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# Functional stirred Yoghurt fortified with buffalo, bovine, mix colostrum and lactoferrin, effect of lactoferrin on pathogenic bacteria and amino acids of buffalo, bovinecolostrum and lactoferrin

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**Abstract**

The aim of this study was to see how adding buffalo, bovine, mixed colostrum, and lactoferrin as natural ingredients affected on the nutritional value and quality of stirred yoghurt. Lactoferrin's influence on harmful microorganisms was investigated. And studying amino acids for buffalo, bovine, colostrum, and lactoferrin. In treatments, buffalo, bovine, and mix colostrum (buffalos and cows1:1) were added at 15% ratios generate a functional stirred yoghurt. On the other hand lactoferrin, was added at a ratio of 5 mg/mL. Samples of stirred yoghurt were chemically, physically microbiologically and organoleptically analyzed. The addition of buffalo and bovine colostrum to stirred yoghurt improves significantly the total solids, protein, and ash content of the yoghurt compared to the control. Stirred yoghurt with buffalo, bovine, mix colostrum and lactoferrin characterized with slightly lower treatable acidity when fresh and during the storage period. Higher viscosity was detected in stirred yoghurt fortified with 15% buffalo, bovine and mix colostrum, compared with the control. Control stirred yoghurt had a significant increasing of lactic acid bacteria counts compared with buffalo, bovine, mix colostrum, and lactoferrin. Molds and yeasts could not be detected in either fresh treatments or during the storage period. There are significant differences between treatments for organoleptic evaluation. The stirred yoghurt fortified with lactoferrin and buffalo colostrumgained higher scores of body and texture and appearance, compared with control. Lactoferrin has been shown to haveantibacterial action against a wide range of Gram-positive and Gram-negative bacteria. Gram-negative Ps. aeruginosa and E. coli isolates were the most resistant. lactoferrin have strong inhibitory effect against B. cereus and Staph. aureus. Essential and non-essential amino acids of buffalo, bovine colostrum, and lactoferrin were identified and compared to FAO / WHO (2007) indicated requirements. Valine had the highest value for essential amino acids in buffalo colostrum (107.72 mg/g protein) bovine colostrum (102.30 mg/g protein) and lactoferrin (102.36 mg/protein). Glutamic acid had the highest values in buffalo colostrum (122.49 mg/g protein) and bovine colostrum (99.92 mg/g protein) while alanine had the highest value in lactoferrin (105.67 mg/g protein) in non-essential amino acids.

**Keywords**: Stirred yoghurt, buffalo colostrum, bovine colostrum, lactoferrin, antibacterial, physic-chemical, sensory quality and essential and non- essential amino acids.

## 1. Introduction

Colostrum is the first milk produced by mammals after birth, and it has a distinct nutritive profile [1,2]. It is the initial milk secreted by mammals' mammary glands within the first 3-4 days after birth. It is a complex biological fluid that delivers all necessary nutrients, immunological and development factors to the baby and differs significantly from mature milk [3]. Colostrum includes greater total solids than mature milk and is high in proteins, lipids, essential fatty acids, and amino acids. It also contains oligosaccharides, lactoferrin, lysozyme, lactoperoxidase, proline-rich polypeptides, growth factors such as insulin, epidermal growth factor,

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transforming growth factor I and II, growth hormone, cytokines, and nucleosides, all of which act as natural anti-microbial agents to help the new-born develop immunity [4,5,6].

Colostrum provides passive immunity to human and cattle new-born, which has sparked interest in bovine colostrum's ability to prevent infection and disease in people [7].Colostrum's immunoglobulins (particularly IgG) provide the immunological balance and minimize inflammation and disease [3].Bovine colostrum is now widely used as a nutritional supplement. Clinical investigations have revealed that colostrum is taken as a supplement by human beings; it shows antioxidative, immunomodulatory and anti-inflammatory activity. So, colostrum has attracted considerable interest as a functional food ingredient [8].

Lactoferrin is a biologically active cationic protein with a molecular weight of 80 kDa that is present in exocrine secretions such as tears, salvia, bile, and neutrophil granules and belongs to the transferrin family. One of the most significant components in milk is lactoferrin [9,10]. Lactoperoxidase, lactoferrin, and lysozyme are the primary antibacterial agents found in raw milk. In most situations, the enzymes activate milk's natural antibacterial system, killing the target microbes. Antimicrobial systems in natural milk are distinguished by their simultaneous attack on the oxidative and lytic mechanisms of microorganisms [11].Lactoferrin's antibacterial effect is mediated by two mechanisms: The first entails Iron sequestration on infection sites deprives germs of iron, resulting in a bacteriostatic effect [12]. The second mechanism of lactoferrin and lactoferrin-derived peptides is direct interaction with microorganisms via positively charged amino acids interacting with anionic molecules on particular bacterial, viral, fungal, and parasitic surfaces, resulting in cell lysis [13,14].

Lactoferrin has high levels of amylase, DNase, RNase and ATPase activity. Therefore, LF can damage the nucleic acids of bacteria through hydrolysis and can inhibit the organism**.** Furthermore, Lactoferrin has a significant impact on enteric pathogens: it inhibits growth and hinders the function of surface-expressed virulence factors, reducing their capacity to adhere and infiltrate mammalian cells [15,16].

