl-5-oxo-, methyl ester	C7H11NO3	9.024	0.923398
Benzene, 1,2,3-trimethyl-	C9H12	10.09	0.899678
Octane, 3,5-dimethyl-	C10H22	10.208	4.304377
Cyclotrisiloxane, hexamethyl-	C6H18O3Si3	11.819	0.454519
Propanoic acid, anhydride	C6H10O3	12.07	2.489516
Cyclohexanone, 5-methyl-2-(1-methylethyl)-, trans-	C10H18O	13.031	0.5756
Cyclohexanol, 5-methyl-2-(1-methylethyl)-, (1.alpha.,2.beta.,5.alpha.)-(.+/-.)-	C10H20O	13.327	4.665625
Carbonic acid, (1R)-(-)-menthyl butyl ester	C15H28O3	15.119	0.986668
Benzenemethanol, .alpha.-[1-(ethylmethylamino)ethyl]-, [R-(R*,S*)]-	C12H19NO	22.361	0.500725
Hexadecanoic acid, methyl ester	C17H34O2	22.5	0.817673
Bis(2-ethylhexyl) phthalate	C24H38O4	27.959	2.071244
Thiophene, 2-bromo-3-methyl-	C5H5BrS	31.16	0.286273
4-Methyl-2,4-bis(p-hydroxyphenyl)pent-1-ene, 2TMS derivative	C24H36O2Si2	31.693	23.195
Acetic acid, cesium salt	C2H3CsO2	31.934	25.08334
Succinic acid, 1,1,1-trifluoroprop-2-yl 2-decyl ester	C17H29F3O4	32.954	0.333214
Ergosta-6,22-dien-3-ol, 5,8-epidioxy-, (3.beta.,5.alpha.,8.alpha.,22E)-	C28H44O3	32.968	0.533806
1,3-Benzodioxole-5-methanamine, N-(4-methoxyphenyl)-	C16H17NO3	33.108	19.34917
1H-1,2,4-Triazol-1-acetic acid, 3-amino-, methyl ester	C5H8N4O2	39.577	0.308771

Table 4. Active Compounds in Yogurt Milk for Starter 495 YO-MIX.

Name	Formula	RT	%
2,2-Dimethoxybutane	C6H14O2	4.53	4.311284
1,3,5,7-Tetroxane	C4H8O4	4.659	1.652644
Propane, 1,1-diethoxy-	C7H16O2	5.697	1.128299
Silane, methylenebis[methyl-	C3H12Si2	5.885	1.304318
Methylal	C3H8O2	6.314	1.664714
Trichloromonofluoromethane	CCl3F	6.742	3.309572
Benzene, 1,3-dimethyl-	C8H10	7.093	3.936842
1H-Imidazole-4-methanol	C4H6N2O	7.64	0.835759
3-(1'-pyrrolidinyl)-2-butanone	C8H15NO	8.928	2.576048
Octane, 3,5-dimethyl-	C10H22	10.21	5.816717
Propanoic acid, anhydride	C6H10O3	12.043	9.33743
Cyclohexanol, 5-methyl-2-(1-methylethyl)-, (1.alpha.,2.beta.,5.alpha.)-(.+/-.)-	C10H20O	13.326	5.305343
(1-Methoxy-pentyl)-cyclopropane	C9H18O	13.899	1.290246

Bicyclo[2.2.1]heptane, 1,7,7-trimethyl-	C10H18	15.114	1.214246
Cyclotetrasiloxane, octamethyl-	C8H24O4Si4	15.333	1.353712
.beta.-D-Glucopyranose, 1,6-anhydro-	C6H10O5	17.646	0.845481
Tolycaine	C15H22N2O3	22.357	0.855906
Hexadecanoic acid, methyl ester	C17H34O2	22.487	1.046954
9-Dodecenoic acid, methyl ester, (E)-	C13H24O2	24.171	1.087633
Bis(2-ethylhexyl) phthalate	C24H38O4	27.957	2.800798
4-Methyl-2,4-bis(p-hydroxyphenyl)pent-1-ene, 2TMS derivative	C24H36O2Si2	31.839	20.3756
2,5-Octadiyne, 4,4-diethyl-	C12H18	33.099	17.98694
Fumaric acid, octyl pent-4-en-2-yl ester	C17H28O4	35.188	0.955183
9,10-Anthracenedione, 2-methyl-1,6-bis[(trimethylsilyl)oxy]-	C21H26O4Si2	36.145	5.995416
trans-3-Trifluoromethylcinnamic acid, 2-fluorophenyl ester	C16H10F4O2	36.188	1.318033

Table 5. Active Compounds in Yoghurt 505 YO-MIX starter.

