



Nutritional, Health-Promoting Properties and Antioxidant Activity of Yemeni Fermented Milk (Laban) and A Laban-Pulicaria Jaubertii Mixture

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ABSTRACT

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Fermented milk is known as a major functional food that has proven health benefits, such as probiotic effects. The fermented milk, laban, is the foremost dairy product in Yemen. This study aimed to evaluate the nutritional and health-promoting properties and antioxidant activity of laban. Nutritional and health-promoting properties were estimated. Antioxidant activity was evaluated by measuring total polyphenol levels, 1,1-diphenyl-2-picrylhydrazyl scavenging activity, reducing power, and total antioxidant capacity. The antioxidant activity of a laban-Pulicaria jaubertii mixture was also determined. Results showed considerable quantities of nutrients, especially fats, in both milk and laban. The composition differences between milk and laban show several significant health-promoting properties for laban. Laban showed higher antioxidant activity than raw milk and the addition of *P. jaubertii* to the laban, increased antioxidant activity. The properties of laban suggested the potential use of laban as a promising functional food with excellent health benefits, especially when mixed with *P. jaubertii*.

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Introduction

Metchnikoff and Tissier were the first to suggest the probiotic use of bacteria, even though the word “probiotic” was not coined until 1960, to refer to substances produced by microorganisms that promote the growth of other microorganisms (Lilly and Stillwell, 1965). Schrezenmeier and de Vrese (2001) defined a probiotic as “a preparation of a product containing viable, defined microorganisms in sufficient numbers, which alter the microflora (by implantation or colonization) in a compartment of the host and by that, exert beneficial health effects in this host.” The Joint FAO/WHO Expert Committee on Food Additives defined probiotics as “live microorganisms which, when consumed in adequate amounts as part of food (water is included as a food), confer a health benefit on the host” (FAO/WHO, 2006).

Probiotic microorganisms are often incorporated in food products in the form of yoghurt and yoghurt-like fermented foods. Recently, probiotic ice cream, cheese, infant formula,

breakfast cereals, sausages, luncheon meats, chocolate, and puddings have all been reported (Pyr and Peh, 2014). In investigations of commercial fermented milk products or probiotic dairy products available in the Western European market, the isolates (with a strain isolation step applied) have been classified as one or two *Lactobacillus* species and one *Bifidobacterium* species (Gueimonde et al., 2004). The majority of reports have suggested potential benefits following the consumption of fermented dairy products containing viable lactic acid bacteria (LAB; Gilliland, 1990; Fujiwara et al., 1997; Gill and Guarner, 2004).

Traditional fermented milk products are widely consumed globally. Several of these traditional products have been industrially manufactured using selected bacterial strains and a standardized process. Laban is a fermented milk product traditionally prepared from cow's milk in Tunisia and other Arab countries (Samet-Bali et al., 2012).

In Tunisia, traditional laban (TL) is produced by the spontaneous fermentation of cow's milk with natural microflora. The fermentation is allowed to proceed for a period of up to 18 h, after which the resulting fermented milk, known as "rayeb," is churned. Traditionally, churning takes place in a goat leather bag known as a "checoua" (Figure 1). Churning is achieved by hanging the "checoua" filled with "rayeb" and vigorously shaking it back and forth until the fat globules coalesce (Samet-Bali et al., 2012). The microflora of traditional Omani laban consists predominantly of mesophilic *Lactococcus* and homofermentative lactobacilli (1.3×10^8 and 2.4×10^6 cfu/mL, respectively), but also contains high numbers of yeast, coliforms, and fecal coliforms (Guizani and Al-Ramadani, 1999). In Egypt, investigation of laban rayeb revealed a high level of enteric bacterial contaminants, with *Enterococcus* as the predominant species (Khalafalla et al., 1988). Laban is the most popular traditional product in North Africa and the Middle East. The chemical and microbiological properties of traditional laban have been investigated, in an effort to standardize the product (Tantaoui-Elaraki et al., 1983; Guizani et al., 2001; Benkerroum and Tamine, 2004).

The genus, *Pulicaria*, is an annual herb producing small bright yellow flowers. It belongs to the Asteraceae family (tribe Inuleae, subtribe Inulinae), and includes more than 77 species widespread throughout the world (Ezoubeyri et al., 2005; Al-Hajj et al., 2014). *P. jaubertii* is indigenous to Yemen and is locally known as "anssif." It is traditionally used as a diuretic and an antipyretic. The flowers of *P. jaubertii* are also used as a spice in food preparation (Dubaie and El-Khulaidi, 2005). *P. jaubertii*, also known as "alkhaoah" in some regions in Yemen, is added to laban to give it distinct organoleptic properties and additional health benefits.

In Yemen, laban is the main traditional fermented milk. It is mainly produced from cow's milk, but can also be made from the milk of sheep, goats, and camels. The production process involves incubation with a traditional starter culture at ambient temperature in Yemen (20–35°C) for 8–14 h in gourds and then separation of the milk fat by a manual churning process. The resulting liquid fermented milk (i.e., laban) is consumed directly.

Laban is widely consumed in Yemen, and it has been suggested to provide important health benefits. The long-term stability of laban without any preservation methods, suggests that it has antimicrobial activities against a wide range of microorganisms. The aim of this study was to investigate the nutritional and health-promoting Properties and antioxidant activity of laban alone and laban mixed with *P. jaubertii*.

