ISSN NO: 2771-8840
Date of Publication: 11-10-2022

Study of the chemical content and antimicrobial activity of Iraqi butter milk of cows and buffaloes

Raghad Saad Al Musa[1,*], Najla Hussen. Al Garory [2]
[1,2]Department of Food Sciences, College of Agriculture, University of Basrah
Correspondence: agripg.raghad.saad@uobasrah.edu.iq Najla.saper@uobasrah.edu.iq

Abstract: This study was conducted to estimate the chemical content of cow and buffalo milk fermented with four types of commercial starters, namely YO-MIX 495, YO-MIX S31, YO-MIX 505, and YO-MIX 511, after activation of the prefix bacteria for the manufacture of yogurt and stir it by electrophoresis and determination of the chemical content using a device. Anlyzer Eko milk, pH, acidity percentage, and carbohydrates percentage by HPLC technique and evaluation of the inhibitory activity of mixed milk against five types of pathogenic bacteria, namely Bacillus cereus, *Staphylococcus aureus*, *E.Coli*, *Enterobacter aerogenes* and *Pseudomonas aeruginosa* The results showed that the fermentation process had an effect on the chemical content, pH, and surface acidity, as the highest value of pH, acidity, protein, fat, ash, solids, and moisture for cow's milk treatments reached 4.53, 0.84, 2.57, 1.20, 0.61, 6.66 and 93.93%, respectively. For buffalo milk treatments, 4.41, 0.85, 2.76, 1.30, 0.72, 7.08, and 93.51%, respectively, the highest value of carbohydrates is for lactose in cow's milk butter milk, 0.80 - 1.96%, and in buffalo's milk buttermilk 2.44-3.30%, the results also showed that the milk has an inhibitory activity against Gram-positive. Negative bacteria were higher in milk made from buffalo than in cow buttermilk. The highest inhibitory diameter was 12 mm for gram-positive bacteria and 9 mm for gram-negative bacteria.

Keywords: shaken milk, antibacterial activity, MacFarland, inhibitory aura, pathogenic bacteria, prefixes.

Introduction

Buttermilk is defined as the by-product of the butter production process. It is a functional food because of its water-soluble components, including proteins, polar lipids, and biologically active milk fat globules (Ali, 2019). Buttermilk is made from milk, one of the most important foodstuffs containing many nutrients. Nutritional ingredients, including proteins classified as GRAS, are Generally Recognized as Safe, which acts as anti-microbial, anti-cancer, anti-viral, and enhance immunity in many food industries (Al-Hatim et al., 2020). Lactic acid bacteria have an inhibitory effect on hypothyroidism's negative effects, reduce total cholesterol and LDL triglycerides levels, and increase the level of high-density lipoprotein (HDL) cholesterol. Therefore, milk and its products fortified with lactic acid bacteria are used to reduce the risks of heart disease and arterial hypertension et al., 2021) Nasser). Milk is fermented with lactic acid bacteria to produce yogurt, which makes it better nutritionally due to the formation of many biologically active compounds resulting from the fermentation (Bhukya and Bhukya, 2021). Then the process of churning the curd and separating the curd from the butter as a result of repeated collisions that break the membrane of the milk fat granule and merge the fat globules and combine them to form butter and separate it from the milk fat (Conway et al., 2014).

Buttermilk contains most of the components of the milk fat granule membrane and small amounts of triglycerides, depending on the conditions of butter manufacturing, as well as containing biological properties from the fat granule membrane because it contains phospholipids that have anti-inflammatory activity, bacterial toxins, and antioxidants (Sakkas et al., 2022). (Sodini et al., 2006) mentioned that the composition of buttermilk is similar to that of curd, except for the fat, which is less in buttermilk. Still, it increases it with its content of phospholipids, which have biological activity and anti-cancer ability, as well as a protective effect against bacterial toxins and infection. Lactic acid bacteria are one of the probiotic strains most suitable for treating and preventing diseases due to their

 ISSN NO: 2771-8840
Date of Publication: 11-10-2022

ability to adhere to intestinal cells and thus treat gastrointestinal diseases. It has an important role in inhibiting pathogenic bacteria such as organic acids and bacteriocins, which provide an acidic environment unsuitable for bacterial growth and thus inhibit their growth (Forestier et al., 2001).
Savadogo et al. (2004) indicated that lactic acid bacteria produce various compounds after fermentation, such as organic acids and bacteriocins, during lactic fermentation, which explains the prolongation of the shelf life of perishable foods such as fermented dairy products. The inhibition diameter is 12 mm, produced by Lactobacillus fermentum. The minimum inhibition diameter is 8 mm.(Røssland et al., 2005)determined the fermentation products, which are lactic acid, work to raise the acidity, which leads to the inhibition of pathogenic bacteria, and that the reason for the increase in the production of these acids by lactic acid bacteria. It is the ability to hydrolyze protein and produce moderate amounts of acetic acid during fermentation, which is a more inhibitory preservative than lactic because of its high Pka value. (George ,2004) indicated that the inhibitory activity of bacteriocins increases when some factors, such as organic acids, are available. The proteolytic enzymes quickly degrade the bacteriocins in the alimentary canal. They are easy to move and target the bacterial cell by sticking to its surface and penetrating the outer cell wall, which causes deterioration in its DNA and inhibition. Synthesis of peptidoglycans and bacteriocins to have a wide inhibitory range of pathogenic and food spoilage bacteria.

