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FUNCTIONAL FERMENTED MILK WITH CAMEL COLOSTRUM FOR HEALTH PROMOTING

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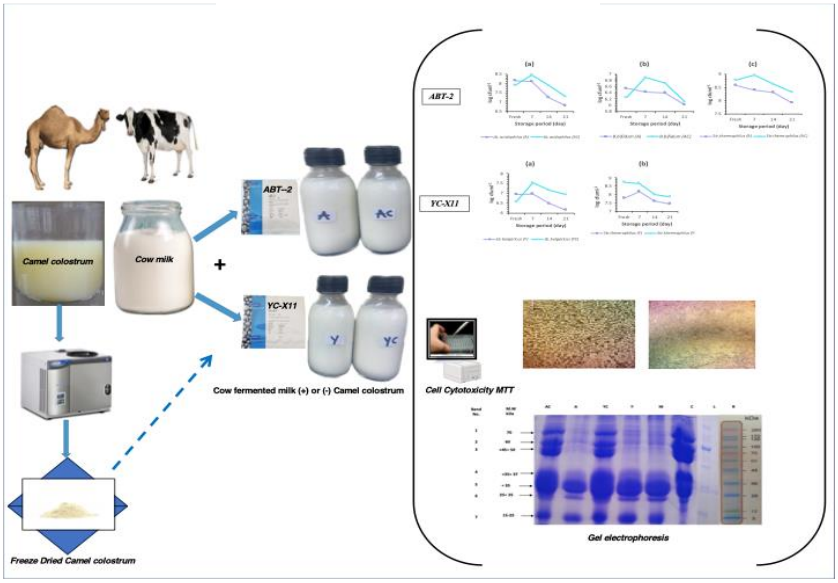
ABSTRACT

This study aims to investigate the in vitro anticancer properties of camel colostrum in fermented milk by comparing the antiproliferative activity of fermented cow milk with freeze-dried camel colostrum to fermented cow milk . The probiotic starter cultures DVS ABT-2 (*Streptococcus thermophilus*, *Lactobacillus acidophilus*, and *Bifidobacterium bifidum*) and YC- X11 (*Streptococcus thermophilus* and *Lactobacillus delbrueckii subsp. bulgaricus*) were used to prepare fermented cow milk separately. The chemical composition of liquid and freeze-dried camel colostrum and immunoglobulin G (IgG) concentration were analyzed. Furthermore, the analysis of fermented milk samples included physicochemical and microbiological assessments, electrophoresis pattern analysis, and sensory evaluation. The findings showed a significant antiproliferative impact on Caco-2 cells with a lower IC_{50} and a rise in lactic acid bacteria availability in colostrum-fermented milk samples. Furthermore, adding freeze-dried camel colostrum enhanced both the textural and sensory characteristics of the resultant fermented milk.

Graphical Abstract



Fermented cow milk fortified by freeze-dried camel colostrum using DVS ABT-2 or DVS YC-X11 as a starter resulted in significantly increased bacterial count, antiproliferative effectiveness, and lower IC_{50} values against Caco-2 cells.

1.Introduction

Consumers inclination towards healthy food products, such as functional foods, has rapidly increased because of the growing desire for a healthy lifestyle for enhancing overall well-being, which are the main factors generating markets for functional food products. Functional food refers to food that provides additional health benefits, including improved physiological performance, such as the regulation of specific body functions and the manipulation of biological defense mechanisms to prevent and/or treat particular disorders along with basic nutritional properties (Shori *et al*. 2019). Supplements including calcium, fibers, omega-3 fatty acids, plant stanols, prebiotics, and lactic acid bacteria (LAB) that produce bioactive peptides can be used as components in the production of functional foods. One particularly successful model of a functional dairy product type is fermented dairy products, which contain probiotic microflora, including yeast and bacteria, such as lactic acid bacteria (LAB) and bifidobacterium, recognized for their health-promoting properties (Behnsen *et al*. 2013). Which are responsible for enhancing food product digestion and the overall health of the human gastrointestinal system, as well as promoting well-being by improving the responsiveness of immune cells and the capacity to boost interferon (INF) production. It also contributes to lowering blood cholesterol, preventing various forms of cancer, and improving cognitive impairments (Bohlouli *et al*. 2021; Sanders *et al*. 2018). Additionally, fermented milk is comparatively simpler to digest than unfermented milk products and is considered a suitable food for those who are intolerant to lactose (Ezzatpanah 2020). The extended shelf life and appealing sensory acceptability of fermented milk enhance its distribution, consumption, and marketability. There is currently enough data to take into account fermented dairy products that include living bacteria when creating dietary plans to enhance health and the fact that it is frequently included in diets across the globe (Akdeniz and Akalin 2019; Behnsen *et al*. 2013).

Colostrum is the main source of nutrition and immunological components for the newborn. The nutraceutical components of colostrum are mainly represented by nutrients, antibodies, vitamins, immune factors, and growth factor contents; these nutrient profiles differ amongst species (Ceniti *et al*. 2023). Camel colostrum is an "early" milk generated by milking glands of she-camel in the first five days after parturition; over the next two days, it changes into mature milk (El-Hatmi *et al*. 2006). Compared to cow, goat, and sheep colostrum, camel colostrum has less lactose and fat but more protein, peptides, non-protein nitrogen, ash, vitamins, and minerals. A significant feature of camel colostrum is that is free of β-lactoglobulin (β-Lg), the principal whey protein in bovine milk that induces allergies in children, alongside a higher concentration of lactoferrin, which enhances the antimicrobial properties of camel colostrum (Benkerroum *et al*. 2004). Furthermore, contains immunoglobulins, which represent the main component of newborns' immunity to infections and is divided into three major subclasses (IgG1, IgG2, and IgG3). The two immunoglobulin subclasses, IgG2 and IgG3, are light chains and have molecular weights of 42 and 45 kDa, respectively (El-Hatmi *et al*. 2006; Konuspayeva *et al*. 2010). This characteristic of camel colostrum's protein composition represents a specific biological action, such as antibacterial, antioxidant, and antihypertensive effects (Jrad *et al*. 2020).

A new generation of competitive food products can be produced through developments in food ingredients and innovative technology. As fermented milk is an active subject of research, therefore, it is necessary to develop innovative new varieties of specialized foodstuffs based on fermented milk enriched with physiologically functional components. Camel milk and its derived products are increasingly being used in human nutrition. Little-known regarding the usage of camel colostrum in food products. Consequently, the aim of this study was to examine in vitro the antiproliferative action against carcinogenic cell line (Caco-2 cells) of fermented cow milk fortified with freeze-dried camel colostrum.

Additionally evaluated fermented milk made with cow milk containing camel colostrum, whether with DVS ABT-2 (*Str. thermophilus, Lb. acidophilus, and Bifidobacterium bifidum*) or YC-X11 (*Str. thermophilus* and *Lb. delbrueckii subsp. bulgaricus*), in terms of bacterial viability throughout a 21-day storage period at 4°C and bioactive peptide content compared to fermented cow milk.

2.Materials and methods

2.1. Materials

DVS ABT-2 (*Str. thermophilus, Lb. acidophilus, and B. bifidum*) and YC-X11 (*Str. thermophilus and Lb. delbrueckii* subsp. *bulgaricus)* starter cultures were obtained from Chr. Hansen's Lab A/S Copenhagen, Denmark. Camel colostrum (CC) was obtained from Berkash Farm, Giza, Egypt. Which were collected from over four multiparous adult female camels (*Camelus dromedarius*) at only one calving season at the beginning of November, and the samples were physically taken from the udder's four quarters after parturition for three days and kept at -18 °C for additional analysis. At the time of colostrum collection, none of the camels that were sampled showed signs of clinical mastitis. Fresh cow milk (11.75% ± 0.05 total solids, 3.30 %± 0.04 fat, 3.41%±0.1 protein, 4.42% ±0.03 lactose, 0.68%± 0.02 ash, 6.77±0.02 pH) was got from the Faculty of Agriculture's dairy unit, Cairo University, Giza, Egypt.

2.2. Methods

2.2.1. Freeze drying camel milk

Camel colostrum is completely dried in a freeze dryer (Snijders Scientific type 2040). The lyophilized camel colostrum was kept at 4°C for additional uses.