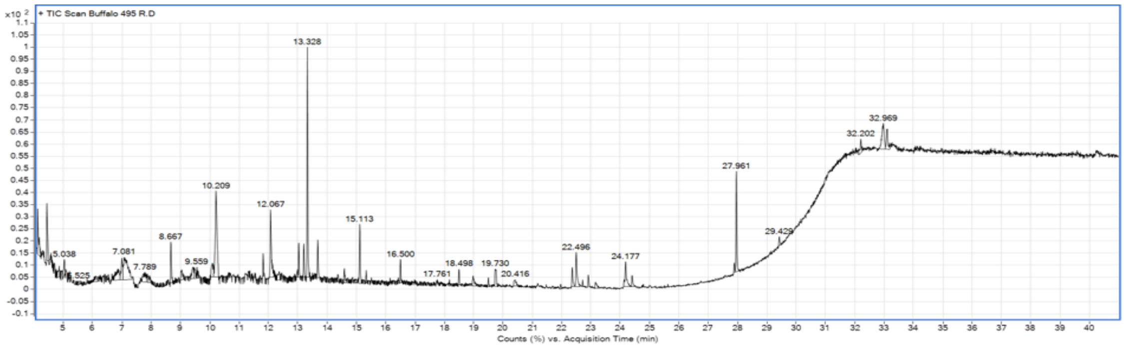

Figure 1. Chromatogram of separated active compounds for the starter495 YO-MIX.

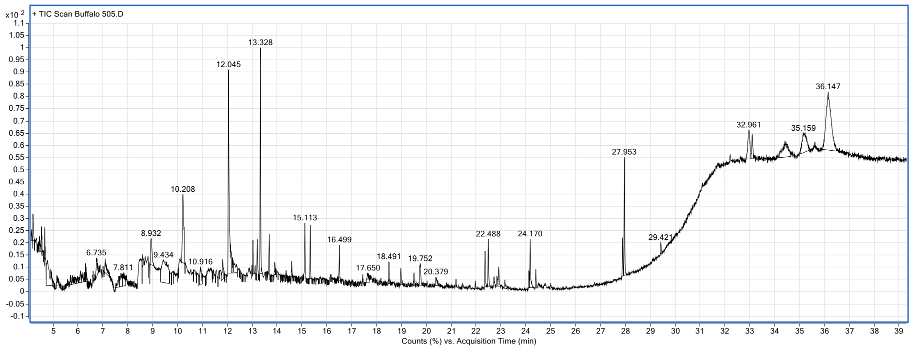

Figure 2. Chromatogram of separated active compounds for starter 505 YO-MIX.

Antioxidant activity of buttermilk

The results in Figure 3 indicate the antioxidant activity of milk shaken by the DPPH root method and compare it with the synthetic antioxidant BHT at a concentration of 5 mg/ml. The results of the statistical analysis indicate that there are no significant differences between the studied samples and the presence of significant differences between the concentrations of the treatments ($P < 0.05$); the effectiveness was increased by increasing the concentration from 61, 70 and 75% at a concentration of 5, 10 and 15% for the buttermilk with the starter YO-MIX 495 and 37, 38 and 50% for the same concentrations for the starter YO-MIX 505. It was also noticed that the buttermilk with the starter YO-MIX495 was more effective as an antioxidant than Initiator YO-MIX 505. The reason for the presence of antioxidant activity in buttermilk is that it contains high amounts of biologically active peptides that show the highest effectiveness in removing free radicals [5]. [35] mentioned that peptides resulting from hydrolysis, enzymatic or thermal hydrolysis have antioxidant properties and were expressed as Trolox equivalent antioxidant capacity (TEAC), i.e., the milk concentration in g/l that produces the same inhibition as trolox. It is a solution similar to vitamin E at 1 mmol/L. These results are consistent with what was found by [36]. It indicated that the highest value was 63.99 for Buttermilk by Lactobacillus acidophilus, and the lowest value was 39.43 for Buttermilk by Streptococcus thermophilus and Lactobacillus delbrueckii ssp. bulgaricus because the initiator strains used in the fermentation of different dairy products analyzed the starter components of milk, such as proteins, carbohydrates and fats, into secondary components, such as free amino acids, active peptides, organic acids and free fatty acids with health benefits.