The aim of the research

Due to the lack of studies and trends for the manufacture and use of this type of dairy product in Iraq, the growing health awareness related to diet, and the desire to produce functional drinks that promote health, the buttermilk. Through identifying the effectiveness of some strains of lactic acid bacteria and their ability to analyze milk components and produce anti-growth of pathogenic microorganisms.

Materials and Methods

Activate starter

The prefixes were activated after sterilization of the sorting milk at a temperature of 121 °C for 15 minutes and cooled to 42 °C. Then inoculated with the initiator 0.05 g/100 ml skimmed milk by direct addition and incubated at 42 °C for 18 hours. The process was repeated three times under sterile conditions in the same proportions and conditions, then Cryopreservation (Dave and Shah, 1996) and (Al-Shihabi et al., 2015).

Buttermilk manufacturing

Making buttermilk after treating raw cow and buffalo milk at pasteurization temperature at 90°C for 5-10 minutes, then cooling to 42°C and inoculating with the commercial starters YO-MIX -495, YO-MIX S31, YO-MIX 505, and YO-MIX 511 with the indicated quantity. The producing company at a rate of 2% and incubated at 42°C for 4 hours until the pH dropped to 4.6-4.3, then cooled to 4°C. for 45 minutes and excluded the resulting butter according to the method (Gandhi et al., 2018).

Physical and chemical examination of buttermilk

Estimation of pH and surface acidity

The pH was estimated by using the Sartorius pH meter in th ebutter milk sample, and the acidity was estimated by slaking with 0.1 N sodium hydroxide solution as mentioned in (A.O.A.C., 2008) and according to the equation:

Precipitation acidity % = **(0.009 x NaOH N 0.1 milliliters number) / (milk sample weight)** × 100

Chemical content of buttermilk

The percentages of buttermilk components, including fat, protein, total solids, moisture, and ash, were estimated using an anlyzer Eko milk device (Dutch origin) (Coroian et al., 2019).

Carbohydrate estimation for a buttermilk

ISSN NO: 2771-8840
Date of Publication: 11-10-2022

The determination of the organic acids and carbohydrates in butter milk was done by using the liquid chromatography technique High-performance HPLC using Dionix Reverse phase using XSELECT™ column / -4.6 x 10 mm CSH™ 130 C 18 3.5 µ The stationary phase is silica gel, and the detection of carbohydrates by UV detector at 25 °C as previously described (Gebreselassie et al. ., 2016).

Mueller-Hinton agar medium

The medium with pH 7.3 was prepared by dissolving 38 g of it in 1 liter of distilled water according to the instructions of the manufacturer Hi-media company to estimate the antibacterial activity (Mohsen, 2011).

McFarland solution

Prepare by dissolving 0.5 ml of barium chloride prepared by dissolving 1.75 gm of barium chloride in 100 ml of distilled water in 99.5 ml of sulfuric acid at a concentration of 1% prepared by adding 1 ml of sulfuric acid (at a concentration of 98%) to 100 ml of distilled water for comparison and giving the number An approximation of bacterial cells is
1.5 × 〖10〗 ^8 bacterial cells/ml by measuring the turbidity at 600 nm (Mohsen, 2011).

Estimation of the pathogenic antimicrobial activity of butter milk

The antimicrobial activity was estimated using 5 types of pathogenic bacteria obtained from Basra University, College of Science, Environment Department: *Bacillus cereus*, *Staphylococcus aureus*, *E.Coli*, *Enterobacter aerogenes*, and *Pseudomonas aeruginosa* after being activated on Nutrient Broth medium for three dilutions and incubated at 37°C. C for 24 hours, after comparing it with McFarland's solution to match the turbidity and choose the optimal dilution after measuring the absorbance at 600 nm. 0.1 ml of it was spread on Petri dishes containing Mueller-Hinton agar medium and left for 30 minutes. The medium was punctured using a 6 mm diameter cork piercer. 0.1 ml of the milk samples were prepared at a concentration of 15 mg/ml distilled water, and the dishes were incubated at 37 °C for 24 hours. Hourly and record the diameter of the growth-free zone according to the method reported by (Gonelimali et al., 2018) and (Zapata and Ramirez-Arcos, 2015).

Statistical analysis

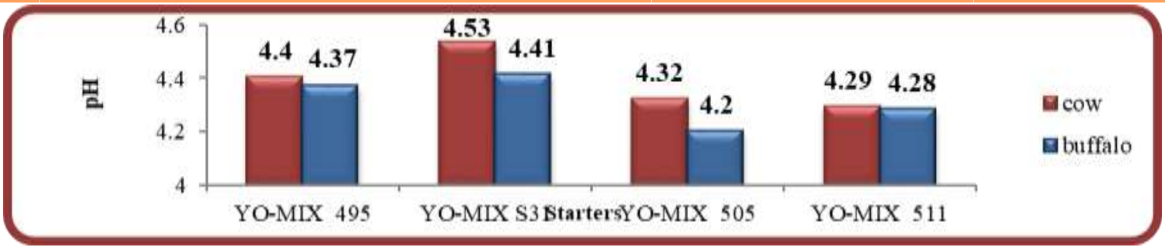
The data were statistically analyzed using the Completely Randomized Designs (CRD), ANOVA, and LSD tests to calculate the significant differences between the mean of the coefficients with a probability level of 0.05 using Genstat software version 12.1 (Schmuller, 2017).