2.2.2. Preparation of functional fermented milk

The steps of producing fermented milk are demonstrated in Figure 1. In short, the raw cow milk (5 kg) was heated to 85 °C for 15 minutes, cooled to 5 °C, after that raised to 40 °C. Four different cow fermented milk samples were produced for analysis (Figure 1). The control samples of fermented cow milk included 0.2 g/L milk of two different types of starters (DVS ABT-2 or DVS YC-X11), which represent A and Y samples, respectively. While AC and YC samples differ from the control samples in the addition of colostrum to each of them at a rate of 3.5% (Tajorudin and Hamirudin 2020). Samples of inoculated milk were placed in 100 ml glass containers and incubated for 3 - 4 h at 42 °C until fully coagulation (pH reaches approximately 4.6). The glasses of fermented milk treatments were stored at 4 °C for 21 days and were examined in triplicate on fresh, 7, 14, and 21 days of cold storage.

2.2.3. Physicochemical analysis

Total solids, fat, total protein, ash, and titratable acidity were measured using the procedure outlined in AOAC (2012). The lactose content was analyzed according to Lawrence (1968). The pH of fermented milk was estimated electrometrically by a pH meter with a plastic electrode and a digital pH meter (JENWAY 3510).

2.2.4. Determination of IgG concentrations by enzyme-linked immunosorbent assay (ELISA)

2.2.4.1. Colostrum whey preparation

Colostrum (100 ml) was added to 50 ml saline. The mixture was centrifuged at 100,000 g for one hour at 4 °C to separate fat. The clarified aqueous layer (cleared colostrum, or "lactoserum") between the surface fatty layer and the particle was collected. After stirring and adding hydrochloric acid to get the pH down to 4, the solution was centrifuged at 30,000 g for 30 minutes at 4°C. After discarding the pellet, the supernatant was neutralized with 2M Tris and centrifuged for 30 minutes at 4°C at 30,000 g. The supernatant was run through a 0.45 μm filter, and the filtrate (colostrum whey) was kept at - 20°C until it was needed after (Azwai *et al*.1996).

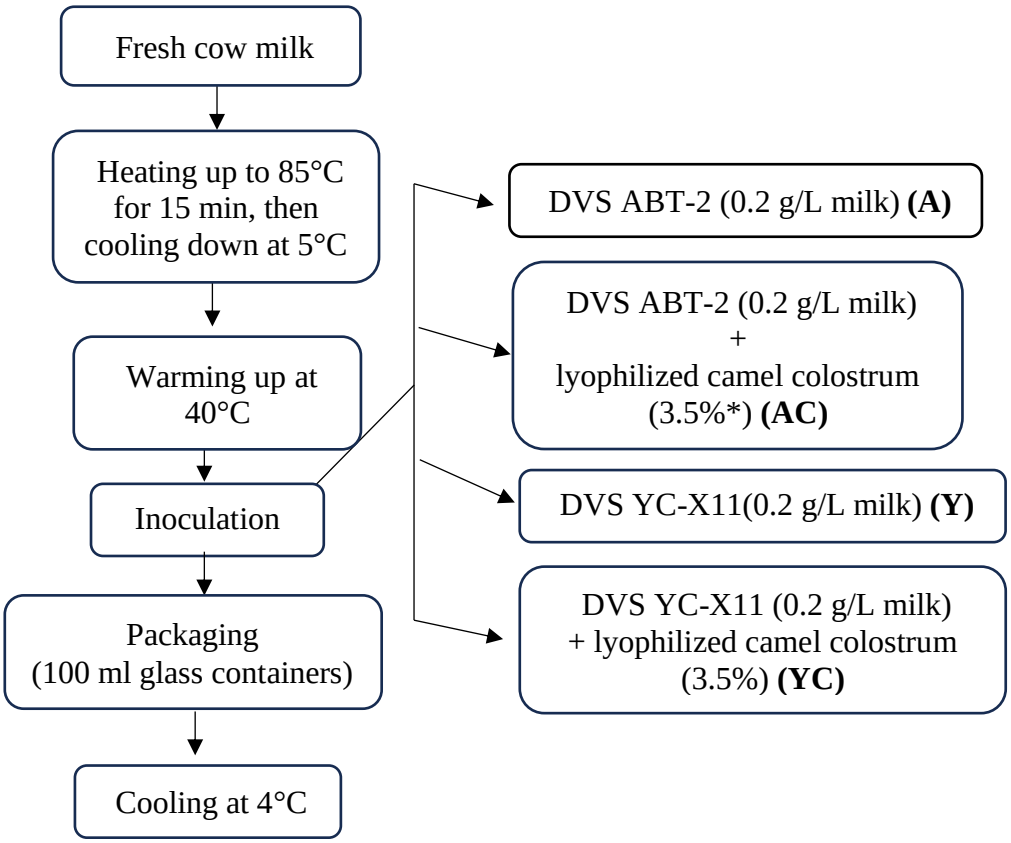


Figure 1. Preparation of functional fermented milk fortified with freeze dried camel colostrum

2.2.4.2. Enzyme-linked immunosorbent assay (ELISA)

For the testing of cross-reactivity of IgG with camel colostrum proteins**.** The antibody generated against IgG was evaluated for its cross-reactivity with a preparation of camel colostrum whey. The samples were diluted 1/800,000 in order to achieve optimal quantification prior to the experiment. The IgG concentration was measured by 2-site ELISA kits (Abcam, Cambridge, UK). The results were interpreted by spectrophotometric measurements at 450 nm wavelength. The antibody level was calculated using 4-parameter logistic curves for quantitative analysis. (Bartkiene *et al*. 2020).

2.2.5. Microbiological changes in functional fermented milk during storage period

The determination of the viable cell count for the *Lb. acidophilus* strain within the mixed culture was conducted by incorporating 1.5% (w/v) bile salt into MRS agar, according to Vinderola and Reinheimer (2000). The enumeration of *Lb. delbrueckii* subsp. *bulgaricus* was conducted utilizing MRS agar, according to Frank and Yousef (2004). Whilst *Str*. *thermophilus* by using M17. The enumeration of *B. bifidum* was conducted using modified MRS agar, which was supplemented with lithium chloride (0.600 g), neomycin sulfate (0.200 g), nalidixic acid (0.030 g), and paromomycin sulfate (0.250 g), all sourced from Merck, Warszawa, Poland, which were then dissolved in 100 ml of distilled water. The pH of the solution was adjusted to 7.3 ± 0.1 using 0.1 M sodium hydroxide (NaOH) and subsequently sterilized via filtration through 0.22 µm millipore filter prior to sterilization. An aliquot of 5 ml from this antibiotic solution was incorporated into 100 ml of MRS agar (pH 6.2 ± 0.1; Oxoid) just before application. The plates were incubated at a temperature of 37 °C for a

duration of 48 hours. Anaerobic conditions were established utilizing anaerobic culture jars with a capacity of 2.5 liters, along with AnaeroGen AN 25 sachets from Oxoid (Dave and Shah 1996). Coliform bacteria were enumerated on Violet Red Bile (VRB) agar following incubation at 37 ˚C for a duration of 48 hours. Colonies exhibiting pink to red-purple coloration, with or without surrounding halos of precipitation, were classified as coliform bacteria (Atlas 2004). Yeast and mold counts were assessed using yeast peptone dextrose agar (Oxoid) that was enriched with chloramphenicol at a concentration of 0.1 g/L (Oxoid) and incubated at a temperature of 30 °C for a duration of 72 hours (Jay *et al*, 2005). All microbial counts were calculated and expressed as log cfu ml^{-1}. The bacteria were counted three times in each experiment.

2.2.6. Sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE)

Polyacrylamide gel electrophoresis was conducted with minor adjustments in accordance with Laemmli (1970). Initially, samples were ultracentrifuged for 30 minutes at 55,000 g, and the high fat surface layer was removed (Goil *et al*.1998). Fat-free samples along with the sample application buffer, which consisted of 2.5 mM Tris-HCl at pH 6.8, 10.0% glycerol, 4.0% SDS, 0.02% bromophenol blue, and 10% mercaptoethanol, were prepared in a 1:2 ratio. The resulting mixtures were subsequently incubated in a water bath at 100 °C for 5 minutes to facilitate protein denaturation. Following this step, 10 µl of the mixture was loaded into the electrophoresis apparatus. Electrophoresis was performed in 15% polyacrylamide separating gel and 4% stacking gel. The gels were subjected to electrophoresis for a duration of 2 hours at a voltage of 100 V. After that, stained with Coomassie Brilliant Blue (CBB) R-250 (Bio-Rad) for 3 hours at ambient temperature to allow the dye to migrate to the bottom of the separating gel and detection of protein bands. Then, the gel is destained until the background is less dark by repeatedly immersing the sample in a solution of methanol, acetic acid, and water in a ratio of 1:1:8. The sample was run in 4 % stacking and 15% separating gel and used the broad-range protein marker (RIS11-prestained protein ladder, Cat. No. PMI11-0500, Volume: 500 µl, Bio- Helix Co., LTD) as the standard of the protein size.