Materials and Methods

Sample Coection and Preparation

Milk and Aban

Fresh milk and laban samples were collected from a private farm in Mafrag Hobaish Directorate, Ibb Province, Yemen. The samples were collected from May–July 2019. Each fresh milk and laban sample was collected from the same source, before and after the fermentation process. Samples were transported and stored in sterilized polyethylene bottles at 4°C until analysis.

To prepare for antioxidant activity assays, 100-mL samples of fresh milk and laban were adjusted to pH 4.0 using trichloroacetic acid. The mixture was then incubated in a water bath at 60°C for 10 min. The supernatant was collected and its pH was adjusted to 7.0 using 0.5 M NaOH, and stored at 4°C until use.

Plant Material

P. jaubertii samples were collected in June 2019 from Bait Al Haidri Village, Al Radhmah Directorate, Ibb Province, Yemen. *P. jaubertii* extracts were prepared by drying the plant, milling it, and storing in polyethylene sacs. One gram of powder was mixed with 40 mL of ethanol:distilled H₂O (1:1). The mixture of laban and *P. jaubertii* extract was then prepared by mixing them in equal volumes (1:1, v/v).

Determination of Nutritional and Health-Promoting Properties of Laban

The Nutritional and Health-promoting Properties of fresh milk and laban samples were determined by measuring cholesterol assimilation and the changes in milk composition by fermentation such as vitamin C and organic Acids production and the modification on proteins, fats, and sugars to produce polysaccharides, amino acids, and fatty acids. These properties were determined by measuring the concentration of various chemical compounds and assessing physical properties, as described below.

Cholesterol Determination

Lipid extracts from milk and laban was prepared using the Folch method. Briefly, 5 mL of milk was mixed with 50 mL of chloroform-methanol solvent (2:1, v/v) and the lipid extract was washed with 20% of its volume of distilled H₂O. One milliliter of the tested solution was added to 1 mL of 33% w/v potassium hydroxide and 2 mL of absolute ethanol. Samples were mixed for 1 min and incubated at 37°C for 15 min. After cooling, 3 mL of the hexane layer was removed and added to 2 mL of distilled H₂O (Zurkowski et al., 1962). Cholesterol determination was performed using a method described previously (Zlatkis et al., 1953; Macintyre et al., 1954; Folch et al., 1954; Zak et al., 1957), with some modifications. Briefly, 50 µL of sample was added to 3 mL of glacial acetic acid and 2 mL of ferric chloride reagent in sulfuric acid. The mixture was allowed to stand for 10 min at ambient temperature and then the absorbance was measured at 560 nm using a UV-VIS spectrophotometer. Cholesterol concentration was calculated according the following equation:

$$\text{Cholesterol conc. } \left(\frac{\text{mg}}{\text{dL}} \right) = \frac{A_{560_{\text{sample}}}}{A_{560_{\text{standard}}}} \times \text{standard conc. } \left(200 \frac{\text{mg}}{\text{dL}} \right)$$

Protein Determination

The concentration of protein in fresh milk and laban samples was determined by the biuret method (Weaver et al., 2005). One milliliter of milk sample (0.1 mL/100 mL distilled H₂O) and 1 mL of protein standard solution was added to 4 mL of biuret solution, mixed, and allowed to stand for 30 min at room temperature. Absorbance was then recorded at 550 nm against a distilled H₂O blank using

a UV-VIS spectrophotometer. Protein concentration was calculated according to the following equation:

$$\text{protein conc.} \left(\frac{\text{mg}}{\text{dL}} \right) = \frac{A_{550_{\text{sample}}}}{A_{550_{\text{standart}}}} \times \text{standart conc.} (250 \text{ mg/dL}).$$

Determination of Sugar Content

The sugar content of fresh milk and laban samples was determined by the phenol-sulfuric acid method (Harshal et al., 2011). In brief, 1 mL of 5% phenol solution was added to 1 mL of sample, after which 5 mL of concentrated H₂SO₄ was added. After 10 min, the absorbance was measured at 488 nm against a blank using a UV-VIS spectrophotometer. Sugar content was expressed as glucose. Sugar concentration was calculated by the following equation:

$$\text{sugar conc.} (\%) = \frac{A_{488_{\text{sample}}}}{A_{488_{\text{standard}}}} \times \text{standard conc.}$$

Determination of Fat Content, Acidity, and Specific Gravity

The fat concentration, total titratable acidity (as lactic acid, TTA), and specific gravity of fresh milk and laban samples were measured according to the AOAC methods for milk and milk products (AOAC, 1999). The TTA (% lactic acid equivalent) was calculated as follows:

$$\text{TTA} = [V (\text{NaOH}) \times N (\text{NaOH}) \times 0.009] / W_{\text{sample}} \times 100$$

Where;

- V = Is the volume of NaOH
- N = Is the normality of NaOH
- W = Is weight of sample

Vitamin C determination

Vitamin C was measured in milk samples using the N-Bromo succinimide method (Aldalaly and Alhakim, 1987; Shehab and Hassan, 1978).