Results and discussion

Physical properties of butter milk

pH and surface acidity

The results show in Figure (1) the pH values of the buttermilk, as the results of the statistical analysis showed that there were significant differences between the treatments according to the different starters and the superiority of cow's milk made using the starter YO-MIX S31 with the highest PH value ($P \leq 0.05$). The PH values are higher in cow's butter than in buffalo buttermilk for the four primers due to the lower percentage of total solids. And the PH values varied according to the different starters used in fermentation, as the PH values of the milk made from cow's milk ranged between 4.29-4.53% and from buffalo milk 4.20-4.41%. The difference is due to the variation in the ability of the initiator bacteria to produce organic acids, including lactic acid, as well as its effect on the percentage of total solids. On the other hand, the positive charges of lactic acid are produced during fermentation until the electrolyte point is reached at PH 4.6. The results agreed with what was found by Gonfa et al., 2001), who noted that the pH of the buttermilk was 4.5-4.3.



LSD Type = 0.1069 LSD Starter = 0.1511 LSD Interaction = 0.2137

Figure (1) pH values for buttermilk treatments

Acidity of buttermilk

Figure (2) shows the acidity values of the buttermilk treatments, estimated in percentage, and the statistical analysis results showed the superiority of buffalo butter milk over cow's milk. There were differences between the starter treatments, as the treatments made from the YO-MIX 511 starter were significantly superior ($P \leq 0.05$). It was observed that the values of the buffalo acidity were inversely proportional to the pH, and it was noted that the acidity of cow's milk buttermilk ranged between 0.67-0.84 %, which is less than that of buffalo milk buttermilk 0.70-0.85%. Initiator bacteria convert lactose into lactic acid and other organic acids, which lowers the value of PKa 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 acidity (Yilmaz-Ersan and Kurdal, 2014). And Benkerrouma and Tamime (2004) indicated that the high acidity is due to the fermentation of lactose sugar and its conversion to lactic acid due to the initiator activity during fermentation. These results agreed with what was found by (Kumar et al. 2015), which indicated a high total acidity in sour curdled milk, which reaches 1%, while in sweet, sour milk, it is 0.15-0.10%.

LSD Type = 0.01609 LSD Starter = 0.02276 LSD Interaction = 0.03219

Figure (2) shows the percentage of acidity of buttermilk treatments

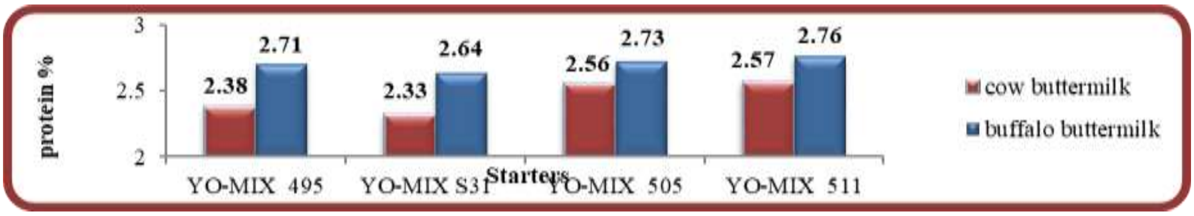
Chemical content of buttermilk

Proteins

Figure (3) indicates the average percentages of protein in the buttermilk treatments. The statistical analysis showed significant differences between the types of butter milk, as it was superior to buffalo butter milk. There were no significant differences between the starter treatments ($P \leq 0.05$). It was noted that the percentage of protein in cow's milk butter milk is less than in buffalo buttermilk for the four primers. Note (Ogrodowczyk et al., 2021) that the reason for the low percentage of protein in stirred milk is that the milk proteins are found in the membrane of the MFGM granule. The results showed that the highest average protein percentage in cow's milk was 2.57% fermented with YO-MIX 511 starter and 2.76% for the same starter in buffalo milk butter milk.

In comparison, the lowest percentage reached 2.33 and 2.64% for both cow's buttermilk and buffalo milk fermented with starter milk YO-MIX S31, respectively, due to the different abilities of primers to hydrolyze the protein (Wati et al., 2018). The hydrolysis of proteins leads to the formation of smaller peptides due to fermentation, which reduces the proportion of proteins (Conway et al.,2013). The results are in agreement with what was mentioned by (Ogrodowczyk et al., 2021), as it was noted that the percentage of protein in milk was reduced by 2.75% compared to milk Raw 3.15%. And to what was found (Skryplonek et al., 2019), the percentage of protein in the buttermilk samples was 3-3.6%.