2.2.7. Cytotoxic activity

2.2.7.1. Water-soluble extract

After adjusting the pH of each fermented milk sample to 4.6 using either 1.0 M hydrochloric acid or 1.0 M sodium hydroxide, the samples were centrifuged at 9,000 x g for a duration of 15 min at 4 °C. The supernatants were filtered through a syringe filter with a pore size of 0.45 µm (mixed cellulose esters membrane, EMD Millipore Corp., Billerica, MA) and were subsequently stored at -20 °C for additional analysis.

2.2.7.2. Cytotoxic effect on human cell line (MTT assay)

Water-soluble extracts were filtered through a Macrosep Advance spin filter (3 kDa; Pall Corp., Port Washington, NY). Filtrates were tested against carcinogenic cell line (Caco-2 cells) using the technique described by Ayyash *et al*. (2018). A 96-well tissue culture plate (Biofloat, Mannheim, Germany) was seeded with 1 x 10^5 cell/ml (100 µl/well) and incubated at 37°C for 24 h to create a complete monolayer sheet. The growth medium was removed from the 96-well microtiter plates when a confluent cell monolayer formed, and the monolayer was subsequently washed twice with wash media. Two-fold dilutions of the tested samples were prepared in RPMI medium containing 2% serum (maintenance medium). 0.1 ml of each dilution was added to separate wells, keeping three wells as controls that contained only maintenance medium. The plate was incubated at 37°C; following that, the cells were examined for any physical indicators of toxicity, such as partial or complete loss of the monolayer, rounding, shrinkage, or cell granulation. A 3-[4,5-dimethylthiazol-2-yl]-2,5-diphenyl tetrazolium bromide (MTT) solution was prepared by mixing 5 mg of MTT with 1 ml of PBS (Bio Basic Canada INC). After that, 20 µl MTT solution was added to each well. The plate was then put on a shaking table and spun at 150 rpm for five minutes to fully incorporate the MTT

into the medium. To enable the MTT to be metabolized, the incubation procedure was then carried out for four hours at 37°C with a 5% CO_2 atmosphere. After draining the culture medium from the plate, they were then dried on paper towels. 200 µl of dimethyl sulfoxide (DMSO) was used to dissolve the formazan compounds that resulted from the reduction of tetrazolium salt. In order to fully integrate the formazan into the solvent, the mixture was then put on a shaking table and stirred at 3.77 g for 5 min. The optical density was measured at a wavelength of 560 nm and subtracted background at 620 nm. Three separate analyses of each sample were conducted. The impact of samples on cell lines was captured using a digital colour microscope camera connected to a microscope (Leitz Labovert, Germany).

2.2.8. Sensory evaluation

Assessment of sensory attributes of all fermented milk samples was organoleptically tested by a 20-member trained and experienced panel consisting of scientists of the Dairy Research Department, FTRI, using fresh samples and samples on the 21 th day of storage that were maintained at 4 °C until the time of testing. The samples were presented in identical plastic cups that were randomly coded. The evaluation of the fermented milk samples encompassed color and appearance, taste and odor, texture, and overall acceptability, employing a 9-point hedonic scale that ranged from 1 (dislike greatly) to 9 (like greatly), as outlined by Lim (2011).

2.2.9. Statistical analysis

The results obtained were subjected to statistical analysis utilizing a software package (Version 2004, SAS Institute, Cary, North Carolina, USA) that employed analysis of variance. In instances where the F-test yielded significant results, the least significant difference (LSD) was computed according to Duncan's method for mean comparisons at a significance level of $P < 0.05$. The data displayed in the tables represent the mean of three measurements, accompanied by the (± standard deviation).

3.Results and discussion

3.1. Chemical composition of camel colostrum

The chemical composition of camel colostrum is shown in Table 1. The amount of total solids TS in camel colostrum is 13.90% this is nearly consistent with the findings of Omer and Eltinay (2008) regarding United Arab Emirates (UAE) camel colostrum (12.90%), but considerably lower than the values reported by Konuspayeva *et al*. (2010) and Ohri and Joshi (1961) for Kazakhstan (KZ) and Indian (IN) camel colostrum (15.61% and 18.4%, respectively). The protein ratio of 4.92% is lower than that of SA and KZ camel colostrum, as reported by Gorban and Izzeldin (1997) and Konuspayeva *et al*. (2010), which were 5.8% and 6.03%, respectively. While in agreement with the results provided by Omer and Eltinay (2008) regarding UAE camel colostrum. The lactose content is 4.70%, exceeding that of camel colostrum from the UAE (4.4%), SA (2.7%), and KZ (3.63%) (Omer and Eltinay 2008; Gorban and Izzeldin 1997; Konuspayeva *et al*. 2010). Abu-Lehia (1989) noted that the lactose concentration during parturition was 2.68% and progressively increased to 4.4% by the third day. The fat content (3.10%) is higher than that reported by Johnson (1978) at 1.36%, Ohri and Joshi (1961) at 0.2-2.8%, and Yagil and Etzion (1980) at 0.15%, but it is comparable to the percentages reported by Gorban and Izzeldin, (1997) and Omer and Eltinay (2008) at values of 3.01 and 3.10%, respectively. Nevertheless, the research conducted by Konuspayeva *et al*. (2010) revealed a finding of 7.88%. The ash content measured at 1.15% is almost equal to the 1.11% reported by Omer and Eltinay (2008). It exceeds the 0.78% found by Gorban and Izzeldin (1997) but is lower than the 2.60% noted by Ohri and Joshi (1961). The average Ig concentration in liquid colostrum was found to be 4.58±0.08%, which is lower than the value reported by El-Hatmi *et al*. (2006) for Tunisian dromedary camel colostrum, which was 10.07 %. The pH level measured at 6.32 exceeds that of UAE and IN camel colostrum, which were recorded at 6.19 and 5.6, respectively, according to Omer and Eltinay

(2008) and Ohri and Joshi (1961). Conversely, this pH is lower than that of SA camel colostrum, which ranged from 6.40 to 6.60, as noted by Abu-Lehia (1989) and Gorban and Izzeldin, (1997), as well as lower than the 6.52 pH found in KA camel colostrum as reported by Konuspayeva *et al*. (2010). The lower pH values observed in colostrum compared to milk may be attributed to the presence of dihydrogen phosphate, citrate, and carbon dioxide in camel colostrum, which are linked to the reduced pH levels found in colostrum (Starkutė *et al*. 2023). Freeze-dried camel colostrum yield is (84.36%± 1.12). The TS content of this colostrum is 96.8%, as detailed in Table 1. Among its components, protein and lactose account for the largest proportions at 34.52% and 32.57%, respectively. This is followed by fat, which constitutes 21.50%, while ash represents the smallest ratio at 8.00%.

Table 1. Composition of liquid and freeze-dried camel colostrum

Properties	Liquid colostrum	Freeze-dried colostrum
Total solids (%)	13.90±0.05	96.80±0.10
Protein (%)	4.92±0.10	34.52±0.20
Fat (%)	3.10±0.10	21.50±0.10
Lactose (%)	4.70±0.05	32.57±0.03
Ash (%)	1.15±0.04	8.00±0.06
IgG (%)	4.58±0.08	28.00±0.19
pH	6.32±0.10	6.48±0.10

3.2. Physicochemical properties of functional fermented milk

Data displaying in Table 2 are the physicochemical characteristics of fermented milk, influenced by the addition of camel colostrum and the type of bacterial starter culture. The results cleared that supplementing with camel colostrum as lyophilized powder led to a significant increase in TS contents. The TS values of fresh samples A, AC, Y, and YC were 11.98, 15.50, 12.00, 15.51%, respectively. However, the TS contents are uninfluenced by the type of bacterial starter culture. A slightly gradual increase in the TS contents of all samples correlated to the prolonging of the cold storage duration. In general, all TS contents obtained in all treatments agree with the legal standard of EOSQ (2010). The pH values of cow milk fermented with the YC-X11 were found to be significantly lower ($P < 0.05$) compared to those fermented with the ABT-2. Additionally, the titratable acidity (TA%) measurements of the fermented milk from each treatment indicated that the acidity level generated by the YC-X11 was significantly higher than that formed by ABT ones either with or without colostrum. This explains the lower acidity rate when using ABT -2 due to the absence of *Lb. delbrueckii* subsp. *bulgaricus*. Similar findings are observed by Akgun *et al*. (2018). Generally, the pH exhibited a significant reduction ($P< 0.05$) and TA % increased ($P < 0.05$) in both treatments fermented by YC-X11 or ABT-2 during the storage period.