Antioxidant Activity Determination

Total Phenolic Content Assay

Total phenolic content was determined as described by Shetty et al. (1995). Briefly, 1 mL of water extract from fresh milk or laban samples transferred to a test tube and mixed with 1 mL of 95% ethanol and 5 ml of distilled H₂O. Then, 0.5 mL of 50% (v/v) Folin-Ciocalteu reagent was added to each sample, followed by a thorough mixing. After 5 min, 1 mL of 5% Na₂CO₃ was added and the reaction mixture was allowed to stand for 60 min. Absorbance was measured at 725 nm using a UV-VIS spectrophotometer. Standard curves were established using various concentrations of gallic acid (5–60 µg/mL) in methanol and were used to convert absorbance values to total phenolic content, expressed in microgram equivalents of gallic acid per gram (µg GAE/g) of sample.

Measurement of Free Radical Scavenging Activity

The free radical scavenging activity (antioxidant capacity) of sample extracts towards the stable radical 1,1-diphenyl-2-picrylhydrazyl (DPPH), was evaluated by the method reported by Unal et al., (2013), with some modifications. In this assay, 1 mL of a sample extract

solution at different concentrations (20, 40, 60, 80, and 100 µg/mL in methanol) was mixed with 1 mL of a DPPH solution (200 mg/mL in methanol). Solution containing an equal amount of methanol and DPPH, without added sample, served as a control. After 30 min at room temperature in the dark, absorbance was measured at 517 nm against a methanol blank using a UV-VIS spectrophotometer, as described above. The percentage free radical scavenging activity was calculated according to the following equation:

$$\text{scavenging activity} (\%) = [(Ac - As) / Ac] \times 100$$

Where;

- Ac = Is the absorbance of control
- As = Is the absorbance of the sample

Total Antioxidant Capacity

The total antioxidant capacity of the extracts was evaluated by the phosphomolybdenum method, according to the procedure described by Prieto et al. (2004). A 0.3-mL aliquot of sample extract at various concentrations (20, 40, 60, 80, and 100 µg/mL) was combined with 3 mL of reagent solution (0.6 M sulfuric acid, 28 mM sodium phosphate, and 4 mM ammonium molybdate). The tubes containing the reaction solution were incubated at 95°C for 90 min. After cooling to room temperature, the absorbance of the solution was measured at 695 nm against a methanol blank, using a UV-VIS spectrophotometer. Total antioxidant activity was expressed as the number of gram equivalents of tannic acid. A calibration curve was prepared by mixing tannic acid with distilled H₂O to concentrations of 20, 40, 60, 80, and 100 µg/mL. All these experiments were performed in triplicate.

Reducing Power Assay

The total reducing power of each extract was determined according to a previously described method (Aliyu et al., 2013). Equal volumes (2.5 mL) of different concentrations of the sample extracts (20, 40, 60, 80, and 100 µg/mL), phosphate buffer solution (0.2 M, pH 6.6), and 1% potassium ferricyanide [K₃Fe(CN)₆] were added to test tubes. The mixture was placed in a water bath at 50°C for 20 min. Then, 2.5 mL of 10% trichloroacetic acid was added and the solution was mixed thoroughly. A 2.5-mL aliquot of this mixture was then added to 2.5 mL of distilled H₂O and 0.5 mL of 0.1% FeCl₃ and the solution was allowed to stand for 10 min. The absorbance of this mixture was then measured at 700 nm using a UV-VIS spectrophotometer. A higher absorbance value of the reaction mixture indicated greater reducing power. Ascorbic acid was used as a positive control. All these experiments were performed in triplicate.

Statistical Analysis

Means and standard deviations (SD) were calculated and data were analysed by one-way ANOVA. Duncan's Multiple Range test was used to determine whether differences were significant, as indicated by P ≤ 0.05. Statistical analyses were performed using the SPSS V21 software package (SPSS Inc., Chicago, IL, USA).

Results and Discussion

Nutritional and Health-Promoting Properties

The Nutritional and Health-promoting Properties of laban were determined by comparing its physical and chemical properties with those of fresh milk. In addition, changes in milk structure due to microorganisms during the fermentation process were also investigated. Figure 1 illustrates the Nutritional and Health-promoting Properties of laban. During laban production, cholesterol assimilation was 16.67%. Lactic acid content increased by 55.22% (w/v), protein content decreased by 22.76%, and vitamin C increased 12.6%. In addition, sugar and fat content were 11.5 and 12% lower, respectively, in laban than in fresh milk. Statistical analysis showed that laban surpassed milk for all tested Nutritional and Health-promoting Properties.

Probiotics are available in many different forms. They are components of foods, usually fermented foods, including those prepared by traditional methods and are also in pharmaceutical products, mainly as capsules or in microencapsulated form. By definition, probiotic strains may even be undefined organisms from fermented foods, that survive passage through the gut and exert positive effects in the gastrointestinal tract (Goktepe et al., 2006). The ideal properties of a probiotic include proven safety in clinical trials and/or a long history of use in foods (Michail and Philip, 2009). Figure 1. Nutritional and Health-promoting Properties of laban.