ISSN NO: 2771-8840
Date of Publication: 11-10-2022

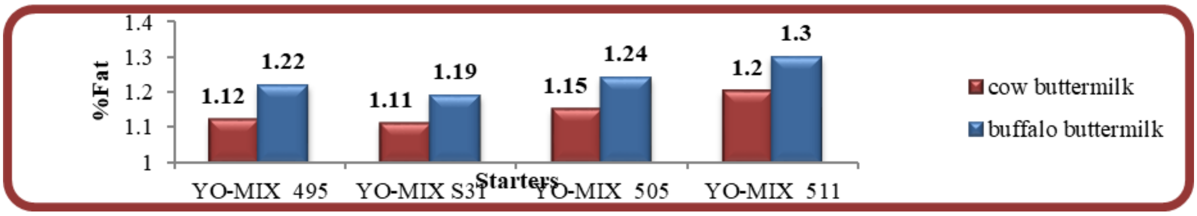


LSD Type = 0.1480 LSD Starter = 0.2093 LSD Interaction = 0.2960

Figure (3) Percentage of protein in buttermilk treatments

Fat

The results in Figure (4) show the average percentages of fat in the buttermilk treatments. The statistical analysis showed significant differences between the two types of butter milk and the treatments of butter milk with different fermented starters, as the milk of fermented buffalo milk with YO-MIX 511 was superior to the highest fat percentage (P≤ 0.05). The fat content of milk made from cow's milk ranged between 1.20-1.11%, and higher than in buffalo milk butter milk between 1.30-1.19%. The presence of a low percentage of fat in the butter milk may be due to the absence of a centrifugal step to remove the fat after shaking, as well as the presence of parts of MFGM, small fat globules, and free fats that cannot be extracted by centrifugation due to their small size (Ali, 2019). The repeated physical collisions resulting from the shaking caused the merging of the fat globules and their transfer to the butter (Conway et al., 2014). The percentages vary according to the different initiators used, as the initiator YO-MIX 511 had the highest fat content and amounted to 1.20 and 1.30% for milk, respectively, compared to the initiator YO-MIX S31, which gave the lowest percentage of 1.11 and 1.19%, respectively due to the difference in the activity of the initiator and its ability to produce The lipolytic enzyme (Wati et al., 2018). The results agree with what was found by (Kumar et al., 2015) that the fat percentage is 0.8% in natural butter milk and 0.60% in sweet cream buttermilk.



LSD Type = 0.03962 LSD Starter= 0.05603 LSD Interaction = 0.07924

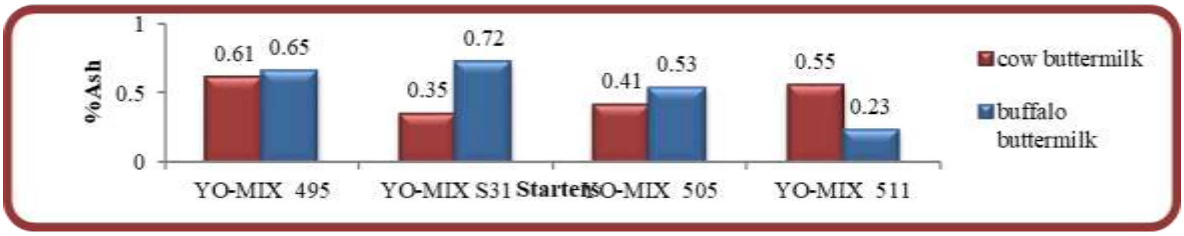
Figure (4) the percentage of fat in buttermilk treatments

Ash

The results in Figure (5) show the average ash percentages in the butter milk treatments. The statistical analysis showed no significant differences between the species and significant differences between the treatments according to the fermented starter treatments, as the YO-MIX 495 starter treatments outperformed all the primers (P≤ 0.05). The percentage of ash in raw milk produced from cow's buttermilk ranged between 0.35 - 0.61% and was higher than it in buttermilk produced from buffalo buttermilk, 0.23 - 0.72%. The ash percentage varied according to the different starters used in fermentation, as the initiator YO-MIX 495 gave the highest rate of ash in milk, buttermilk cow's milk, at 0.61%. The starter YO-MIX S31 gave the highest percentage of ash in buttermilk buffalo milk at 0.72%, depending on the percentage of salts in the milk and its relationship to the moisture content, as the ash percentage increased with a decrease in moisture. The reason for the high percentage of ash is that after removing fat, it rises Moisture percentage, which causes a reduction in the number of dissolved salts and, thus, a decrease in the percentage of ash (Aziznia et al., 2008). As for (Wati et al., 2018), it was shown that when adding different starters to the fermentation of goat milk used in his study, which are 11, 21, 25, 29, and 41, Effect in Mac Milk kernels, as the initiator 21 gave the highest water content of 91.21%, which negatively affected the ash content of 0.70% due to the decrease in

ISSN NO: 2771-8840
Date of Publication: 11-10-2022

the activity of this initiator, which reduced the percentages of solid materials and gave the highest water content, which reduced the ash, as the high content of ash indicates the presence of a high rate of inorganic substances.