The incorporation of camel colostrum resulted in a fermented milk product exhibiting a higher TA% and a lower pH value compared to the fermented milk that did not contain camel colostrum.

Lactic acid bacteria (LAB) metabolize lactose in milk for their growth and produce metabolites, mainly lactic acid, and energy for their growth and keeping up, resulting in a reduction in the pH of fermented milk (Wang *et al*. 2021). Logically, the prolonging of the cold storage period caused an increase in TA% and hence a corresponding decrease in pH value, as the longer presence time of live bacteria in the product means more time available for the LAB to metabolize the milk lactose, and so more lactic acid was produced, which contributed to a much lower pH surrounding the LAB. Also, the results demonstrate that fermented milk fortified with camel colostrum led to a fermented milk

with TA% slightly higher and pH value lower than those that occurred in fermented milk without camel colostrum. The variations noted in pH and acidity are influenced by the composition of the substrate, a relationship that can be linked to the presence of fermentable sugars in camel colostrum including lactose, along with glucose, fructose, glucosamine, galactosamine, N-acetylneuraminic acid, and oligosaccharides (Fukuda *et al*. 2010). The results regarding the total acidity percentage are consistent with the research conducted by (Ayyash *et al*. 2018), which demonstrated that *Lactococcus lactis* KX881782 exhibited a greater ability to generate organic acids, reflected in a higher total acidity percentage, when fermenting camel milk compared to cow milk. We hypothesize that the elevated percentage of TA in camel colostrum fermented camel milk could be associated with the strain's adaptation to camel colostrum, as well as the presence of specific amino acids and peptides in camel colostrum that promote the growth of this bacteria. On the other hand, colostrum is characterized by high buffering capacity (Lucey *et al*.1993) as the high buffering capacity is related to the increased protein content, which is considered a principal buffering component, similar findings are observed by Varghese and Mishra (2008).

3.3. Microbiological changes in functional fermented milk

To examine the effect of camel colostrum on fermentation, DVS YC-X11 (*Lb*. *bulgaricus* and *Str*. *thermophilus*) or DVS ABT-2 starter (*Lb*. *acidphilus*, *B. bifidium*, and *Str*. *thermophilus*) were inoculated in cow milk. While the same analysis was performed as before, but without adding colostrum as a control. The bacterial numbers (log cfu ml^{-1}) of ABT-2 in colostrum fermented milk or fermented milk without colostrum treatments stored at 5 °C, is shown in Figure 2 (a, b, and c). In fresh samples, significantly elevated number of counts ($P < 0.05$) was observed in ABT fermented milk fortified with colostrum. Generally, there was a slight raise in the count of viable cells during the first seven days of refrigerated storage, following a gradual decrease. Xu *et al*. (2021) observed that the count of viable fermented milk organisms initially rose following production, peaked at a certain point, and subsequently declined during the period of refrigerated storage. *Str*. *thermophilus* emerged as the predominant starter culture, with its population surpassing 7.5 logs at the onset of the storage period. This observation aligns with the manufacturer's specifications for the DVS ABT-2 starter culture provided by Chr. Hansen. A significant rise ($P< 0.05$) in the counts of *Str*. *thermophilus* was observed on the seventh day between control and colostrum-fortified fermented milk. The difference in the number of streptococci between colostrum fermented milk and the control group increased throughout the storage period, eventually reaching a value of 0.4 log cycles by the end of the storage experiment. This may be due to the stimulatory effect of the nutrients found in colostrum, which can enhance the growth of LAB (Fasse *et al*. 2021). However, *Str*. *thermophilus* counts were found to be within the range of (7.92±0.28 to 8.32±0.13 log cfu ml^{-1}) after 21 d of storage at 5 °C. It should be noted that the initial count of *Lb. acidphilus* 0.7 log cycle lower than for *Str. thermophilus*. However, a significantly higher viable count of *Lb*. *acidophilus* was recorded during cold storage at 5 °C in comparison to *B. bifidum*, this is due to the resistance of *Lb. acidophilus* to acidity in contrast to *B.bifidium* (Lourens-Hattingh and Viljoen 2001). Nevertheless, a decrease in *Lb. acidophilus* count was observed after that. The initial counts of *B.bifidium* ranged between 7.25-7.43 log cfu ml^{-1}. The count of bifidobacterium noted between days 7 and 14 was greater than that recorded on day 0 in both types of fermented ABT milks. There were significant differences ($P< 0.05$) between the numbers of bifidobacterium in fermented milk fortified with colostrum compared to fermented milk without it; this may be due to oligosaccharides and lacto-N-biose in camel colostrum which act as prebiotics and stimulate the growth of bifidobacteria (Fukuda *et al*. 2010).

Table 2 Chemical properties of functional fermented milk fortified with freeze-dried camel colostrum

Properties	Cold storage (days)	Treatments			
		A	AC	Y	Y C
Total solids (%)	Fresh	11.98 ± 0.01 b, a, b	15.50 ± 0.01 a, a, b	12.00 ± 1.00 b,a,b	15.51 ±0.01 a,,a,b
	7	12.04 ± 0.04 b, a, ab	15.57 ±0.02 a, a, ab	12.07 ± 0.01 b, a, ab	15.58 ±0.02 a, ,a,ab
	14	12.19 ±0.01 b, a, a	15.74 ±0.04 a, a, a	12.20 ± 0.01 b, a, a	15.73 ± 0.03 a ,a, a
	21	12.27 ±0.01 b, a, a	15.82 ±0.02 a, a, a	12.28 ±1.00 b, a, a	15.81 ±0.01 a, a, a
Fat (%)	Fresh	3.20 ±0.20 b, a, a	4.2 ±0.10 a, a, a	3.20 ±0.20 b, a, a	4.20 ±0.20 a, a, a
	7	3.20 ± 0.20 b, a, a	4.2 ± 0.20 a, a, a	3.20 ± 0.20 b, a, a	4.20 ± 0.10 a, a,a
	14	3.30 ±0.30 b, a,a	4.3 ±0.10 a, a, a	3.30 ±0.30 b, a, a	4.30 ±0.10 a, a,a
	21	3.30 ± 0.10 b, a, a	4.3 ± 0.10 a, a, a	3.30 ± 0.20 b, a, a	4.30 ±0.20 a, a, a
Protein (%)	Fresh	3.40 ±0.03 b, a, b	4.64 ±0.02 a, a, b	3.40 ±0.01 b, a, b	4.64 ±0.04 a, a, b
	7	3.41 ±0.01 b, a, b	4.66 ± 0.01 a,a,b	3.42 ± 0.02 b, a, b	4.67 ± 0.1 a, a, b
	14	3.43 ± 0.03 b, a, ab	4.70 ± 0.04 a, a, ab	3.45 ± 0.01 b, a,ab	4.71 ± 0.02 a, a, ab
	21	3.48 ± 0.04 b, a, a	4.73 ± 0.01 a, a, a	3.49 ±0.01 b, a,a	4.75 ±0.05 a, a, a
Lactose (%)	Fresh	4.62 ±0.02 b, a, b	5.61 ± 0.01 a, a, b	4.63 ± 0.10 b,a,b	5.62 ± 0.02 a, a, b
	7	4.66 ± 0.1 b, a, b	5.64 ± 0.04 a, a, b	4.68 ±0.10 b, a, b	5.65 ± 0.05 a, a, b
	14	4.68 ± 0.02 b, a, ab	5.66 ± 0.01 a, a, ab	4.67 ±0.10 b, a, ab	5.64 ±0.04 a, a, ab
	21	4.69 ± 0.1 b, a, a	5.69 ± 0.01 a, a, a	4.68 ± 0.10 b, a, a	5.67 ± 0.07 a, a, a
Ash (%)	Fresh	0.76 ±0.02 b, a, b	1.05 ±0.01 a, a, b	0.77 ±0.01 b, a, b	1.05 ± 0.05 a,a ,b
	7	0.77 ± 0.01 b, a, b	1.07 ± 0.02 a, a, b	0.77 ±0.01 b, a, b	1.06 ±0.01 a, a, b
	14	0.78 ± 0.02 a, a, ab	1.08 ±0.02 a, a, ab	0.78 ±0.01 b, a, ab	1.08 ± 0.02 b, a,ab
	21	0.80 ± 0.1 b, a, a	1.10 ±0.1 a, a, a	0.81 ±0.10 b, a, a	1.09 ±0.01 a, a, a
pH	Fresh	4.73 ±0.03 a, a, a	4.72 ±0.02 b, a, a	4.63 ±0.03 a, b, a	4.60 ±0.10 b, b, a
	7	4.60 ± 0.05 a, a, ab	4.55 ± 0.01 b,a,ab	4.51 ± 0.01 a, b, ab	4.42 ±0.02 b, b,ab
	14	4.51 ±0.01 a, a, b	4.43 ±0.03 b, a, b	4.40 ±0.1 a, b, b	4.33 ±0.03 b, b, b
	21	4.34 ±0.03 a, a, c	4.28 ±0.02 b, a, c	4.25 ±0.02 a, b, c	4.17 ±0.01 b, b,c
T.A. (%)	Fresh	0.75 ±0.01 b, b, c	0.77 ±0.01 a, b, c	0.81 ±0.01 b, a, c	0.85 ±0.03 a, a, c
	7	0.86 ±0.02 b, b, b	0.88 ±0.01 a, b, b	0.93 ±0.01 b, a, b	0.98 ±0.01 a, a, b
	14	0.91 ±0.01 b, b, ab	0.96 ±0.01 a, b, ab	1.02 ±0.02 b, a,ab	1.12 ±0.01 a, a, ab
	21	1.00 ±0.1 b, b, a	1.09 ±0.01 a, b, a	1.10 ±0.1 b, a, a	1.23 ±0.01 a ,a,a