Dairy consumption has been linked to a variety of health benefits in addition to its nutritional worth. Over the last 25 years, dairy products have been used as transporters for functional food additives due to their perceived health benefits, such as phytochemical and probiotics. Yoghurt is one of the most widely consumed fermented milk products in the world, not only because of its excellent sensory qualities, but also because of its great nutritional and medicinal value. It comes in a range of varieties in terms of fat and total solids content, body shape (stirred, set-style, frozen, concentrated), additives, probiotic microorganisms, and different flavours [17].As yoghurt is a popular fermented dairy product of Egypt, manufacture of yoghurt usually involves milk fortification with dairy ingredients to increase nutritional value or produce functional yoghurt due to image they possess and their unique nutritional attributes, in addition for refreshing taste, palatability and therapeutic values. Due to consumer opposition, the use of colostrum or any of its constituents in functional dairy meals is currently relatively limited.

Due to the bioactive components of colostrum, buffalo colostrum, bovine colostrum, and lactoferrin were used as natural ingredients in this study to boost the nutritional value and health advantages of stirred yoghurt as a functional dairy food and evaluate the action of lactoferrin on pathogenic microorganisms and enteric pathogens, with the goal of determining the protective potential of lactoferrin - as a natural component of milk - to limit the growth of harmful microbes in meals. In addition to studying the physical-chemical, sensorial and functional characteristics to develop a product which may gain greater demand and consumer appeal.

## 2. Experimental

*Materials*

Fresh buffalo milk (6.8% fat), buffalo colostrum and cow’s colostrum used in this study were obtained from the dairy farm Qutour, Gharbia Governorate, Egypt. Lactoferrin was obtained from National Research Centre, Dokki, Giza, Egypt.

*Methods*

*Preparation stirred yoghurt*

Stirred yoghurt manufactured according to Tamime and Robinson [18].Milk was fortified with 15 % (v/v) of buffalo colostrum, bovine colostrum and mixed colostrum (buffalos and bovine1:1), heated at 90 ºC for 15min. followed by cooling at 40±3ºC, and with added 2% yoghurt starter. Yoghurt mixes of the different treatments were filled in 100 ml plastic cups, and incubated at 42ºC until complete coagulation. After coagulation, to manufacture the stirred yoghurt, the yoghurt was blended for 1 minute in a sterile stainless steel blender. The resulting stirred yoghurt was kept at 5±1 ºC for 10 days. The samples were tested when they were fresh, as well as after 5 and 10 days of storage.

Buffalo colostrum Bovine colostrum Lactoferrin

Control stirred yoghurt was prepared without additions of colostrum, while the other examined treatments were indicated as follows:
C: control
BuCSY: Buffalo colostrum stirred yoghurt (15%), BOCSY: Bovine colostrum stirred yoghurt fresh colostrum (15%)., MCSY: Mix colostrum (1buffalo:1 bovine) stirred yoghurt (15%), LSY: lactoferrin stirred yoghurt (5mg/mL)

1= control sample2= stirred yoghurt fortified with buffalo colostrums 3= stirred yoghurt fortified with bovine colostrum 4=stirred yoghurt fortified with mix colostrum 5= stirred yoghurt fortified with lactoferrin

***Chemical analysis***

Total solids, fat, protein, and ash concentrations of stirred yoghurt samples were chemically tested as specified in the AOAC[19].Minerals content (Ca, Cu, Fe, K,Na and Zn)were determined by instrument Inductively Coupled Plasma (ICP) model Optima 7000 DV according to the method of James [20].

***Physical properties of stirred yoghurt***

*Determination of pH and Titratable acidity:* The pH of stirred yoghurt samples was determined by using pocket pH meter (model pH 211; Hanna Instruments, Woonsocket, RI)at 5 ºC. pH values were determined when fresh and after5, and 10days, after calibration with standard buffers. Titratable acidity in terms of % lactic acid was measured according to BSI [21], following the addition of 10 g of stirred yoghurt to 10 mL of hot distilled water and titrating with 0.1N NaOH using 0.5 percent phenolphthalein indicator.

*Determination of viscosity*: Brookfield DV-E Viscometer was used to measure viscosity. All samples were run on Spindle No 3 at 30 rpm, as previously reported by Denin-Djurdjevic *et al*.[22] The viscosity of the product was measured during storage at 5°C when it was fresh, as well as after 5 and 10 days.

***Microbiological characteristics of stirred yoghurt***

Lactic acid bacteria were counted by pour plating in MRS agar. Count all colonies on the plate after anaerobic incubation at 36°C for 48h according to Devriese *et al.* [23].

*Str.thermophilus* counts were determined using $M_{17}$ agar. The plates were incubated aerobically at 37°C for 72 h according to [23].

*L. bulgaricus* were counted on MRS agar medium duplicate plates at 45°C/48 h which pH of agar medium was adjusted to 5.2 [24].Mold and Yeast were estimated according to Sahana,*et al..* [7] as described by Ehirim and Onyeneke [25] using potato dextrose agar medium, after incubation for up to 5 days 25˚C.

***Sensory evaluation***

The organoleptic properties were evaluated according to Tamime and Robinson [18]. Flavor was scored out of 50-point body, texture 40 points and appearance of 10 points. The organoleptic properties were assessed by 10 panelists from the staff members of Home Economic Faculty Al-Azhar University.