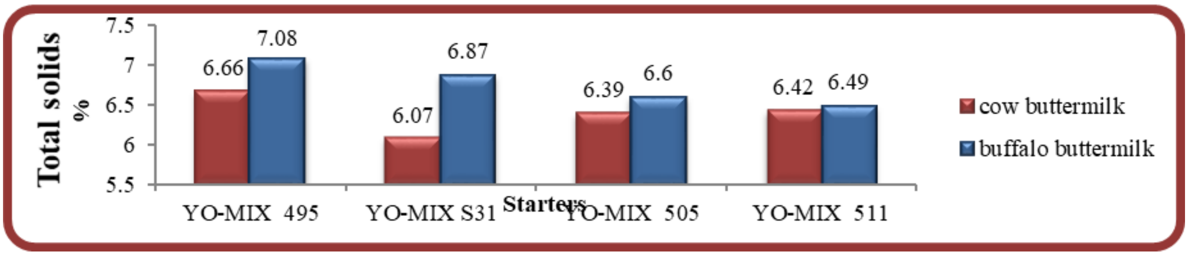


LSD Type = 0.0900 LSD Starter = 0.1273 LSD Interaction = 0.1800

Figure (5) Percentage of ash in butter milk treatments

Total solids

The results in Figure (6) refer to the percentages of solids in the buttermilk treatments. The statistical analysis showed significant differences between the two types of buttermilk manufactured from cows and buffalo milk, as the superiority of buffalo buttermilk was significant. There were differences between the fermented treatments according to different starters, as it was noted that the treatments were superior Fermented with YO-MIX 495 starter on the rest of the treatments (P≤ 0.05). The percentage of solids in the buttermilk made from cow's milk was 6.66-6.07%, which is less than the treatments manufactured from buffalo milk, 7.08-6.49%. It was noted that the values of the treatments varied according to the difference. The starter used where the highest percentage in milk was 6.66% for milk of cows fermented with the starter YO-MIX 495 and for milk of 7.08% for milk of buffalo milk fermented with the same starter due to the ability of these starters to decompose milk components by decomposing enzymes. And between (Ogrodowczyk et al., 2021) the decrease in the percentages of milk components such as lactose, protein, and fat negatively affects the dry matter in the buttermilk. The results agree with what was mentioned (Kumar et al., 2015), that the percentage of solids ranged between 9.75 and 4%.



LSD Type = 0.2670 LSD Starter = 0.3776 LSD Interaction = 0.5340

Figure (6) shows the percentage of total solids in butter milk treatments

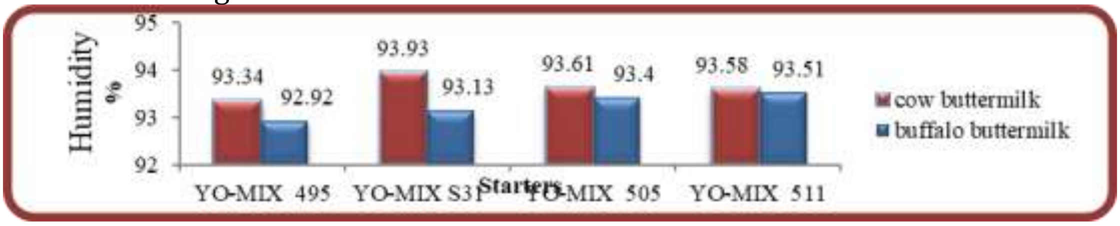
Humidity

The results of Figure (7) show the percentage of moisture in the buttermilk. The results of the statistical analysis indicated that there were significant differences between the buttermilk of cow's milk and buffalo's milk, the superiority of milk of cow's buttermilk significantly over the buttermilk of buffalo milk and that there were no differences in the treatments according to the prefixes (P≤ 0.05). It was noticed that the moisture percentage was higher compared to the raw milk made from it. The moisture content in the treatments of cow's buttermilk, which ranged between 93.93-93.34 %, is higher than that in the buttermilk of buffalo milk, which ran between 93.51-92.92% due to the high percentage of total solids in the milk of butter milk Buffalo milk compared to cow's milk butter milk. The moisture content varied in the treatments according to the different starters used, as the starter YO-MIX S31 gave the highest moisture content in cow's buttermilk milk, with 93.93%.

In comparison, the starter YO-MIX 511 gave the highest moisture percentage in buffalo milk butter milk, 93.51%. Water has a negative relationship with solids. It was found (Wati et al., 2018) when

ISSN NO: 2771-8840
Date of Publication: 11-10-2022

using several initiators for milk fermentation that starter 11 gave the lowest water content of 90.48%, the highest solids content, and the highest percentage of lactic acid, which led to the precipitation of proteins, curd formation and moisture reduction at the end of fermentation, while the initiator 21 It gave the highest water content of 91.21%, which resulted in a decrease in the activity of this initiator, which reduced solids, as the initiators with high activity work to raise the percentage of organic acids when fermenting milk components, thus reducing the water percentage and raising the percentage of solids to increase the decomposition of milk components and the occurrence of coagulation. These results agree with what was mentioned (Skryplonek et al., 2019), which noted that the moisture content of butter milk ranged between 90.89-89.26%.



LSD Type = 0.3062 LSD Starter = 0.4330 LSD Interaction **= 0.6124**

Figure (7) Moisture percentage in butter milk treatments

Determination of carbohydrates in butter milk by HPLC technique

The results in Table (1) refer to the percentages of the average concentration of lactose, glucose, galactose, the retention time RT, and the percentage of the peak area % in the treatments of cow and buffalo butter milk for the four starters, as it was noted that the fermentation process and the type of initiator used affected the concentrations of lactose, glucose, and galactose. It was pointed out that the concentration of carbohydrates in the milk of buffalo milkshake compared to the milk of cow's buttermilk according to the type of milk made from it, as well as the superiority of lactose sugar in the highest percentage compared to glucose and galactose sugar. -2.44% the percentages varied according to the different starters and their ability to ferment these sugars and were less than the percentage of lactose in milk. This is because lactose is the main carbohydrate source in milk and its concentration in cow's milk is 4.6 g/100 ml, but when fermentation, it decreases by about a third due to its transformation into lactic acid. Mediated by lactic acid bacteria, Schaafsma (2008). It was found (Sharma et al., 2021) that the carbohydrate content of the stirred milk decreased from 3.5% in milk to 2.6% when fermented with lactic acid bacteria, and exopolysaccharides produced increased the viscosity after their interaction with proteins. This is because the initiator bacteria fermented lactose and used only a portion of glucose during fermentation to reach the required pH, and lactose was not fermented (Narvhus et al., 2021). Glucose enters the process of formation of organic acids after the decomposition of lactose during the fermentation process by the action of lactic acid bacteria (Martinez et al., 2013). As for galactose sugar, the results showed a decrease in its concentration compared to lactose and galactose, as it ranged in butter milk made from buffalo milk 0.13-0.09% and less than in butter milk made from cow's milk 0.07-0.10%, and the percentages vary according to the different starters used in fermentation. The results agreed. With what was found by Gebreselassie et al. (2016) noticed that lactose was the highest, which ranged between 4.77-2.51 g / 100 g in butter milk due to the difference in the degree of fermentation. The percentage of lactose decreased to 364 µg / g, and the glucose concentration reached 59.9 µg / g.