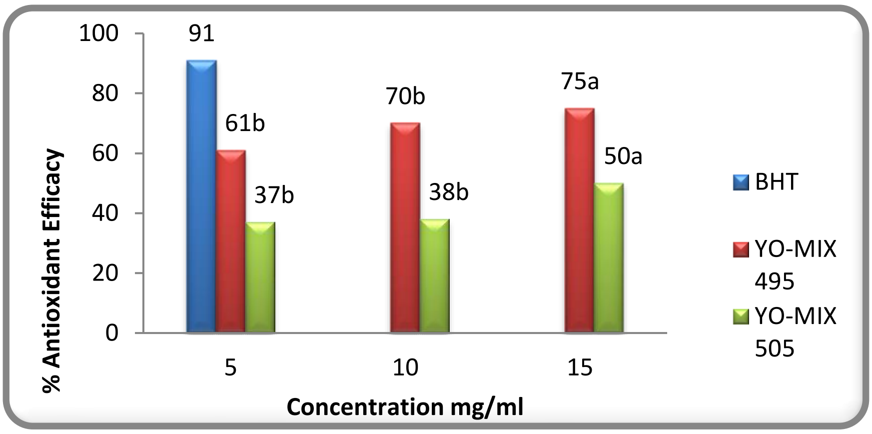

Figure 3. Antioxidant activity of the DPPH root method. Different letters indicate the presence of significant differences, and similar letters indicate no significant differences between the treatments at the probability level (P<0.05).

Physical tests of ice cream for ice cream

The pH, Titration Acidity, spreadability and melting rate of ice cream were tested when it was manufactured with three mixtures of 0:1, 1:3 and 1:1 from mixed milk to raw milk and compared with the control sample made from buttermilk only. The results in Table 6 showed significant differences in pH and Titration Acidity values and no significant differences in diffusion time and melting rate at the probability level of $P < 0.05$ between the ice cream treatments. It was noted that the pH values decreased, and the Titration Acidity of the ice cream increased in the 1:1 mixture compared to the control sample. It was also noted that the Titration Acidity was lower than the milk made from it due to the addition of sweeteners. The pH values of the ice cream made from buttermilk fermented with the starter YO-MIX 495 ranged between 5.51-6.38, and for the starter YO-MIX 505, 5.40-6.36, and the Titration Acidity of the treatments increased from the control sample, which ranged between -0.36 and 0.64% for both starters. The pH values increased, and the Titration Acidity decreased in the 1:1 and 1:3 mixtures compared to the 0:1 mixture as the replacement ratio increased as a result of the higher percentage of solids in the yogurt made from skimmed milk and buffalo buttermilk compared to ice cream made from buttermilk alone.

Furthermore, [37] stated that the increase in Titration Acidity and milk coagulation at a pH between 5.0-5.4 and the formation of lactic acid led to a decrease in the pH to 4.75-4.92, which led to an increase in Titration Acidity to 0.7-0.8%, which is higher than in the buttermilk used in manufacturing. The results are in agreement with what was mentioned by [38], who found that the pH values of the ice cream made from cultured buttermilk ranged between 5.16-5.66 and the precipitation

Titration Acidity to 0.56-0.58 %, and the values in sweet milkshake were 6.53-6.66 and 0.18 -0.24%, respectively, depending on the type of milk used in manufacturing, heat treatment, agitation, fermentation and solids. It was also observed that the spreadability increased from 125 mm in the control sample to 165, 151 and 140 mm for the 0:1, 1:3 and 1:1 mixtures, for the YO-MIX 495, 162, 149 and 139 mm for the YO-MIX 505, initiator for the same mixtures, respectively. It was also observed that the spreadability decreased with the increase in the replacement ratio, where the highest value reached 165 mm for the YO-MIX 495 starter ice cream samples in the 1:1 mixture, and the lowest value was 139 mm for the YO-MIX 505 starter samples in the 1:1 mixture after 10 minutes. The reason is the increase in the percentage of solids with an increase in replacement [16]. Note [39] The solubility decreases when adding fruit due to the high percentage of total solids of ice cream with fruit, with a percentage ranging between 38.06-39.08% compared to the control sample, 36.69%, which led to high viscosity and low solubility. It was also noted that the melting rate in the ice cream treatments with the three mixtures increased compared to the control sample's melting rate. It decreased with the increase in the percentage of milk replacement, as it reached 55.8% in the control sample. It reached 98.8, 88.8 and 78.8% in the mixtures 1:1, 1:3, and 1:1, respectively, for the starter YO-MIX 495 also amounted to 98.5, 88.4 and 78.6% for the starter YO-MIX 505 for the same mixtures, respectively, after 50 minutes. The results were close to what was found by [16] who estimated the melting rate of about 97.2% and 2.5% foam, while the control sample was 80.2% and 19.8% foam, and the melting rate increased than the regular ice cream, as milk fat plays a distinctive role in reducing The melting point of ice cream increases viscosity and encourages the formation of ice crystals. [40] explained the differences in the melting rate to the instability of the fat and the size of the ice crystals, which leads to an increase in the volume of the trapped air content and, consequently, an increase in the apparent viscosity and, consequently, the lack of fusion. [41] showed that the water content of the milky ice mixture turns into ice crystals, which leads to an increase in the average size of ice crystals, which ranges between 20-55 m. The melting rate is also affected by several factors, including the amount of retained air, the fat content, the yield, the size of the ice crystals, the concentration of stabilizers, the temperature and the viscosity of the product [42].