LABs in milk have been shown to possess proteolytic enzymes, such as proteinases, peptidases, and aminopeptidases at extracellular and intracellular levels (Osaana et al., 2007). Bioactive peptides can be liberated from milk proteins by enzymatic hydrolysis with digestive enzymes or by the fermentation of milk using proteolytic starter cultures and the subsequent action of enzymes derived from these microorganisms. After these peptides are released during food processing, they may exert various physiological effects (Sarmadi and Ismail, 2010). The decrease in protein content in laban compared with fresh milk in this experiment indicated that some of the proteins had converted into peptides and amino acids by the actions of bacteria during the fermentation process. This may be due to the proteolytic activity of the laban starter culture, since the protein profile of milk has been shown to change with all starters used. The greatest amount of proteolysis is observed when both *Lactobacillus delbrueckii* subsp. *bulgaricus* 286 and 287 are used as co-starters (Aleksandrova et al., 2013). The proteolytic activities of LAB, including yoghurt starter bacteria and probiotic organisms, have been studied extensively and their proteolytic enzymes have been isolated and characterized (Wohlrab and Bockelmann, 1992; Shihata and Shah, 2000). Biologically active peptides are generated during milk fermentation by proteolytic enzymes produced by various species of LAB, such as *L. helveticus*, *L. lactis* subsp. *Cremoris* FT4, and *L. delbrueckii* ssp. *Bulgaricus* SS1 (Gobbetti et al., 2002). These biologically active peptides include hypotensive peptides that inhibit angiotensin I-converting enzyme; opioid agonist and antagonist peptides; and mineral binding, immunomodulatory, antibacterial, and antithrombotic peptides (Shah, 2000; Pihlanto and Korhonen, 2003).

The high acid content of laban seen in this study is likely due to the production of lactic acid, amino acids, and

fatty acids from sugar, proteins, and lipids, respectively. That was confirmed by the finding that sugar and fat content decreased during laban production.

Fermentation in food processing involves the conversion of carbohydrates to alcohol and carbon dioxide or organic acids by bacteria (William, 2011). The organisms most commonly used for the fermentation of foods are acid-forming bacteria, such as the LAB genera, *Lactobacillus*, *Lactococcus*, *Leuconostoc*, *Enterococcus*, *Streptococcus*, *Aerococcus*, and *Pediococcus* (Chelule et al., 2010). Lactic acid fermentation of food has been found to reduce the risk of contamination with pathogenic microorganisms (Abdel et al., 2009).

The main species of microorganisms that can potentially be used as probiotic cultures in dairy products include, *Lb. acidophilus* and/or *Lb. johnsonii*, *Lb. delbrueckii* subsp. *bulgaricus*, *Lb. delbrueckii* subsp. *lactis*, *Lb. casei*, *Lb. gasserii*, *Lb. helveticus*, *B. breve*, *E. faecalis*, *E. faecium*, and *P. acidilactici* (Tamime, 2005).

Cholesterol assimilation was observed during the milk fermentation process in this study. There is some data reported in the literature suggesting that LAB can reduce the quantity of cholesterol during the production of fermented dairy beverages. Some of the putative health benefits of probiotics include a reduction in total cholesterol and the assimilation of cholesterol is one of the proposed mechanisms (Gibson and Beaumont, 1996).

Juskiewicz and Panfil-Kuncewicz (2003) showed that bacteria included in thermophilic starter cultures possess the ability to reduce cholesterol content in milk during its fermentation. In that study, cholesterol assimilation by a classic yoghurt starter culture was 22.2% and 19.8% in yoghurt containing 4% and 8% fat, respectively. This exceeded the degree of cholesterol assimilation seen in laban in this study. However, another study used another yoghurt starter culture with a similar qualitative composition, showed cholesterol assimilation abilities of 11.3% and 15.4% in yoghurt containing 4% and 8% fat, respectively. In agreement with this study, another study found that *Pediococcus plantarum* L14/1, *P. acidilactici* L25, *Lb. plantarum* L26, *Lb. pentosus*, and *E. faecium* N15 were able to remove 15–17% of cholesterol from the culture medium without bile acid (Monthon et al., 2014). In starter cultures used for the production of traditional yoghurt, consisting of *Streptococcus salivarius* subsp. *thermophilus*, and *Lactobacillus delbrueckii* subsp. *bulgaricus*, the quantity of assimilated cholesterol did not exceed 27% of its initial content (0.7 g in 1 dm³; Ziarno, 2007). LAB from a commercial yoghurt culture was shown to bind 111 µg of cholesterol in 1 cm³ of MRS broth medium during 18 h of incubation at 37°C. The *Lb. delbrueckii* subsp. *bulgaricus* strain showed the highest assimilation ability among all the cultures tested in that study. It was able to bind 276 µg of cholesterol in 1 cm³ of medium (Rasic et al., 1992).

Chemical and Physical Properties of Milk Products

In general, cow's milk is mainly composed of water, with approximately 4.8% lactose, 3.2% protein, 3.7% fat, 0.19% nonprotein nitrogen, and 0.7% ash (Banks and Dalgleish, 1990; Fox and McSweeney, 2006). The results of our physicochemical analyses of fresh milk and laban are presented below

Total Acidity

The results presented in Figure 2 show the total acidity, measured as lactic acid, for fresh milk and laban samples. It is clear from these data that lactic acid is three-fold higher in laban than in fresh milk and this difference was statistically significant ($P \leq 0.05$).