Table (1) carbohydrates in butter milk

	Carbohydrate	Starter	RT/ min	Area %	Concentration%
Cow buttermilk	**Lactose**	**YO-MIX 495**	2.21	78799	1.96
		YO-MIX S31	2.15	38729	0.96
		YO-MIX 505	2.21	32267	0.80
		YO-MIX 511	2.18	34491	0.86
	Glucose	**YO-MIX 495**	1.70	1904095	0.10

		YO-MIX S31	1.72	2004976	0.11
		YO-MIX 505	1.81	2569990	0.14
		YO-MIX 511	2.07	1343272	0.07
	Galactose	YO-MIX 495	2.12	2470124	0.10
		YO-MIX S31	1.38	1948107	0.08
		YO-MIX 505	1.11	2130565	0.09
		YO-MIX 511	1.16	1636341	0.07
Buffalo buttermilk	Lactose	YO-MIX 495	2.12	121722	3.02
		YO-MIX S31	2.24	103659	2.57
		YO-MIX 505	2.36	133124	3.30
		YO-MIX 511	2.21	98379	2.44
	Glucose	YO-MIX 495	1.79	2135284	1.11
		YO-MIX S31	1.71	2726590	0.15
		YO-MIX 505	1.85	2693651	0.14
		YO-MIX 511	1.84	2275225	0.12
	Galactose	YO-MIX 495	1.31	2230588	0.09
		YO-MIX S31	1.15	2433603	0.10
		YO-MIX 505	1.15	2237692	0.09
		YO-MIX 511	1.21	3278642	0.13

Pathological antimicrobial efficacy of butter milk

The effectiveness of cow and buffalo butter milk fermented with four primers was tested against five types of pathogenic bacteria, namely *Bacillus cereus*, *Staphylococcus aureus*, *E.Coli*, *Enterobacter aerogenes*, and *Pseudomonas aeruginosa*. Figure (8) shows the diameter of the growth-free zone for the pathological microorganisms of the shaken milk treatments. It was noticed that the inhibitory activity of buffalo buttermilk treatments is higher than that of cow buttermilk. And all samples gave an inhibitory activity that varied according to the different primers used due to the difference in the degree of decomposition and fermentation between the starters and the amount of production of organic acids and active compounds for buffalo milk compared to cow's milk. The organic acids formed due to the fermentation of lactose add the positive hydrogen ion and thus lower the pH inside the cytoplasm of the bacterial cell, which causes stopping or prevents molecular interactions inside these cells (Manab et al., 2011). (Ricke, 2003) showed that the organic acids produced by fermentation with lactic acid bacteria could show anti-bacterial properties. The pH is a basic determinant of effectiveness, as the acids have the ability to penetrate the fatty membrane of the bacterial cell and reduce the pH. It requires the disposal of excess protons energy consumption ATP Which leads to the depletion of energy in bacteria cells (Wali and Abed., 2019) mentioned that the organic acids in milk have health benefits, such as acetic acid, which is an antimicrobial for bacteria resistant to different types of antibiotics due to its effect on the bacterial cell membrane because the permeability of cell membranes increases at low pH (Tamime et al., 2006).

The results indicated that the curd treatments inhibited the growth of gram-positive and gram-negative bacteria. The diameter of the inhibition halo of gram-positive bacteria Bacillus cereus and Staphylococcus aureus was higher than *E. Coli*, *Enterobacter aerogenes*, and *Pseudomonas aeruginosa*, which reached 12 mm, and the most increased diameter of positive bacteria. 9 mm for negative bacteria. The reason is that the negative bacteria cell wall contains a layer estimated at 80% of the components of the cell wall consisting of polysaccharides, lipids, and proteins. While these substances are less in positive bacteria, which leads to the breaking of the glycosidic bond of the building blocks of these despite the increase in its thickness with positive bacteria, thus destroying the cell wall and inhibiting the growth of these bacteria (Mine et al., 2004)). The results agreed with what was mentioned by (Savadogo et al., 2004), where the highest inhibition diameter was 12 mm produced by Lactobacillus fermentum bacteria, and the lowest inhibition diameter was 8 mm made by Leuconostoc

ISSN NO: 2771-8840
Date of Publication: 11-10-2022

mesenteroides subsp. Against *Enterococcus faecalis* and *E. coli* due to the ability of lactic acid bacteria to produce various compounds such as organic acids and bacteriocins during fermentation, which explains the prolongation of the shelf life of perishable foods such as dairy products.