***Antibacterial activity of Lactoferrin***

Five pathogenic bacteria; *Salmonella typhi*, *Escherichia coli*, and *Pseudomonas aeruginosa* as Gram negative organisms as well as *Staphylococcus aureus* and *Bacillus cereus* as Gram positive ones were used for antibacterial assay. The tested bacterial species were grown on Tryptic soya broth (TSB) tubes and incubated at 35ºC. until they achieve or exceeds the turbidity of the 0.5McFarland standards after four hours of incubation). A uniform bacterial layer was developed on the surface of solidified nutrient agar plates using the adjusted bacterial suspension and sterile cotton swabs and left to dry. Whatman filter paper no. 1 is used to prepare discs 6 mm; which impregnated with tested Lactoferrin at two concentrations (100 mg/ml and 50 mg/ ml). The impregnated discs were applied on the surface of streaked nutrient agar plates. Dimethyl sulfoxide (DMSO) was used as negative control, while a 1mg/ml Ceftriaxone was used as positive control. The plates were inverted and incubated at35ºC for 16- 18hr.After incubation, the inhibitory zones, as well as the disc's diameter, were measured. A ruler is placed on the back of the inverted Petri plates and used to measure zones to the closest millimeter. All treatments were carried in triplicates, and the results were expressed as mean ±SD as mentioned by[26].

***Determination of minimum inhibitory concentration (MIC)of lactoferrin***

The MIC was determined by utilizing the tube dilution method [27]. To achieve a $10^8$cfu ml$^{-1}$ inocula, a 24h culture of the investigated bacterial species was diluted in 10 ml of tryptic soy broth (TSB) with reference to the 0.5 McFarland standards. In a culture tube filled with nine concentrations of lactoferrin extract (20.0, 10.0, 5.0, 4.0, 3.0, 2.0, 1.0, 0.5, and 0.25 mg ml$^{-1}$ in DMSO) were created. Each tube was injected with 100 mL of bacterial cell suspension and incubated for 24 hours at 37°C. The turbidity of the broth indicates inoculum growth, and the lowest concentration that inhibited the test organism's growth was chosen as the minimal inhibitory concentration (MIC).

***Determination of amino acids***

HPLC Pico-Tag method to evaluate amino acid composition: The amino acid content of experimental materials was determined using HPLC Pico-Tag method according to [28]. Pico-Tag is a method described by [29,30,31]. Waters Associates commercialized the Pico-Tag technology, which was an integrated methodology for amino-acid analysis. Pre-column derivatization was done with phenyl isothiocyanate (PITC, or Edman's reagent), and the phenyl thio carbamyl (PTC) derivatives were separated using reversed-phase gradient elution high-performance liquid chromatography (HPLC). The protein ratio sample was weighted into a 25 mm hydrolyzed tube with 6 N HCl and set in a 110ºC oven for 24 hours, after which the tube was removed from the oven and allowed to cool. The contents of the tube were transferred quantitatively to a volumetric flask and diluted with HPLC grade water. The diluted hydrolysate was filtered through 0.45 um sample filter. Aliquots of hydrolysate, together with appropriate standards. The sample was placed into Pico-Tag amino acids Workstation (Waters, USA) for sample preparation (Drying, redrying and derivatization) using Waters reagents. The chromatographic analysis was carried out using HPLC with Pico-Tag amino acids method. At 38°C, flow rate 1 ml/min, the liquid chromatographic equipment was fitted with a 600 E Multi solvent Delivery System and the following Pico- Tag solvent A and B (Waters Eluent A & B) gradient. Using linear gradient elution, 20 microliters of material were injected and put onto the Pico-Tag amino acids column (150x3.9 mm, stainless steel). UV absorption measurements at a fixed wavelength of 254nm (2489 UV/Vis Detector) are used to detect PTC compounds. Two injections of lysine standards were used to calibrate the illustrated before injecting the sample.

***Statistical Analysis***

Data were assessed using SPSS 20 for Microsoft Windows. Statistical results were analyzed by Duncan’s multiple-range test at the P < 0.05 level of significance.

## 3. Results and Discussion

***Chemical composition of stirred yogurt***

Results presented in Table (1) show that the total solids, fat, protein and ash contents of the stirred yoghurt treatments increased in samples fortified with colostrum. There are significant differences between all treatments The highest value of TS, fat, protein and ash is for BuCSY. Similar outcomes were obtained by [32], who reported that fat, ash, protein and TS were higher with the rate of colostrum than control. This could be due to high content of total solids in colostrum, as compared to normal milk [33]. The obtained results are also in agreement with [34], they discovered that protein and fat contents in samples slightly risen in conjunction with increasing the concentration of colostrum in both yogurt and kefir samples.

Table 1. Chemical composition of stirred yogurt fortified with buffalo, bovine, mix colostrum and lactoferrin

| Chemical composition (%) | C | BuCSY | BoCSY | MCSY | LSY |
|---|---|---|---|---|---|
| Total solids | $17.42^{e}$ | $18.72^{a}$ | $18.22^{c}$ | $18.47^{b}$ | $17.91^{d}$ |
| Fat | $6.52^{c}$ | $6.75^{a}$ | $6.42^{d}$ | $6.58^{b}$ | $6.51^{c}$ |
| Protein | $4.20^{e}$ | $5.01^{a}$ | $4.91^{c}$ | $4.97^{b}$ | $4.68^{d}$ |
| Ash | $0.78^{d}$ | $0.95^{a}$ | $0.80^{c}$ | $0.85^{b}$ | $0.79^{cd}$ |

[a-d] There is a significant difference between any two means, within the same raw have the same superscript letter (Duncan’s test P<0.05)