Figure 2. Total acidity of fresh milk and laban. Results are presented as mean \pm standard deviation, $n=3$ per group.

The increase in the acidity of laban was due to the production of lactic acid from lactose by the starter culture, as indicated by the decrease in the sugar content of laban and the reduction of proteins to amino acids and fats to fatty acids and glycerol. The titratable acidity of laban in this study was lower than that of yoghurt and probiotic yoghurt, which have been reported as 0.96% and 1.04%, respectively (Yangilar and Çakmakci, 2017).

Protein Content

Figure 3 shows the protein concentration of fresh milk and laban. The protein concentration of fresh milk (4.13%) was higher than that of laban (3.19%). The protein content of laban observed in this study was lower than that reported for yoghurt and probiotic yoghurt, which are 3.80% and 3.76%, respectively (Yangilar and Çakmakci, 2017).

Figure 3. Protein content of fresh milk and laban. Results are presented as mean \pm standard deviation, $n = 3$.

Milk proteins are regarded as a source of energy and essential amino acids, which are needed for growth and the maintenance of physiological functions (Sarmadi and Ismail, 2010). Our results agree with that of a previous study showing that the concentration of protein in fresh and fermented buffalo milk is 0.817 mg/mL and 0.501 mg/mL, respectively and peptide concentrations are 0.4 mg/mL and 0.805 mg/mL before and after fermentation, respectively (Hussein et al., 2015).

Fat Content

Fresh milk samples in this study were shown to have higher quantities of fat compounds than laban (Figure 4).

These differences between the two milk products were statistically significant ($P \leq 0.05$).

Figure 4. Fat content of fresh milk and laban. Results are presented as mean \pm standard deviation, $n = 3$.

Fat content observed in laban in the present study was higher than the fat content of yoghurt and probiotic yoghurt, reported to be 3.40% and 3.50%, respectively (Yangilar and Çakmakci, 2017). This difference is attributed to the higher fat content in the fresh milk samples prior to fermentation in our study.

Vitamin C Content

Figure 5 shows the concentration of vitamin C in fresh milk and laban samples. The mean vitamin C concentration in laban samples was 4.2 mg/100 mL, which was higher than the concentration in fresh milk samples (3.7 mg/100 mL).

Figure 5. Vitamin C content of fresh milk and laban. Results are presented as mean \pm standard deviation, $n = 3$.

Vitamin C acts as an oxygen scavenger. Milk and milk products supply only 10–15% of the daily requirements of vitamin C (Rasic and Kurmann, 1978). In addition to vitamin C, milk also contains other water-soluble vitamins, such as B vitamins, in variable quantities (Miller et al., 2000). Andersson and Oste (1994) reported that vitamin C concentrations in unpasteurized milk are highest in March and August (20–27 mg/L) and lowest in October (12 mg/L). The mean content of vitamin C in cow’s milk is 2.11 mg/100 g (range 1.65–2.75 mg/100 g; Walstra and Jenness, 1984). This is lower than that in the Yemeni milk used in this study. The vitamin C content of laban in this study was also higher than that of fermented milk products produced using a kombucha starter and fermented on sweetened wild thyme extract, which was 12.36–31.19 mg/L (Malbaša et al., 2014).

These results indicate that the microorganisms in laban possess a robust ability to produce vitamin C in fermented milk, which enhances the nutritional value and bioactivity of these products.

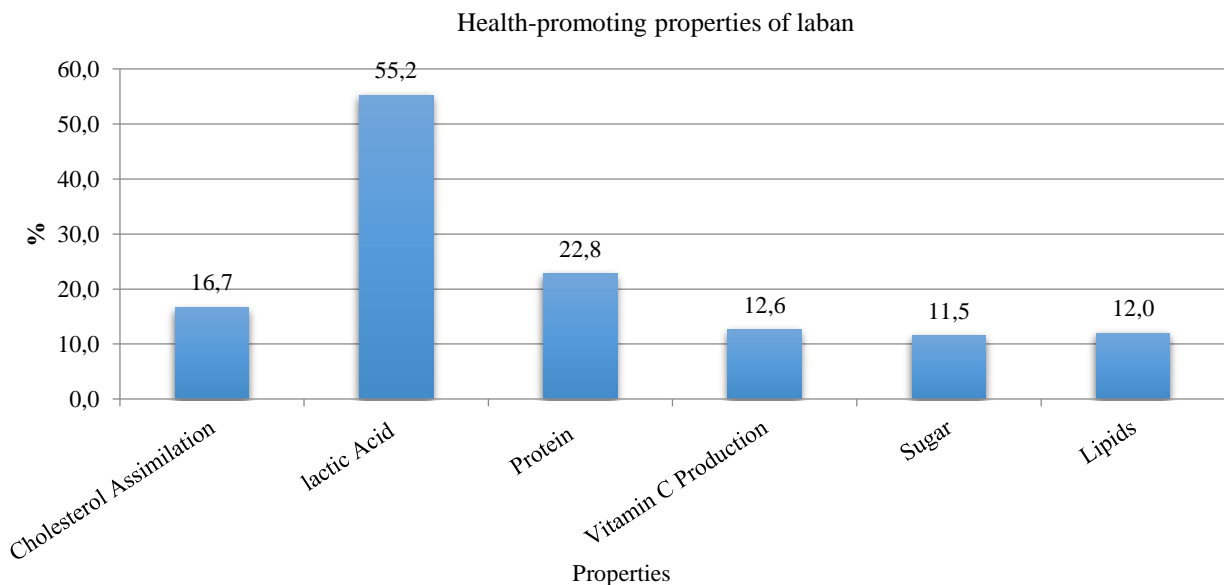


Figure 1. Nutritional and Health-promoting Properties of laban.