The letters preceding the comma indicate the presence of camel colostrum factor, whereas those following the comma represent the factors associated with the starter and storage periods, respectively. Means sharing the same letter at any position do not exhibit significant differences (P>0.05). All values are presented as means ±SD, n = 3. (A) Fermented milk with ABT-2; (AC) Colostrum fermented milk with ABT-2; (Y) Fermented milk with YC- X11; (YC) Colostrum Fermented milk with YC -X11.

Additionally, it was noted that *Str. thermophilus* has the potential to enhance the growth and viability of bifidobacterium when utilized in conjunction within starter cultures through creating an anaerobic environment by acting as an oxygen scavenger (Lourens-Hattingh and Viljoen 2001). However, the decrease in the number of bifidobacterium on the 21st day of storage is due to this low tolerance to acidity, and thus their survival rate decreases at low levels of pH (less than 4.6) (Lee and Salminen 2009).

Data presented in Figure 2 (d & e) shows changes in fermented milk with YC-X11 (*Lb. delbrueckii* subsp. *bulgaricus* and *Str. thermophilus*) in fresh samples and during storage period. The survival rate of *Str. thermophilus* is often reported to be high, exceeding 8 log cfu ml^{-1} in fermented milk products that have been refrigerated for a duration of up to 6 weeks. (Varga *et al*. 2014). Research indicates that the relative abundance of *Str*. *thermophilus* within the overall acidifying microflora of yogurt exceeds that of *Lb. bulgaricus*, despite both being inoculated in equal proportions (Bielecka and Majkowska, 1998). The proto-cooperation is partly due to the ability of *Str. thermophilus* to rapidly metabolize lactose, resulting in the production of acids that decrease the pH to a level conducive to the growth of *Lb*. *bulgaricus*. Additionally, *Lb*. *bulgaricus* may be further stimulated by various metabolites generated by *Str. thermophilus*, as previously noted (Sieuwerts *et al*. 2008). Concurrently, *Lb*. *delbrueckii* subsp. *bulgaricus* synthesizes vital amino acids during the fermentation process, which are necessary for the proliferation of *Str. thermophilus* (Lourens-Hattingh and Viljoen 2001). Moreover, *Lb. bulgaricus* was better adapted to the acidic environment and dominated the fermentation process subsequently until the endpoint (Gasser *et al*. 2022). The viable counts of *Str*. *thermophilus* and *Lb*. *bulgaricus* in colostrum fermented milk were significantly higher ($P < 0.05$) compared to those in free colostrum fermented milk. This enhancement may be attributed to the presence of amino acids and peptides in camel colostrum, which are known to promote the growth of these bacterial species (Fenster *et al*. 2019).

The population of *Str. thermophilus* in ABT-fermented samples exceeded that of the YC-X11 starter, both in fresh samples and throughout the storage period. This phenomenon can be attributed to the fact that the ABT culture generated lower acidity in the fermented milk compared to the YC-X11 starter, resulting in a reduced loss of survival values for the former relative to the latter. These findings align with the observations made by Ismail (2015). The diminished bacterial population observed in ABT -2 or YC-X11 within fermented milk containing colostrum in fresh samples may be attributed to the fact that camel colostrum contains more natural antibacterial compounds than cow's milk. These components, which include immunoglobulins, lactoferrin, lactoperoxidase, lysozyme, and cytokines, are known to impede the rapid proliferation of bacterial cultures. Furthermore, they exhibit bacteriostatic rather than bactericidal effects on probiotic strains (Ali *et al*. 2023; González-Navarro *et al*. 2022). So, we assume that both starter cultures take more time to overcome this effect. In general, the counts of different microbial groups for all fermented milk treatments pronounced decreased within storage. The decline was evidently attributed to a reduction in pH below the optimal level, resulting from lactic acid production by proliferating lactic acid bacteria. This alteration affects the intracellular pH of the LAB, thereby inhibiting enzyme activity, ion transport, and nutrient absorption, which subsequently inhibits bacterial growth and reduces LAB populations at elevated concentrations (Soleymanzadeh *et al*. 2016). However, all fermented milk conforms to requirements by food regulations the starter microorganisms must remain viable, active, and in sufficient numbers (at least 10^6) in the product to the date of minimum durability to maintain their health promoting effects (CODEX STAN 243-2003).