C=control sample

BuCSY= stirred yoghurt fortified with buffalo colostrum

BoCSY= stirred yoghurt fortified with bovine colostrum

MCSY= stirred yoghurt fortified with mix colostrum

LSY= stirred yoghurt fortified with lactoferrin

***The minerals content of stirred yoghurt***

The minerals content of stirred yoghurt fortified with buffalo, bovine, mix colostrums, and lactoferrin were presented in Table (2). Stirred yoghurt fortification with lactoferrin were rich in Ca, Fe, K and Na, compared to stirred yoghurt fortified with buffalo, bovine colostrums, and with control sample. The content of calcium in stirred yoghurt fortified with lactoferrin seems very high, compared with in all treatments, since it is equal to 80.84 (mg/ L) against 19.10, 17.84, 16.35 and 14.18 (mg/L) in stirred yoghurt fortified with buffalo, mix and bovine colostrum and control sample respectively. These results indicated that lactoferrin was a good natural source of calcium. Cu and Zn were higher in stirred yoghurt fortified with bovine colostrum than those in all other treated samples. Stirred yoghurt fortified with bovine colostrum contained 2.682 mg/L Cu and 0.410 mg/L Zn. Zinc is particularly important for cellular replication and immune response development. Zinc is also vital for growth; it affects over 300 enzymes by partaking in their structure, as well as their catalytic and regularity functions [35]. These findings are partially in line with those obtained by [32], who found that the results of mineral analysis revealed that the Ca, Na, Fe, Mg, K and Zn contents of stirred yoghurt with colostrum had higher values when fresh compared to control yoghurt samples and this was proportional to the amount of colostrum added. This also due to the higher contents of colostrum for minerals.

Table (2). The minerals content (mg/L) of stirred yoghurt fortified with buffalo, bovine, mix colostrum and lactoferrin

| Minerals, (mg/L) | C | BuCSY | BoCSY | MCSY | LSY |
|---|---|---|---|---|---|
| Ca | 14.18 | 19.10 | 16.35 | 17.84 | 80.48 |
| Cu | 1.940 | 0.910 | 2.682 | 1.190 | 1.850 |
| Zn | 0.409 | 0.338 | 0.410 | 0.313 | 0.098 |
| Fe | 0.287 | 0.631 | 0.308 | 0.613 | .8650 |
| K | 8.841 | 12.20 | 9.476 | 11.26 | 47.32 |
| Na | 2.839 | 3.945 | 2.851 | 3.425 | 17.74 |

C=control sample
BuCSY= stirred yoghurt fortified with buffalo
BoCSY= stirred yoghurt fortified with bovine colostrum
MCSY= stirred yoghurt fortified with mix colostrum
LSY= stirred yoghurt fortified with lactoferrin

Regarding the iron content in the examined treatments, results showed that high increase of iron content of stirred yoghurt fortified with lactoferrin (0.865 mg/L), compared with control (0.287 mg/L). Also, the iron content in stirred yoghurt fortified with bovine colostrum and stirred yoghurt fortified with buffalo colostrum was observed in range of 0.308 to 0.631mg/L.The addition of colostrum and lactoferrin to the tested therapies clearly increased the iron content. Iron transports oxygen to cells and is required for energy production, collagen formation, and the immune system's correct functioning. Iron deficiency anemia, on the other hand, is thought to be responsible for 20% of all maternal deaths in Africa [36]. The amount of K shows great variability in all treatments, with highest values in stirred yoghurt fortified with lactoferrin, and lowest in control sample. Results presented in Table (2) revealed that the sodium concentrations of stirred yoghurt fortified with lactoferrin, the highest concentration of sodium (17.74 mg/L) while lower concentration was in control sample (2.839 mg/ L).

***Physical properties of stirred yoghurt***

Fig. 1 and 2show the pH and titratable acidity values of stirred yoghurt samples. Stirred yoghurt with buffalo, bovine and mixed (buffalo and bovine) colostrum samples had slightly low treatable acidity when fresh and during storage period, it may be because of the presence antimicrobial agents of lactoferrin and colostrum which decrease the chances of fermentation. Also, due to the presences of bioactive components of colostrum and its effective utilization [37].The pH values of all the produced yoghurt took an opposite trend to that of the acidity [38].

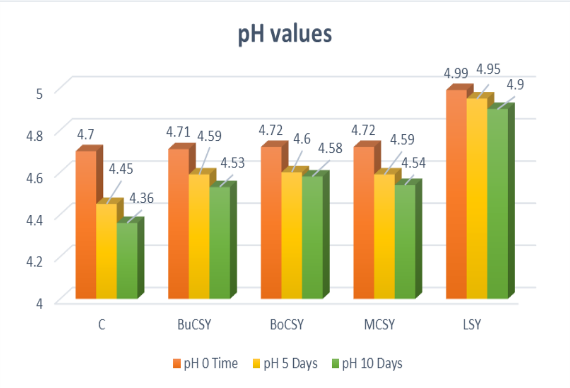



Fig.1.pH values of stirred yoghurt fortified with buffalo, bovine,mix colostrum and lactoferrin during storage period (C=control sample BuCSY= stirred yoghurt fortified with buffalo colostrum BoCSY= stirred yoghurt fortified with bovine colostrum MCSY= stirred yoghurt fortified with mix colostrum LSY= stirred yoghurt fortified with lactoferrin )