Table (2) Anti-pathogenic activity of microorganisms

	Starter	Diameter Halo of inhibition (mm)				
		E.coli	*Staphylococcus aureus*	*Bacillus cereus*	*Enterobacter aerogenes*	*Pseudomonas aeruginosa*
Cow buttermilk	YO-MIX 495	7	7	9	6	7
	YO-MIX S31	7	9	7	7	6
	YO-MIX 505	6	7	10	7	8
	YO-MIX 511	7	12	9	7	7
Buffalobutter milk	YO-MIX 495	8	10	12	6	8
	YO-MIX S31	9	10	10	8	7
	YO-MIX 505	9	10	10	7	8
	YO-MIX 511	9	12	9	8	7

YO-MIX495 YO-MIXS31 YO-MIX511 YO-MIX 505

Escherichia coli

YO-MIX 511 YO-MIX505 YO-MIX S31 YO-MIX495

Staphylococcus aureus

YO-MIXS31` YO-MIX511 YO-MIX 505 YO-MIX495

Bacillus cereus

YO-MIX505 YO-MIX511 YO-MIX S31 YO-MIX495

Enterobacter aerogenes

ISSN NO: 2771-8840
Date of Publication: 11-10-2022

YO-MIXS31 YO-MIX505 YO-MIX511 YO-MIX495

Pseudomonas aeruginosa

Figure (8) Inhibitory diameter against pathogenic microorganisms in butter milk

Conclusion

The types of buttermilk produced varied according to the different starters used, as the fermentation process affected its chemical content, pH, and acidity. It was noted that the best starter in terms of sensory and manufacturing terms is YO-MIX 495 for the production of some dairy products such as yogurt due to the high percentage of solids, its thick and smooth texture, and moderate flavor And the stability of the pH. The highest value of carbohydrates was estimated by the HPLC device in fermented milk, mainly lactose, compared to the rest of the carbohydrates. Buttermilk also showed high inhibitory activity against gram-positive and gram-negative bacteria that cause food spoilage and diseases. Diseases, including indigestion.

References

1. Al-Hatim, R. R., Al-Rikabi, A. K., & Ghadban, A. K. (2020). The Physico-chemical properties of bovine and buffalo whey protein milk using ultrafiltration membrane Technology. Basrah Journal of Agricultural Sciences, 33(1): 122-134.
2. Ali, A. H. (2019). Current knowledge of buttermilk: Composition, applications in the food industry, nutritional and beneficial health characteristics. International Journal of Dairy Technology, 72(2): 169-182.
3. Al-Shihabi, M., Hamed, F., Abu Ghara, S. (2015). Effect of the type of initiator and the rate of inoculation on the characteristics of the labneh manufactured by the direct method and its comparison with the labneh manufactured by the traditional method. The Syrian Journal of Agricultural Research 2(2). : 55-68.
4. Association of Official Analytical Chemists A.O.A.C. (2008). Official Methods of Analysis 16th ed. Association of Official Analytical Chemists International Arligton, Virginia, U.S.A.
5. Aziznia, S., Khosrowshahi, A., Madadlou, A., & Rahimi, J. (2008). Whey protein concentrate and gum tragacanth as fat replacers in nonfat yogurt: chemical, physical, and microstructural properties. Journal of dairy science, 91(7): 2545-2552.
6. Benkerroum, N., & Tamime, A. Y. (2004). Technology transfer of some Moroccan traditional dairy products (lben, ben, and smen) to a small industrial scale. Food Microbiology, 21(4): 399-413.
7. Bhukya, K. K., & Bhukya, B. (2021). Unraveling the probiotic efficiency of bacterium Pediococcus pentosaceus OBK05 isolated from buttermilk: An in vitro study for cholesterol assimilation potential and antibiotic resistance status. Plos one, 16(11): e0259702.
8. Conway, V., Gauthier, S. F., & Pouliot, Y. (2013). Antioxidant activities of buttermilk proteins, whey proteins, and their enzymatic hydrolysates. Journal of agricultural and food chemistry, 61(2): 364-372.
9. Conway, V., Gauthier, S. F., & Pouliot, Y. (2014). Buttermilk: much more than a source of milk phospholipids. Animal Frontiers, 4(2): 44-51. Gandhi, K., Reddy, S., & Singh, I. (2018). Quality Issues during Production and Distribution of Buttermilk. Available at SSRN 3168999.
10. Coroian, A., Raducu, C., Miresan, V., Cocan, D., Balta, I., Longodor, A. L. & Marchiis, Z. (2019). Physico-chemical composition and antioxidant capacity of buffalo milk. Scientific Bulletin. Series F. Biotechnologies, 23: 2285-1364.