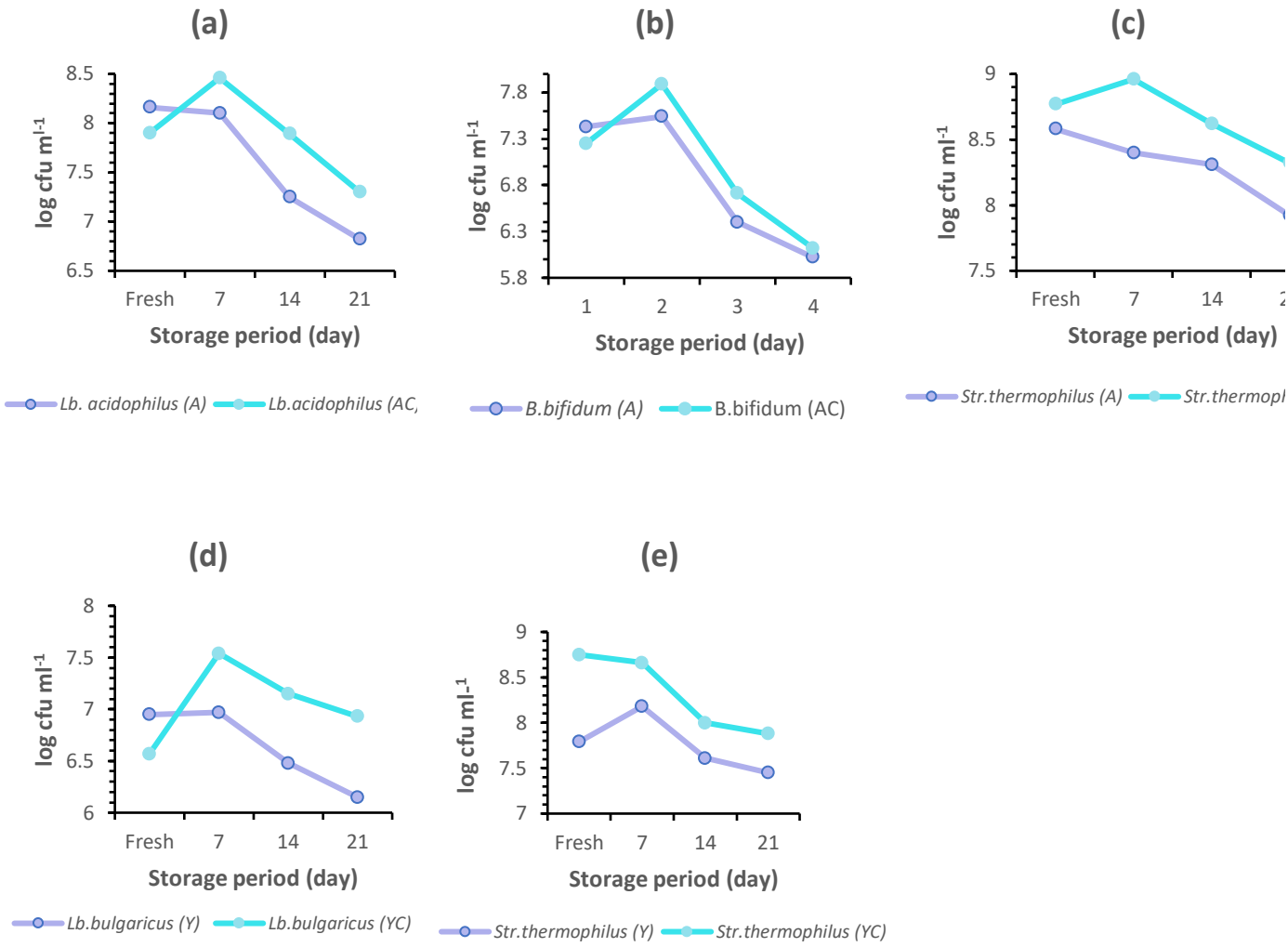


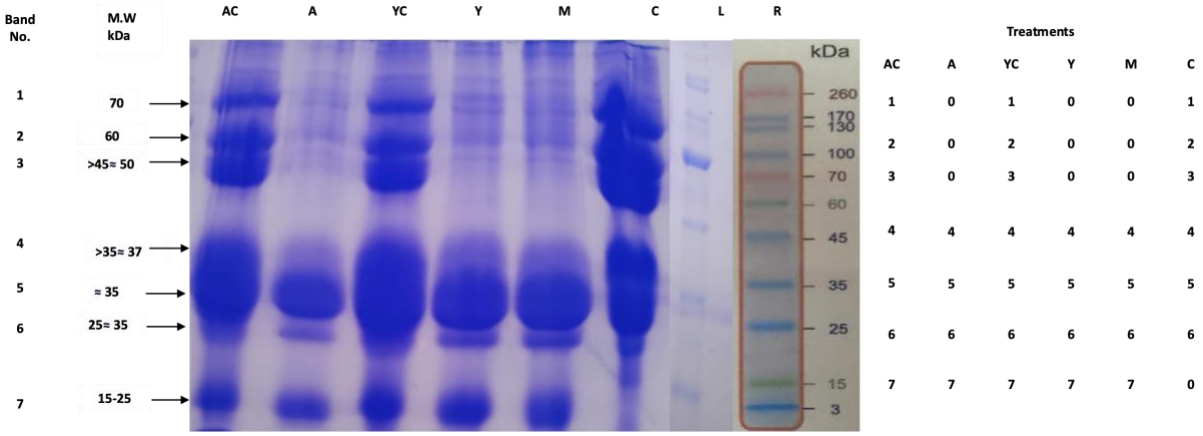
Figure 2 Effect of using camel colostrum on starter bacteria counts of fermented milk.
(A) Fermented milk with ABT-2; (AC) Colostrum fermented milk with ABT-2; (Y) Fermented milk with YC- X11; (YC) Colostrum fermented milk with YC-X11.

This results from the particular dietary needs that lactic acid bacteria (LAB) and bifidobacteria have for growth and function (Hébert *et al*. 2004). The availability of nutrients in the growth environment, including short peptides, free amino acids, and oligosaccharides, influences the growth and viability of starter cultures (Fenster *et al*. 2019). The camel colostrum provides the fermentation media with the nutrients that starter bacteria need to grow and maintain their viability. Moreover, the decline in viability during storage was significant in the control fermented milk. The findings indicate that colostrum has a beneficial impact on improving the viability of ABT and YC starter cultures.

No proliferation of yeast and mold, or coliform was observed in either the colostrum-fermented ABT milk or the controls throughout the entire storage duration. This can be associated with the characteristics of fermented milks, which include low oxygen levels, high acidity, and the synthesis of antimicrobial compounds by the starter bacteria (Surono and Hosono 2011).

3.4. Electrophoretic patterns of functional fermented milk

The proteins bands of fresh fermented milk treatments as well as cow milk and freeze-dried camel colostrum are presented in descending order based on their relative molecular weights are shown in Figure 3. It is clear that there were more bands of protein in the range of 50 to 70 kDa in colostrum fermented milk and freeze-dried colostrum (treatment C) compared to free colostrum samples and cow milk. These bands may be associated with IgM (band 1), IgA (band 2), IgG1, IgG2 and IgG3 (band 3) with molecular weight of 70, 63, 50, 46, and 42 kDa, respectively. The findings align with those of Azwai *et al*. (1996), who indicated that the molecular weight for IgM, IgA and IgG1, IgG2, IgG3 have the same range of molecular weight. Furthermore, a specific biological activity, such as antibacterial, antioxidant, and antihypertensive properties, was reported for proteins with a molecular weight of 42-45 kDa (Jrad *et al*. 2020). The data indicated that a peptide with MW of 25≈30 kDa present in all tested samples, which may correspond to lactoferrin (Gaspar-Pintiliescu *et al.* 2020). All tested samples except colostrum exhibited a band indicative of light chains within the 15-25 kDa range. These results agree with the observations in the literature (Costa *et al*. 2014), who indicated that the proteins α S2- casein (CN), α S1- CN, β- CN, and κ- CN exhibit molecular weights ranging from 35 to 24 kDa, while β- lactoglobulin and α- lactalbumin display molecular weight bands of 18 kDa and 14.2 kDa, respectively, in cow milk.



Treatments

	AC	A	YC	Y	M	C
1	1	0	1	0	0	1
2	2	0	2	0	0	2
3	3	0	3	0	0	3
4	4	4	4	4	4	4
5	5	5	5	5	5	5
6	6	6	6	6	6	6
7	7	7	7	7	7	0

Figure 3 Electrophoretic patterns of fermented milk samples, cow milk, and freeze-dried colostrum migrated along with prestained protein ladder. **(**Lane A**)** fermented milk with ABT-2; (lane AC) colostrum fermented milk with ABT-2; (lane Y) fermented milk with YC- X11; (lane YC) colostrum fermented milk with YC-X11; (lane C) Freeze-dried colostrum; (lane L) prestained protein ladder; (R) molecular weight marker.