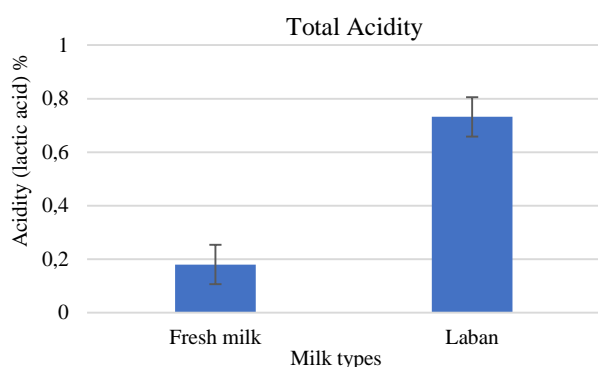


Figure 2. Total acidity of fresh

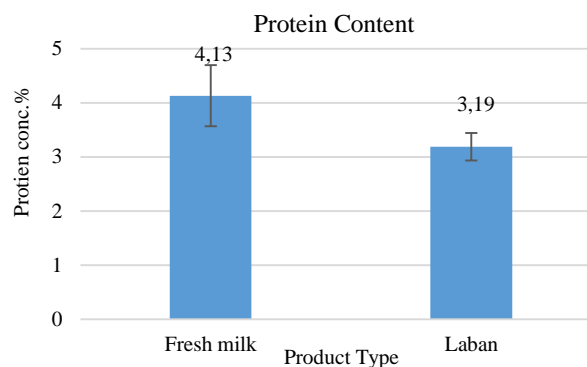


Figure 3. Protein content of fresh milk and laban.

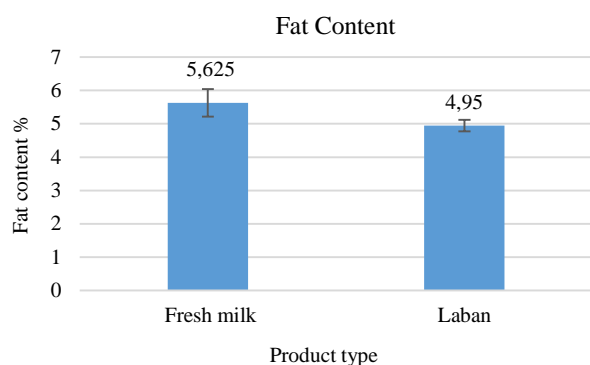


Figure 4. Fat content of fresh milk and laban.

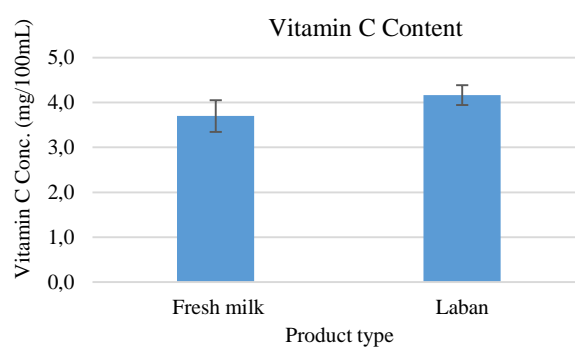


Figure 5. Vitamin C content of fresh milk and laban.

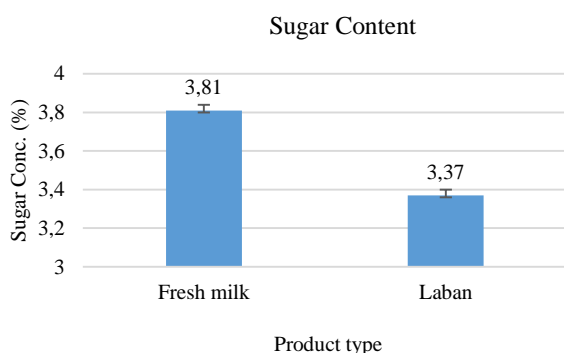


Figure 6. Sugar content of fresh milk and laban.

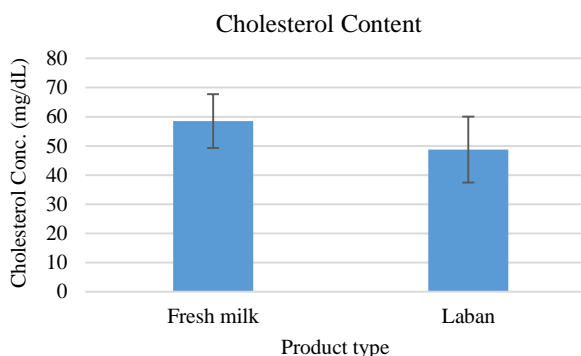


Figure 7. Cholesterol content of fresh milk and laban.