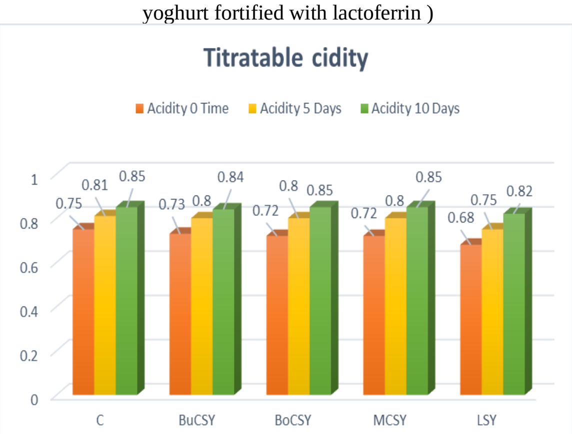



Fig. 2. Titratable acidity of stirred yoghurt fortified with buffalo, bovine, mix colostrum and lactoferrin during storage period (C=control sample BuCSY= stirred yoghurt fortified with buffalo colostrum BoCSY= stirred yoghurt fortified with bovine colostrum MCSY= stirred yoghurt fortified with mix colostrum LSY= stirred yoghurt fortified with lactoferrin)

Stirred yoghurt sample fortified with lactoferrin was of the highest pH and the lowest acidity when fresh and during storage. This might be due to the partial inhibition of lactic acid producing microorganisms LF[39].
Values of pH decreased during storage in all samples and acidity values increased, which came in agreement with[32],who found that stirred yoghurt with colostrum was of low titratable acidity when fresh and during storage, compared with control as mentioned by [39],who found that pH values of yoghurt supplemented with lactoferrin were higher, compared with control, and decreased during storage period (30 days).Similar results were also obtained in the presence of lactoferrin by[40].
Results presented in Fig. 3 show that the stirred yogurt fortified with buffalo and bovine colostrums characterized with the highest values of viscosity, while when fresh and during storage period. Meanwhile the control stirred yoghurt sample was of the lowest value of viscosity. Increase viscosity of stirred yoghurt with buffalo and bovine colostrum could be due to presence of α-lactoglobulin which plays a major role as a gelatinizing agent due to presence of free sulfhydryl groups. Increased whey proteins in colostrum might have improved the rheological characteristics of stirred yoghurt with colostrum as these parameters are associated with the forces involved in the internal bonds of the produced products [41].Ayar *et al.*[34] reported that high protein and fat contents of colostrum may could the primary cause of viscosity changes in general; viscosity rises as the solid content of liquid foods rises.

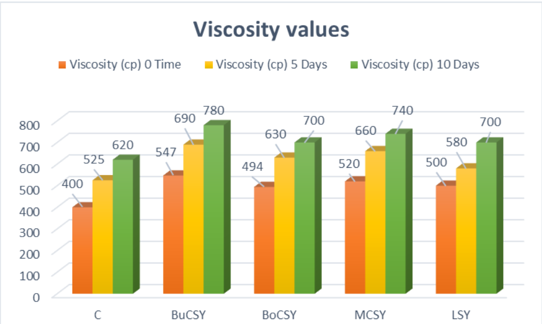


Fig. 3. Viscosity values of stirred yoghurt fortified with buffalo, bovine, mix colostrum and lactoferrin during storage period (C=control sample BuCSY= stirred yoghurt fortified with buffalo colostrum BoCSY= stirred yoghurt fortified with bovine colostrum MCSY= stirred yoghurt fortified with mix colostrum LSY= stirred yoghurt fortified with lactoferrin)

***Microbiological characteristics of stirred yoghurt***
Data in Table (3) show that the lactic acid bacterial, *Str. thermophilus*, *L. bulgaricus* and molds and yeasts counts of stirred yoghurt were affected by the addition of colostrum, lactoferrin and storage period (10 days). According to the obtained results, Lactic acidbacteria count of control stirred yoghurt were higher than the stirred yoghurt fortified with buffalo, bovine, mix colostrum and lactoferrin in fresh and throughout the entire storage period. This could be due to the higher antibacterial content of colostrum and lactoferrin, which reduce the growth and activity of lactic acid bacteria in yoghurt cultures [32,39]. The count of the control and all other treatments grew as the storage periods progressed with significant difference.

Table3. Microbiological characteristics ($\log_{10}$ cfu $g^{-1}$) of stirred yoghurt fortified with buffalo, bovine, mix colostrum and lactoferrin during storage at 5°C for 10 days

| Treatments | Storage Period (Days) | | | Means |
|---|---|---|---|---|
| | Fresh | 5 | 10 | |
| *Lactic acid bacteria* | | | | |
| C | 7.95 | 8.15 | 8.35 | 8.14$^{a}$ |
| BuCSY | 7.74 | 7.90 | 8.13 | 7.93$^{b}$ |
| BoCSY | 7.80 | 7.85 | 8.08 | *7.91$^{b}$* |
| MCSY | 7.78 | 7.88 | 8.11 | *7.91$^{b}$* |
| LSY | 7.70 | 7.83 | 8.05 | *7.86$^{b}$* |
| Means | 7.80$^{c}$ | 7.92$^{b}$ | 8.15$^{a}$ | |
| *Str.thermophilus* | | | | |
| C | 7.71 | 7.86 | 8.10 | 7.89$^{a}$ |
| BuCSY | 7.51 | 7.65 | 7.88 | 7.67$^{b}$ |
| BoCSY | 7.55 | 7.71 | 7.94 | 7.73$^{b}$ |
| MCSY | 7.53 | 7.68 | 7.90 | 7.71$^{b}$ |
| LSY | 7.47 | 7.62 | 7.85 | 7.64$^{b}$ |
| Means | 7.55$^{c}$ | 7.71$^{b}$ | 7.93$^{a}$ | |
| *L. bulgaricus* | | | | |
| C | 7.61 | 7.76 | 7.98 | 7.78$^{a}$ |
| BuCSY | 7.45 | 7.60 | 7.83 | 7.68$^{ab}$ |
| BoCSY | 7.53 | 7.60 | 7.83 | 7.66$^{ab}$ |
| MCSY | 7.50 | 7.58 | 7.80 | 7.63$^{ab}$ |
| LSY | 7.44 | 7.51 | 7.74 | 7.56$^{b}$ |
| Means | 7.51$^{c}$ | 7.64$^{b}$ | 7.84$^{a}$ | |
| Mold and Yeast | | | | |
| C | ND | | | |
| BuCSY | ND | | | |
| BoCSY | ND | | | |
| MCSY | ND | | | |
| LSY | ND | | | |