3.5. Cytotoxic activity of functional fermented milk

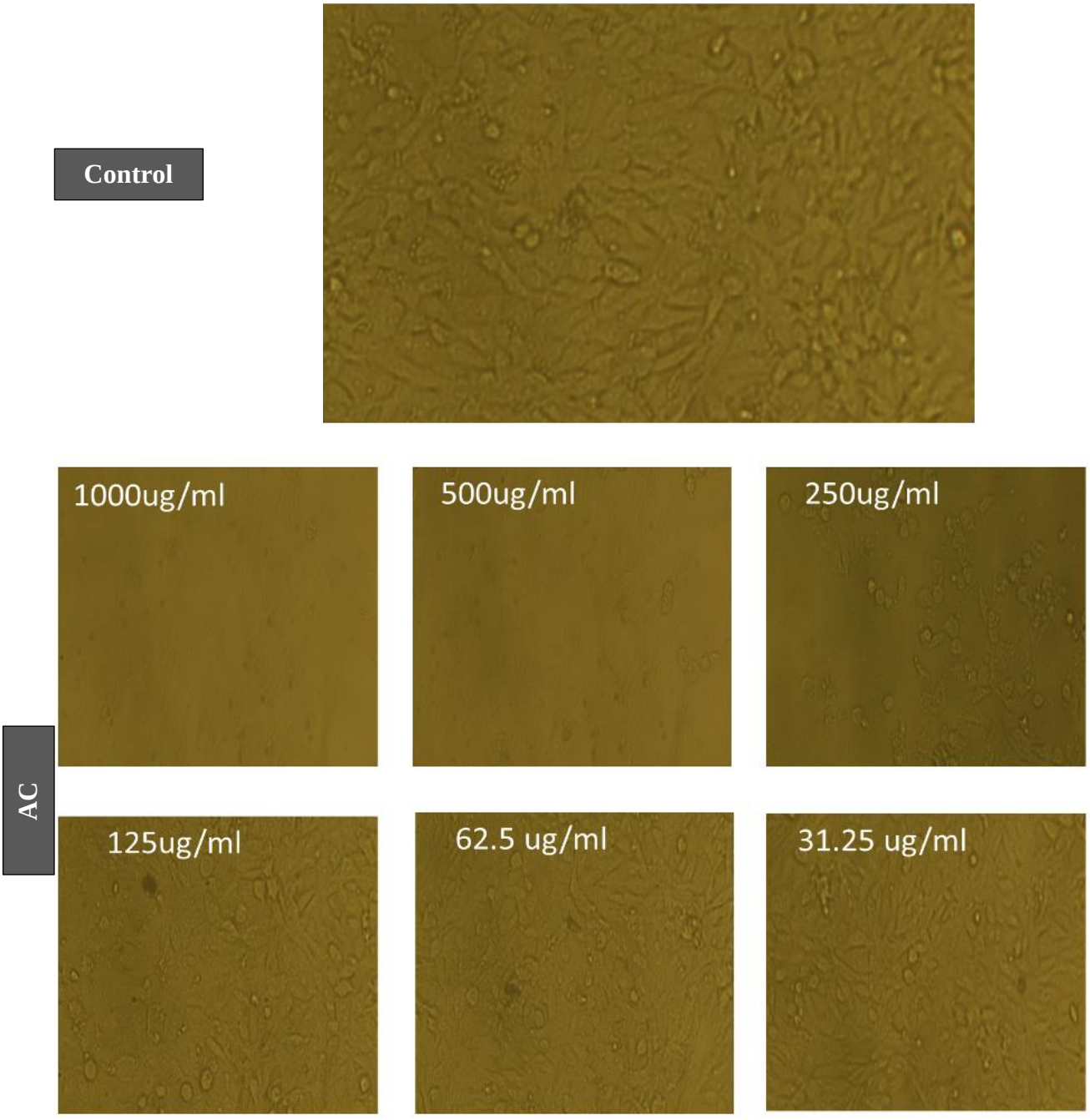
The anticancer effect of different concentrations and the IC_{50} value (refers to the concentration of a substance that results in a 50% mortality rate of cells within a 48-hour exposure period) of fermented milk and colostrum fermented milk samples against the carcinogenic cell lines (Caco-2 cells) are illustrated in Table 3 and Figure 4. The present results confirmed that the carcinogenic cells maintained their viability when cultivated in the standard growth conditions and exhibited no cytotoxic effects. The data further indicated a reduction in the viability of carcinogenic cells when cultured in growth media supplemented with the fermented milk samples. The cytotoxic activity against Caco-2 cells, as demonstrated in Table 3 and Figure 4, generally increased with higher concentrations of each sample. Specifically, the lowest cytotoxic activity was observed at the minimal concentration of 31.25 µg/ml, whereas the most significant antiproliferative cytotoxic effect was recorded at

the maximum concentration of 1000 µg/ml. The findings additionally indicated that the YC sample exhibited the most significant cytotoxic activity ($P < 0.05$), as reflected by a reduced IC_{50} value. Whereas the YC sample inhibited the viability and growth of half of the cultured Caco-2 cells using 74.62 µg/ml of its extract, followed by Y (79.51 µg/ml), AC (158.1µg/ml), and A (204.52 µg/ml). The current results support previously published information about the therapeutic properties of camel colostrum, especially its effectiveness as an anticancer agent (El-Kattawy *et al*. 2021). The findings align with those of Gaspar-Pintiliescu *et al*. (2020), who indicated that fermented colostrum extracts and their associated peptide fractions exhibited good cytocompatibility in fibroblast-treated cells. Nevertheless, additional investigations indicated that the existence of small peptides derived from fermented milk promoted the proliferation of murine spleen cells and augmented their immunomodulatory capabilities (Qian *et al*. 2011).

Table 3. Cytotoxic activity of functional fermented milk on Caco-2 cell lines

ID	µg/ml	Viability %	Toxicity %	IC_{50} µg/ml
Negative control (Caco2)	-------	100.00	0.00	
A	31.25	100.00 ± 1.00 ^{a,a,a}	0.00 ± 0.00 ^{b,b,f}	204.52 ± 1.87^{a}
	62.50	100.00 ±1.00 ^{a,a,b}	0.00 ±0.00 ^{b,b,e}	
	125	93.28 ±0.02 ^{a,a,c}	6.72 ± 0.13 ^{b,b,d}	
	250	30.09 ± 0.17 ^{a,a,d}	69.91 ±0.10 ^{b,b,c}	
	500	10.80 ± 0.01 ^{a,a,e}	89.20 ±0.10 ^{b,b,b}	
	1000	3.99 ± 0.01 ^{a,a,f}	96.01 ± 0.08 ^{b,b,a}	
AC	31.25	99.72 ± 0.20 ^{b,a,a}	0.28 ± 0.01 ^{a,b,f}	158.1 ± 1.63^{b}
	62.5	92.81 ± 0.01 ^{b,a,b}	7.19 ± 0.04 ^{a,b,e}	
	125	51.65 ± 0.05 ^{b,a,c}	48.35 ± 0.17 ^{a,b,d}	
	250	20.17 ± 0.06 ^{b,a,d}	79.83 ± 0.01^{a,b,c}	
	500	2.69 ± 0.04 ^{b,a,e}	97.31 ± 0.10 ^{a,b,b}	
	1000	2.50 ± 0.08 ^{b,a,f}	97.50 ± 0.09 ^{a,b,a}	
Y	31.25	99.17 ± 0.06 ^{a,b,a}	0.83 ± 0.03 ^{b,a,f}	79.51 ± 0.94 ^{c}
	62.5	51.69 ± 0.01 ^{a,b,b}	48.31± 0.11^{b,a,e}	
	125	21.28 ± 0.14 ^{a,b,c}	78.72± 0.09 ^{b,a,d}	
	250	10.20 ± 0.07 ^{a,b,d}	89.80 ± 0.12 ^{b,a,c}	
	500	3.01 ± 0.09 ^{a,b,e}	96.99 ± 0.11 ^{b,a,b}	
	1000	2.92 ± 0.18 ^{a,b,f}	97.08 ± 0.12 ^{b,a,a}	
YC	31.25	92.44 ± 0.16 ^{b,b,a}	7.56 ± 0.16 ^{a,a,f}	74.62 ± 0.38^{d}
	62.5	45.94 ± 0.03 ^{b,b,b}	54.06 ± 0.10 ^{a,a,e}	
	125	9.92 ± 0.19 ^{b,b,c}	90.08 ± 0.17 ^{a,a,d}	
	250	5.70 ± 0.10 ^{b,b,d}	94.30 ± 0.17 ^{a,a,c}	
	500	2.60 ± 0.04 ^{b,b,e}	97.40 ± 0.19 ^{a,a,b}	
	1000	2.55 ± 0.11 ^{b,b,f}	97.45 ± 0.30 ^{a,a,a}	

The letters preceding the comma indicate the presence of camel colostrum factor, whereas those following the comma represent the factors associated with the starter and storage periods, respectively. Means sharing the same letter at any position do not exhibit significant differences (P >0.05). All values are presented as means ±SD, n = 3 (A) Fermented milk with ABT-2; (AC) Colostrum fermented milk with ABT-2; (Y) Fermented milk with YC- X11; (YC) Colostrum Fermented milk with YC -X11.