ISSN NO: 2771-8840
Date of Publication: 11-10-2022

11. Dave, R.I. and Shah, N.P. (1996). Evaluation of media for selective enumeration of Streptococcus thermophilus, Lactobacillus delbrueckii ssp. bulgaricus, Lactobacillus acidophilus, and bifidobacteria. Journal of Dairy Science, 79 (9): 1529-1536.
12. Forestier, C., De Champs, C., Vatoux, C., & Joly, B. (2001). Probiotic activities of Lactobacillus casei rhamnosus: in vitro adherence to intestinal cells and antimicrobial properties. Research in microbiology, 152(2): 167-173.
13. Gebreselassie, N., Abrahamsen, R. K., Beyene, F., Abay, F., & Narvhus, J. A. (2016). Chemical composition of naturally fermented buttermilk. International Journal of dairy technology, 69(2): 200-208.
14. George, S. S. (2004). Detection of E.Coli O157:H7 in fresh minced beef and study of the effect of some biological preservation materials on its viability under cold storage conditions. PhD thesis, College of Agriculture, University of Basra.
15. Gonelimali, F. D., Lin, J., Miao, W., Xuan, J., Charles, F., Chen, M., & Hatab, S. R. (2018). Antimicrobial properties and mechanism of action of some plant extract against food pathogens and spoilage microorganisms. Frontiers in microbiology, 9:1639.
16. Gonfa, A., Foster, H. A., & Holzapfel, W. H. (2001). Field survey and literature review on traditional fermented milk products of Ethiopia. International Journal of Food Microbiology, 68(3): 173-186.
17. Kumar, R., Kaur, M. , Garsa, A. K., Shrivastava, B., Reddy, V. P., & Tyagi, A. (2015). Natural and cultured buttermilk. Fermented milk and dairy products: 203-225.
18. Manab, A.; Sawitri, M. E.; Al Awwaly, K. U. and Purnomo, H. (2011). Antimicrobial activity of whey protein-based edible film incorporated with organic acids. African Journal of Food Science, 5(1): 6-11.
19. Martinez, F. A. C., Balciunas, E. M., Salgado, J. M., González, J. M. D., Converti, A., & de Souza Oliveira, R. P. (2013). Lactic acid properties, applications, and production: A review. Trends in food science & technology, 30(1): 70-83.
20. Mine, Y., Ma, F., & Lauriau, S. (2004). Antimicrobial peptides released by enzymatic hydrolysis of hen egg white lysozyme. Journal of Agricultural and Food Chemistry, 52(5): 1088-1094.
21. Mohsen, R. T. (2011). Study the inhibitory activity of some organic extracts of the cancer plant against Pseudomonas aeruginosa and Staphylococcus aureus ex vivo. Anbar Journal of Agricultural Sciences. 9(2), 329-322.
22. Narvhus, J. A., Bækkelund, O. N., Tidemann, E. M., Østlie, H. M., & Abrahamsen, R. K. (2021). Isolates of Pseudomonas spp. from cold-stored raw milk show variation in proteolytic and lipolytic properties. International Dairy Journal, 123: 105049.
23. Nasser, E. K., Majeed, K. R., & Ali, H. I. (2021). Effect of Some Strains of Lactic Acid Bacteria and Their Mixture on the Level of Fats and Cholesterol in Albino Rats (*Rattus norvegicus*) Male with.
24. Ogrodowczyk, A. M., Kalicki, B., & Wróblewska, B. (2021). The effect of lactic acid fermentation with different bacterial strains on the chemical composition, immunoreactive properties, and sensory quality of sweet buttermilk. Food Chemistry, 353: 129512.
25. Ricke, S. C. (2003). Perspectives on the use of organic acids and short-chain fatty acids as antimicrobials. Poultry Science, 82(4): 632-639.
26. Røssland, E., Langsrud, T., Granum, P. E., & Sørhaug, T. (2005). Production of antimicrobial metabolites by strains of Lactobacillus or Lactococcus co-cultured with Bacillus cereus in milk. International Journal of Food Microbiology, 98(2): 193-200.
27. Sakkas, L., Evageliou, V., Igoumenidis, P. E., & Moatsou, G. (2022). Properties of Sweet Buttermilk Released from the Churning of Cream Separated from Sheep or Cow Milk or Sheep Cheese Whey: Effect of Heat Treatment and Storage of Cream. Foods, 11(3): 465.
28. Savadogo, A., Ouattara, C. A., Bassole, I. H., & Traore, A. S. (2004). Antimicrobial activities of lactic acid bacteria strain isolated from Burkina Faso fermented milk. Pakistan Journal of Nutrition, 3(3): 174-179.

ISSN NO: 2771-8840
Date of Publication: 11-10-2022

29. Schaafsma, G. (2008). Lactose and lactose derivatives as bioactive ingredients in human nutrition. International Dairy Journal, 18(5): 458-465.
30. Schmuller, J. (2017). Statistical Analysis with R For Dummies. John Wiley and Sons.111, River Street, Hoboken, Canada. 438p.
31. Sharma, A., Noda, M., Sugiyama, M., Ahmad, A., & Kaur, B. (2021). Production of functional buttermilk and soymilk using Pediococcus acidilactici BD16 (alaD+). Molecules, 26(15): 4671.
32. Skryplonek, K., Dmytrów, I., & Mituniewicz-Małek, A. (2019). The use of buttermilk as a raw material for cheese production. International Journal of Dairy Technology, 72(4): 610-616.
33. Sodini, I., Morin, P., Olabi, A., & Jiménez-Flores, R. (2006). Compositional and functional properties of buttermilk: A comparison between sweet, sour, and whey buttermilk. Journal of Dairy Science, 89(2): 525-536.
34. Tamime A Y, Skriver A and Nilsson L-E (2006) .Starter cultures. In: Fermented Milk, pp. 11–25. Tamime A Y, ed. Oxford, UK: Blackwell Pu.
35. Wali, M. K., & Abed, M. M. (2019). Antibacterial activity of acetic acid against different types of bacteria causes food spoilage. Plant Archives, 19(1): 1827-1831.
36. Wati, A. M., Lin, M. J., & Radiati, L. E. (2018). Physicochemical characteristics of fermented goat milk added with different starters of lactic acid bacteria. Jurnal Ilmu dan Teknologi Hasil Ternak (JITEK), 13(1): 54-62.
37. Yilmaz-Ersan, L., & Kurdal, E. (2014). The production of set-type-bio-yogurt with commercial probiotic culture. International Journal of Chemical Engineering and Applications, 5(5): 402.