Mean values with the same letters along the row are not significantly different ($P < 0.05$)
C=control sample
BuCSY= stirred yoghurt fortified with buffalo colostrum
BoCSY= stirred yoghurt fortified with bovine colostrum
MCSY= stirred yoghurt fortified with mix colostrum
LSY= stirred yoghurt fortified with lactoferrin
ND=Not Detected

Table 4. Sensory evaluation of stirred yoghurt fortified with buffalo, bovine, mix colostrum and lactoferrin

| Components | C | BuCSY | BoCSY | MCSY | LSY |
|---|---|---|---|---|---|
| Flavor (50) | 47.30$^{c}$ | 47.50$^{b}$ | 47.70$^{a}$ | 47.20$^{e}$ | 47.24$^{d}$ |
| Body and texture (40) | 35.20$^{e}$ | 37.90$^{a}$ | 36.65$^{c}$ | 36.70$^{b}$ | 37.48$^{d}$ |
| Appearance (10) | 7.22$^{e}$ | 9.26$^{b}$ | 8.08$^{d}$ | 8.22$^{c}$ | 9.46$^{a}$ |

$^{a-d}$ There is a significant difference between any two means, within the same raw have the same superscript letter (Duncan's test P<0.05)
C=control sample
BuCSY= stirred yoghurt fortified with buffalo colostrum
BoCSY= stirred yoghurt fortified with bovine colostrum
MCSY= stirred yoghurt fortified with mix colostrum
LSY= stirred yoghurt fortified with lactoferrin

On the other hand, coliform bacterial, molds and yeasts could not be detected either when fresh or during the storage periods up to 10 days in all treatments, which might be due to the severity of heat treatments of milk and the preventive action of lactic acid bacteria.

***Sensory evaluation of stirred yoghurt***

Consumer acceptability of healthy food products is largely determined by sensory perception. The popularity of yoghurt is heavily reliant on its sensory characteristics, which aid in yoghurt marketing and consumer acceptability[42,17]. The sensory properties of the stirred yoghurt were evaluated by panels and the results in Table (4) revealed that, there were significant differences in flavor scores among all stirred yoghurt treatments. The stirred yoghurt fortified with lactoferrin and buffalo colostrum had higher scores of Body and texture and appearance than control sample with significant differences. Furthermore, the addition of colostrum and lactoferrin improved the look. As a result, adding colostrum and lactoferrin to stirred yoghurt improves its sensory qualities with significant differences. These findings are consistent with those reported by[41], who found that curd samples fortified with colostrum whey powder scored much higher than the control in terms of body and texture, colour, appearance, and overall acceptability, although flavour scores did not alter significantly when compared to the control.

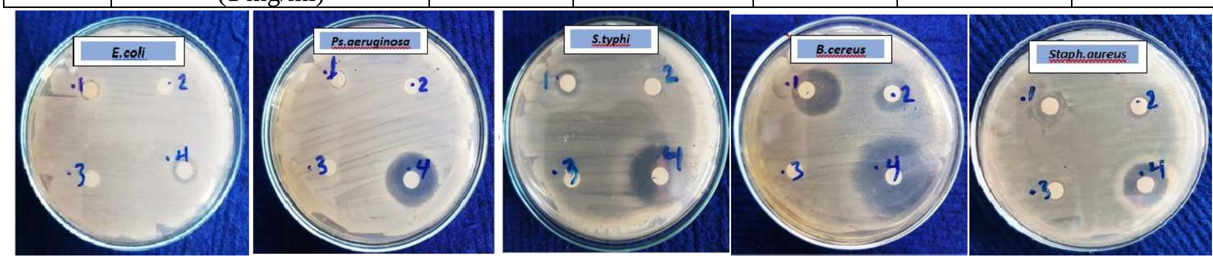
***Antimicrobial activity of lactoferrin***

Lactoferrin's antibacterial activity has been established against a wide spectrum of Gram-positive and Gram-negative bacteria[43]. Some microorganisms have emerged as emerging pathogens in recent years [44] may be lactoferrin activity against them is little knowledge. In recent years, natural antimicrobials have been proposed as food preservatives, combining them with non-thermal or lower treatments to preserve the nutritional and organoleptic properties of food [45]. The inhibitory zone diameters recorded with the agar diffusion are presented in Table 5 and Fig 4.