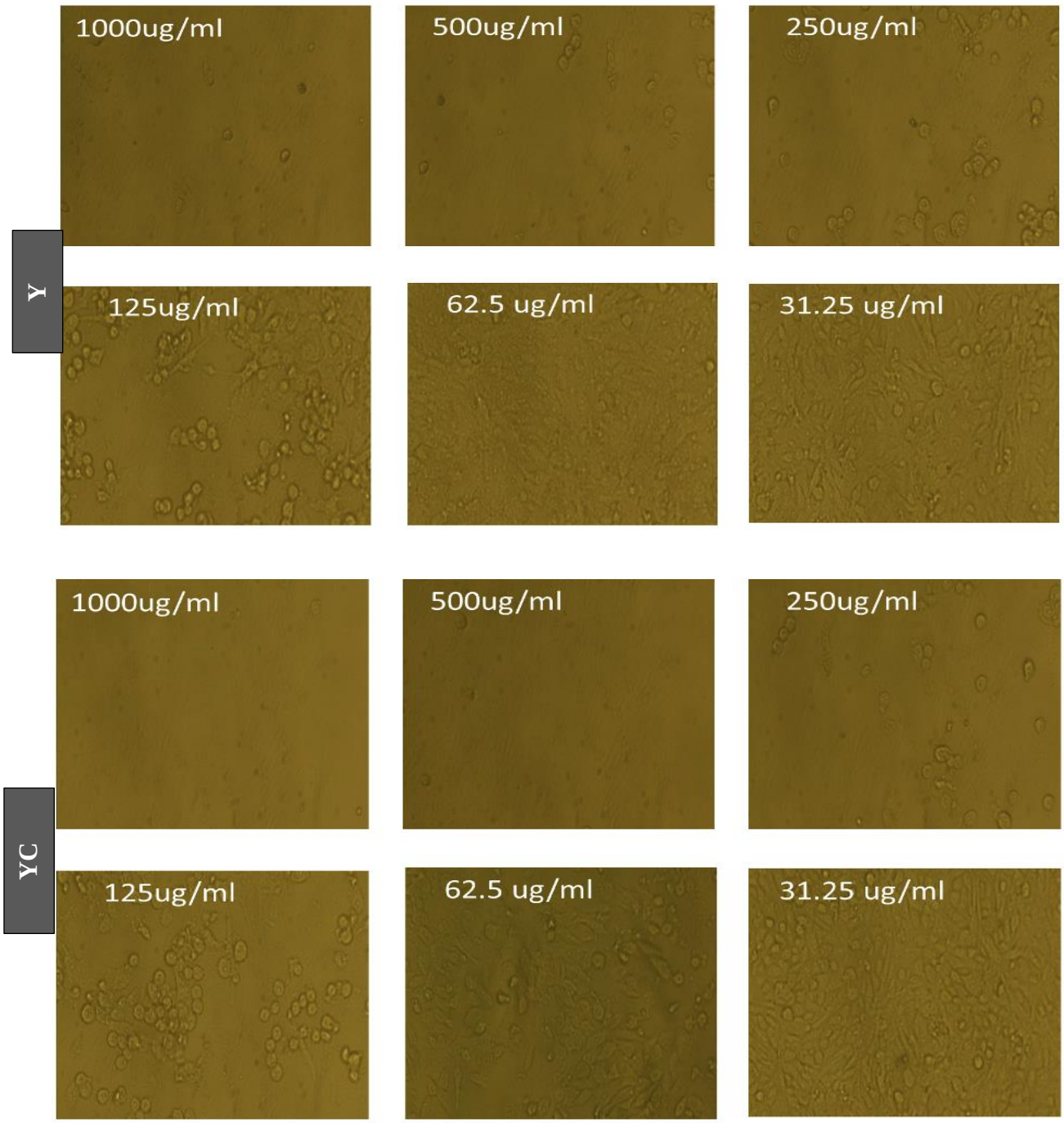


Figure 4. Effect of camel colostrum fermented samples on the Caco-2 cell line. **(**A) fermented milk with ABT-2; (AC) colostrum fermented milk with ABT-2; (Y) fermented milk with YC- X11; (YC) colostrum fermented milk with YC -X11.

3.6. Organoleptic quality of functional fermented milk

Table 4 presents the scores of fermented milks, both in their fresh and following a 21- day cold storage period, highlighting the influence of freeze-dried camel colostrum fortification and the specific type of bacterial starter used (ABT-2 or YC -X11). In terms of color and appearance ratings, all samples exhibited no differences, regardless of whether they were fortified with ABT-2 or YC-X11. However, colostrum fermented milk samples recorded higher scores (P <0.05) compared to the other treatments and the same result was observed both in fresh

samples and those stored for 21 days. Regarding the panelist score of texture data, it indicated that fermented milk containing colostrum received a significantly higher score (P <0.05) compared to fermented milk samples devoid of colostrum. This enhancement in texture is likely attributable to the increased viscosity conferred by the addition of colostrum, a finding that aligns with the results reported by Ayar *et al*. (2016) in their studies on yogurt and kefir formulations. Nevertheless, the texture consistency was affected by the end of cold storage period. Concerning the taste and odor scores, the fortification with colostrum led to a higher score than those without colostrum; it can be a consequence of the higher ratio of TS, fat, and overall protein content present in colostrum, which contribute significantly to the flavor and sensory characteristics of milk products (Silva *et al.* 2022*)*. Moreover, fermented milk made by ABT-2 gained a palatability score higher than those made by YC-X11; these findings align with Gallardo-Escamilla *et al.* (2005). However, a lower flavor score (P<0.05) was observed in the fermented milk produced using the YC-X11 starter after 21 days of storage, which may be attributed to an increase in acidity. For overall acceptability, the statistical analysis indicated that the fermented milk produced with camel colostrum achieved a significantly higher total score (P<0.05) in comparison to samples free of colostrum, regardless of the use of ABT or YC-X11 starter. Generally, the sensory attributes assessed in the camel colostrum samples consistently received scores exceeding the acceptability threshold of 70% (6.30 points), indicating favorable acceptance of this fermented food among consumers (Gularte 2009).

Table 4 Sensory evaluation of functional fermented milk fortified with camel colostrum

Sample	**A**	**AC**	**Y**	**YC**
Storage period (day)	Color and appearance			
Fresh	8.56 ±0.13 ^{b,a,a}	8.68 ±0.02 ^{a,a,a}	8.45 ±0.15 ^{b,a,a}	8.51 ±0.06 ^{a,a,a}
After 21 days	8.10 ±0.09 ^{b,a,b}	8.29 ±0.09 ^{a,a,b}	8.21 ±0.10 ^{b,a,b}	8.35 ±0.11 ^{a,a,b}
	Taste and odor			
Fresh	8.68 ±0.04 ^{b,a,a}	8.72 ±0.08 ^{a,a,a}	8.43 ±0.08 ^{b,b,a}	8.56 ±0.09 ^{a,b,a}
After 21 days	8.13 ±0.03 ^{b,a,b}	8.34 ± 0.10 ^{a,a,b}	7.94 ±0.06 ^{b,b,b}	8.29 ±0.03 ^{a,b,b}
	Texture			
Fresh	8.30 ±0.05 ^{b,b,a}	8.46 ±0.01 ^{a,b,a}	8.52 ±0.09 ^{b,a,a}	8.60 ±0.15 ^{a,a,a}
After 21 days	7.91 ±0.04 ^{b,b,b}	8.21 ±0.04 ^{a,b,b}	8.39 ±0.06 ^{b,a,b}	8.44 ± 0.06 ^{a,a,b}
	Overall acceptability			
Fresh	8.51 ± 0.19 ^{b,a,a}	8.62 ±0.03 ^{a,a,a}	8.47 ± 0.05 ^{b,a,a}	8.56± 0.05 ^{a,a,a}
After 21 days	7.98 ± 0.10 ^{b,a,b}	8.28± 0.14 ^{a,a,b}	8.24 ± 0.13 ^{b,a,b}	8.36 ± 0.08 ^{a,a,b}

The letters preceding the comma indicate the presence of camel colostrum factor, whereas those following the comma represent the factors associated with the starter and storage periods, respectively. Means sharing the same letter at any position do not exhibit significant differences (P>0.05). All values are presented as means ±SD, n = 3. (A) Fermented milk with ABT-2; (AC) Colostrum fermented milk with ABT-2; (Y) Fermented milk with YC- X11; (YC) Colostrum Fermented milk with YC -X11.

4.Conclusion

Freeze-dried camel colostrum CC (3.5%) promoted the ABT-2 and YC-X11 starter counts in fermented cow milk. Significant antiproliferative activity and lower IC_{50} values were demonstrated in CC fermented milk samples against Caco-2. Free CC samples showed higher IC_{50} values compared to using CC. Fermented milk samples with CC displayed low MW peptides, mainly IgG, IgM, and IgA, with potential health effects. The incorporation of freeze-dried camel colostrum enhanced both the texture and the flavor profile, including taste and aroma, of the resulting fermented milk. The findings indicate the potential utilization of

freeze-dried camel colostrum in the formulation of functional dairy products.

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Conflict of interest statement

There are no conflicts of interest declared by the Authors.