Sugar Content

The results presented in Figure 6 show that the sugar content of fresh milk (3.81%) was higher than that of laban (3.37%). Figure 6. Sugar content of fresh milk and laban. Results are expressed as mean \pm standard deviation, $n = 3$.

Cholesterol Content

The cholesterol content of fresh milk and laban were determined by the ferric chloride method. As shown in Figure 7, the cholesterol content of fresh milk was 58.5%, but his decreased to 48.75% in laban.

Figure 7. Cholesterol content of fresh milk and laban. Results are presented as mean \pm standard deviation, $n = 3$.

The normal value of cholesterol in milk is 3.3 mg/g of fat (Fox and McSweeney, 2006). Many experiments have been performed *in vitro* and *in vivo* to investigate the hypocholesterolemic effect of LAB. These studies have concluded that fermentation of dairy products with the appropriate strain(s) of bacteria may result in a decrease in the concentration of circulating cholesterol (Tamime, 2005).

Antioxidant Activity

Total Polyphenol Content

Total polyphenol quantities were highest in the laban-*P. jaubertii* mixture, followed by laban alone, and then fresh milk (Figure 8). Statistical analysis showed that the difference in polyphenol content between the laban-*P. jaubertii* mixture and the other two milk products was highly significant ($P \leq 0.05$), but there was no significant difference between fresh milk and laban.

Figure 8. Total polyphenol content of fresh milk, laban, and a laban-*P. jaubertii* mixture. The letters a, b, and c indicate significant differences between treatments ($P \leq 0.05$). GAE, gallic acid equivalents; L-P, laban-*P. jaubertii* mixture.

Total Antioxidant Capacity

Total antioxidant capacity of the different milk products is shown in Figure 9. The laban-*P. jaubertii* mixture showed the highest antioxidant capacity, followed by laban alone and then fresh milk. Statistical analysis showed that the difference in antioxidant capacity between

the laban-*P. jaubertii* mixture and the other two milk products was highly significant ($P \leq 0.05$), but there was no significant difference between fresh milk and laban.

Figure 9. Total antioxidant capacity of fresh milk, laban, and a laban-*P. jaubertii* mixture. The letters a and b indicate significant differences between treatments ($P \leq 0.05$). L-P, laban-*P. jaubertii* mixture.

A previous study has shown that Bulgarian yoghurts have antioxidant probiotic properties (Aleksandrova et al., 2013). The antioxidant activity of laban, as with other fermented milk products, may be attributed to the presence of antioxidant compounds, such as proteins, peptides and amino acids, uric acid, vitamin C and A, carotenoids, coenzyme Q10, and enzymes, combined with the antioxidant activity of LAB (Pihlanto, 2006; Jiménez et al., 2008; Zulueta et al., 2009).

DPPH-Scavenging Activity

The DPPH-scavenging activities of fresh milk, laban, and the laban-*P. jaubertii* mixture are illustrated in Figure 10. The laban-*P. jaubertii* mixture had the highest DPPH-scavenging activity, followed by laban alone, and then fresh milk.

Statistical analysis showed that the difference in DPPH-scavenging activity between the laban-*P. jaubertii* mixture and the other two milk products was significant ($P \leq 0.05$), but there was no significant difference between fresh milk and laban. DPPH-scavenging activity of fresh milk, laban, and a laban-*P. jaubertii* mixture. The letters a, b, and c indicate significant differences between treatments ($P \leq 0.05$). L-P, laban-*P. jaubertii* mixture.

A previous study has shown that TAC levels are in the range of 13.33 to 25.93 mg TE per 100 mL of human milk,

when using the ABTS method and 2.45 to 17.66 mg TE per 100 mL, when using the DPPH test (Dorota and Weronika, 2012). The highest DPPH-radical-scavenging ability was found for yoghurts (0.19 mM TE/kg) and kefirs (0.17 mM TE/kg), followed by buttermilk, cultured milks, and ayran (Najgebauer-Lejko and Sady, 2015). However, kombucha-fermented milk products containing wild thyme showed different antioxidant responses to DPPH and hydroxyl radicals, with DPPH antioxidant activity at 1.36–36.12% and OH antioxidant activity at 3.11–5.12% (Malbaša, 2014). Another study showed that probiotic yoghurt extracts possessed very high DPPH-scavenging activities, ranging from 90 to 93% (Unal et al., 2013). The high antioxidant activity of the mixture of laban and *P. jaubertii* agrees with the findings of Najgebauer-Lejko and Sady (2015), who found that flavored fermented milk products have strong antioxidant properties, which differed according to the type and quality of the product. This has also been confirmed by Al-Naqeb (2015), who found that the scavenging activity of *P. jaubertii* gradually increased to 84% of the total free radicals. The author suggested that the high antioxidant and antimicrobial activity of *P. jaubertii* makes it a potential component of new pharmacological products.

Reducing Power

Figure 11 shows the reducing power of milk, laban, and the laban-*Pulicaria jaubertii* mixture. The results show that the highest reducing power activity was observed in the laban-*Pulicaria jaubertii* mixture followed by laban and fresh milk. Reducing power of milk, laban and a laban-*P. jaubertii* mixture. The letters a, b, and c indicate significant differences between treatments ($P \leq 0.05$).