Results show that all extracts of lactoferrin were effective against all tested microbial strains. Comparison of the sensitivity of tested bacteria towards the lactoferrin showed that, Gram-negative *Ps. aeruginosa* and *E. coli* isolates were the most resistant. Characterized by the presence of zones of growth inhibition with diameters were 6.5 and 6.0 mm respectively, by using 50 mg/ml concentration of lactoferrin. Similar results were also obtained by[9],who tested bovine colostrum lactoferrin's antibacterial efficacy against *P. aeruginosa and E. coli* (DH5)

**Table 5. Zone inhibition (mm) of lactoferrin against tested microorganisms**

| No. | Sample | Inhibition zone in mm (Mean ± SD) | | | | |
|---|---|---|---|---|---|---|
| | | *E. coli* | *P. aeruginosa* | *S. typhi* | *B. cereus* | *Staph. aureus* |
| 1 | 100 mg/ml | 8±0.00 | 8±0.00 | 10.5±0.71 | 14±2.83 | 12.5±0.71 |
| 2 | 50 mg /ml | 6±0.00 | 6.5±0.71 | 8.5±0.71 | 10.5±0.71 | 10.5±0.71 |
| 3 | Negative Control | - | - | - | - | - |
| 4 | Ceftriaxone (1 mg/ml) | 13±0.0 | 15.5±0.7 | 22±0.0 | 24±1.4 | 16±1.4 |




A B C D E

Fig. 4. Zone inhibition (mm) of lactoferrin against tested microorganisms
A= *E. coli* B=*P. aeruginosa* C= *S. typhi* D=*B. cereus* E= *Staph. aureus*
1-100 mg/ml 2- 50 mg /ml 3- Negative Control 4- Ceftriaxone (1 mg/ml)

Their findings revealed that lactoferrin concentrations of 400 g/mL had the least inhibitory effect, with 35 percent and 29 percent growth inhibition on *P. aeruginosa and E. coli*, respectively, and lactoferrin concentrations of 700 g/mL had the highest inhibitory effect, with 86 percent and 66 percent growth inhibition on *P. aeruginosa and E. coli*, respectively. On the other hand, the most sensitive bacterium was *B. cereus* (14.0 mm by using 100 mg/ml concentration of lactoferrin). lactoferrin have strong inhibitory effect against all the tested bacteria especially against *B. cereus* and *Staph. aureus*, respectively. 100 mg/mL concentration of lactoferrin had the highest inhibitory effect on *P. aeruginosa* and *E. coli*, 14.0 and 12.5 mm respectively. This activity is linked to its ability to interact with bacterial membranes, causing their instability, affecting bacterial metabolic processes, and eventually killing them[46].

***Minimum inhibitory concentration of lactoferrin***

The results of MIC obtained against tested bacteria strains have been given in Fig. 5. The lowest MIC values which gave very high antimicrobial susceptibility of lactoferrin against *E. coli*, *P. aeruginosa*, *S. typhi*, *Bacillus cereus* and *Staph. aureus* were in a range of 0.4–2.3 mg/ml. *Bacillus cereus* and *Staph. aureus* were the most sensitive strains. Similar findings have been observed in other studies of lactoferrin's antibacterial action.

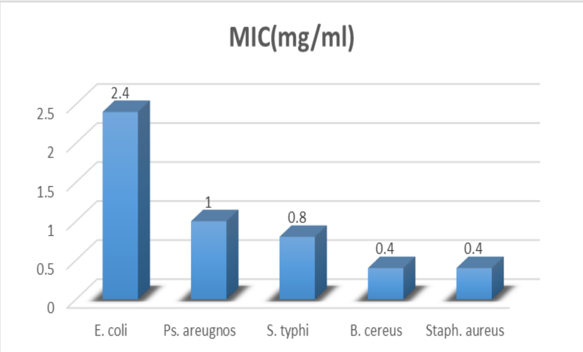



Fig. 5. Minimum inhibitory concentration of lactoferrin against tested microorganisms

The lowest MIC values led to very high antimicrobial susceptibility of lactoferrin against *Bacillus cereus* and *Staph. aureus* were 0.4 mg/m. MIC for *E. coli*, *P. aeruginosa* and *S. typhi* were 2.3, 1 and 0.8 respectively. Although lactoferrin is effective on both gram positive and gram-negative bacteria, it had a greater effect on the gram-positive bacteria.The main reason for greater effect of the lactoferrin on gram-positive bacteria than gram-negative might be the protective role of the gram-negative membrane's outer membrane, which acts as an effective barrier, is to blame[47,48].Another reason is that gram-positive bacteria have a single membrane, which renders them more accessible to permeation by lactoferrin. The MIC and MBC of several types of lactoferrin have been examined in multiple research. Gram-positive bacteria were more sensitive to bovine lactoferrin than gram-negative bacteria, according to a study[49].

***Amino acids content of buffalo, bovine colostrum and bovine lactoferrin compared to WHO/ FAO suggested requirements***

The comparison of amino acids content of buffalo, bovine colostrum and bovine lactoferrin to WHO/FAO[50], requirements are shown in Table (6). It could be noticed that buffalo, bovine colostrum and bovine lactoferrin are rich in total essential amino acids. Also, data showed that, the total amino acids of bovine colostrum, buffalo colostrum and bovine lactoferrin were 952.04, 950.04 and 939.65 mg/g protein, respectively. It is clearly shown that, total essential amino acids contents of buffalo, bovine colostrum and lactoferrin were higher compared to those given by the recommended pattern [50], for all age groups.

Valin is the highest value for BuC, BoC and L in essential amino acids. These results are in according with [51], who compared amino acids between human and bovine milk.