Starter	Mixtures	pH	Titration Acidity %	response time/min	melting rate %
Control		6.50	0.29	125	55.8
YO-MIX 495	0:1	5.51^{c}	0.58^{a}	165^{a}	98.8^{a}
	1:3	5.63^{b}	0.47^{b}	151^{a}	88.8^{a}
	1:1	6.38^{a}	0.36^{c}	140^{a}	78.8^{a}
YO-MIX 505	0:1	5.40^{c}	0.64^{a}	162^{a}	98.5^{a}
	1:3	5.59^{b}	0.50^{b}	149^{a}	88.4^{a}
	1:1	6.36^{a}	0.37^{c}	139^{a}	78.6^{a}

Table 6. Physical tests for ice cream.

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

Sensory evaluation

Table 7 shows the results of sensory evaluation of 1:1, 1:1 and 1:3 ice cream mixtures made from milk mixed with YO-MIX starter 495 and YO-MIX starter 505 in the characteristics of color, appearance, texture, taste, flavor, oral feel and general acceptance. The statistical analysis results showed significant differences between the samples for color, texture, oral feeling, and general acceptance, and there were no significant differences in taste at the level of probability < 0.05. Mixtures manufactured with YO-MIX starter 495 outperformed those manufactured with YO-MIX starter 505 in the best sensory receptivity. It was also noted that the 1:1 mixture was superior in taste and general acceptance, with values of 14.0 and 14.6, respectively, due to the increase in the proportion of replacing raw milk with buttermilk, but the 1:3 mixture. It gave good results in color, texture and oral feel: 14.6, 32.0 and 18.3%, and its health excelled due to the low-fat percentage. The 0:1 mixture obtained the lowest degree of acceptance among the three mixtures due to the low percentage of fat that gives the ice cream acceptable sensory qualities because it is made of 100% pure milk and has high acidity. These results agreed with what was mentioned by [38] in the decrease in the sensory characteristics of the ice cream made from sweet, which obtained a degree of 4.19 and 4.26, respectively, compared to the control sample of 4.426. The ice cream made from buttermilk was distinguished by its higher acidity than natural ice cream. [15] When studying the effect of adding inulin and sugar on the sensory properties of ice cream made from yogurt and probiotics found that the increase in the sugar percentage caused an improvement in texture, flavor and aroma and that adding inulin had no effect on the sensory properties of ice cream Also, its flavor was not noticeable due to the high acidity of the ice cream. The increase in consumer demand and the need to consume products that reduce many health problems is one of the most important reasons for studying the manufacture of low-calorie, nutritional and healthy ice cream as one of the desired snacks by the consumer, As well as the health benefits gained after fermenting milk due to lactic acid bacteria and the low percentage of saturated fats.

Starter	concentration	Color and appearance15	texture 35	Taste and flavor 15	Oral feeling 20	general acceptance 15	Total 100
Control		15.0	31.0	14.0	18.0	14.0	92.0
YO-MIX 495	0:1	13.0^{b}	30.0^{b}	13.6^{a}	15.0^{b}	13.3^{ab}	84.9
	1:3	14.6^{a}	32.0^{a}	12.0^{a}	18.3^{a}	14.0^{a}	90.9
	1:1	14.3^{a}	27.3^{b}	14.0^{a}	17.0^{a}	14.6^{a}	87.2
YO-MIX 505	0:1	12.6^{b}	26.0^{ab}	12.0^{a}	13.6^{b}	13.0^{ab}	77.2
	1:3	14.0^{a}	31.0^{a}	12.6^{a}	18.3^{a}	13.6^{a}	89.5
	1:1	14.3^{a}	28.6^{a}	13.6^{a}	14.6^{a}	14.0^{a}	85.1

Table 7. Sensory evaluation of ice cream mixtures.

Conclusions

Buttermilk was produced after churning the yogurt made from buffalo milk, separating the fat from it, and determining its chemical content, pH, and Titration Acidity in the buttermilk. It was observed that compounds formed after the fermentation process affect the quality of this product. These compounds showed a noticeable variation in their concentrations after the decomposition of the basic compounds, organic acids, and the production of effective compounds in varying proportions with health-promoting properties, such as antioxidants, to the local market need for this product.

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Conflict of interest
The authors declare no conflict of interest.

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