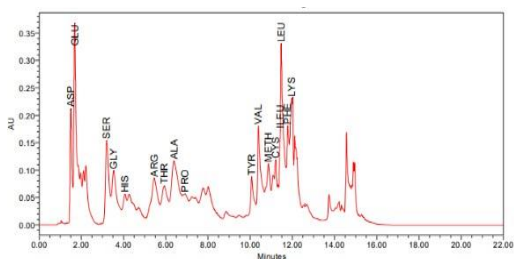
Buffalo colostrum and lactoferrin had the highest leucine content it was 80.96 mg/g protein for buffalo colostrum and 76.31 mg /g protein for lactoferrin. From the same table, it could be seen that, Methionine+ Cysteine, Phenylalanine+ Tyrosine, Threonine and valine were higher in buffalo, bovine colostrum and lactoferrin compared to those given by the recommended pattern [50] for all age groups.

We detected 7 non-essential amino acids in buffalo, bovine colostrum and bovine lactoferrin Table(6). The non-essential amino acids such as aspartic, glutamic and glycine were existed in relatively high amounts. They found to be 84.97, 122.49 and 26.71 mg/g protein for buffalo colostrum, respectively. Meanwhile, they found to be 54.41, 99.92 and 35.17mg/g protein for bovine colostrum and10.10, 11.42 and 12.37 mg/g protein for bovine lactoferrin, respectively. Glutamic acid and aspartic acid were formerly thought to be excitatory amino acids, according to previous research.Bjorn-Yoshimoto and Underhill[52], they found increased levels of each, particularly glutamic acid, in our data.

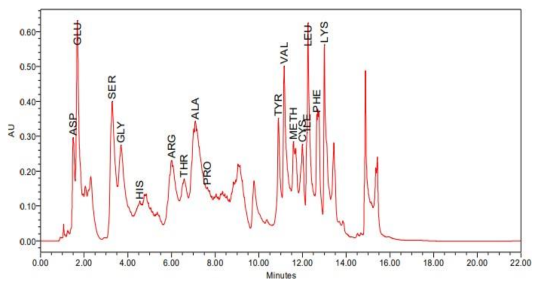
Bovine colostrum had higher levels of serine (SER) and glycine (GLY) than buffalo colostrum and bovine lactoferrin. SER contributes to the synthesis of antibodies and offers crucial information about biological functions linked with maintaining a healthy immune system, among other things [53].In a similar way, GLN improves human immunity while also increasing immune cell development, maturation, and advancement [54].

Table 6. Comparison of amino acids content (mg/g protein) of buffalo, bovine colostrum and bovine lactoferrin compared to WHO/FAO suggested requirements

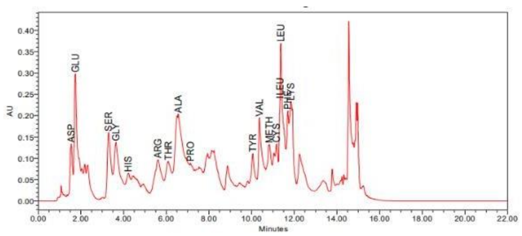
| Amino acids | Buffalo, colostrum | Bovine colostrum | lactoferrin | WHO / FAO | | |
|---|---|---|---|---|---|---|
| | | | | 0.5-2 years | 3-18 years | Adults |
| Histidine | 40.54 | 52.70 | 46.06 | 18-20 | 16 | 15 |
| Isoleucine | 12.81 | 9.51 | 12.93 | 31-32 | 30-31 | 30 |
| Leucine | 80.96 | 55.98 | 76.31 | 63-66 | 60-61 | 59 |
| Lysine | 43.02 | 55.66 | 46.04 | 52-57 | 47-48 | 45 |
| Methionine+ Cysteine | 89.29 | 81.12 | 83.81 | 26-28 | 23-24 | 22 |
| Phenylalanine+ Tyrosine | 99.11 | 121.61 | 104.24 | 46-52 | 40-41 | 30 |
| Threonine | 54.39 | 49.58 | 53.89 | 27-31 | 24-25 | 23 |
| Valine | 107.72 | 102.30 | 102.36 | 42-43 | 40 | 39 |
| Total essential amino acids | 527.84 | 528.46 | 525.64 | 305-329 | 280-286 | 263 |
| Alanine | 63.29 | 84.32 | 105.67 | | | |
| Arginine | 33.74 | 37.70 | 32.88 | | | |
| Aspartic acid | 84.97 | 54.41 | 63.59 | | | |
| Glutamic acid | 122.49 | 99.92 | 90.21 | | | |
| Glycine | 26.71 | 35.17 | 32.78 | | | |
| Proline | 40.42 | 50.75 | 44.24 | | | |
| Serine | 50.58 | 61.31 | 44.64 | | | |
| Total non essential amino acids | 422.2 | 423.58 | 414.01 | | | |
| Total amino acids | 950.04 | 952.04 | 939.65 | | | |




Auto-Scaled Chromatogram of buffalo colostrum




Auto-Scaled Chromatogram of bovine colostrum




Auto-Scaled Chromatogram of lactoferrin

## Conclusion

It could be concluded that colostrum and lactoferrin is a good source to fortify yoghurt with calcium, zinc, iron and sodium. Addition of colostrum and lactoferrin improved the flavor, body and texture and appearance. Increasing demand for natural food also increases the importance of natural inhibitors such as lactoferrin. Thus, colostrum and lactoferrin produce good nutritional and functional stirred yoghurt. The habitual consumption of LF stirred yogurt may contribute to the prevention of illness for gave very high antimicrobial against pathogenic bacteria.Buffalo, bovine colostrum and bovine lactoferrin are good sources of essential and non-essential amino acids, and can be used to complement foods that are lacking in any of these amino acids for healthy child growth and adult health.

## Conflicts of interest

There are no conflicts to declare.

## Formatting of funding sources

No funding source to declare

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