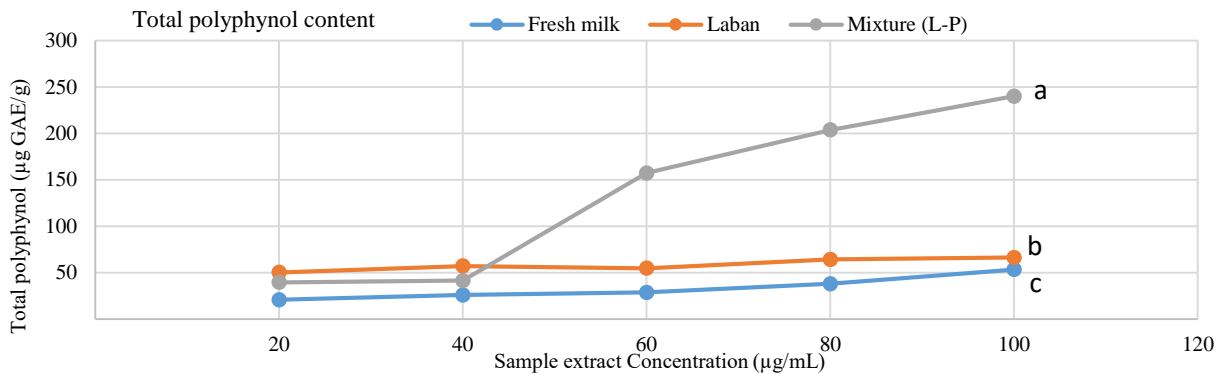


Figure 8. Total polyphenol content of fresh milk, laban, and a laban-*P. jaubertii* mixture

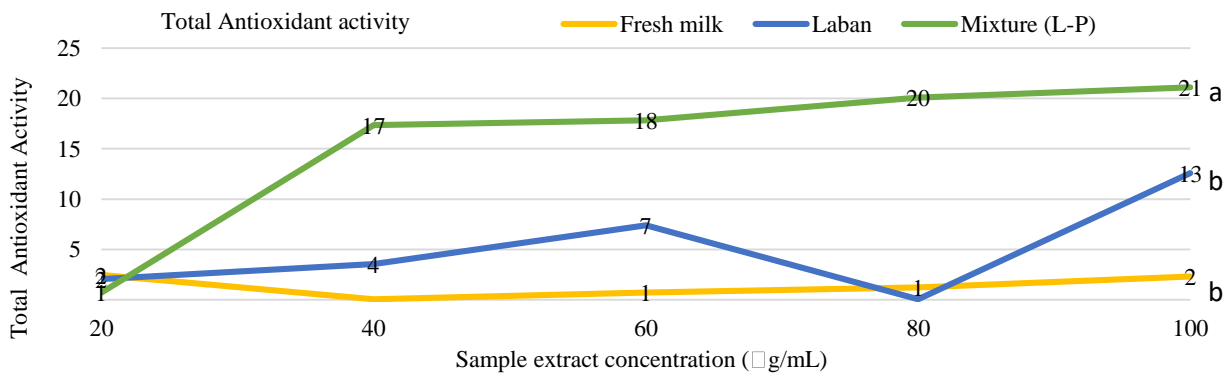


Figure 9. Total antioxidant capacity of fresh milk, laban, and a laban-*P. jaubertii* mixture

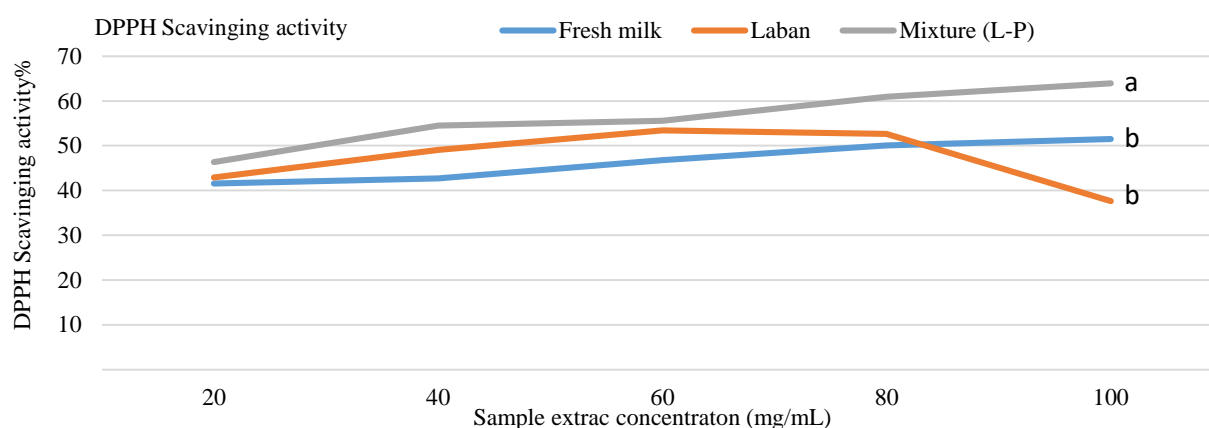


Figure 10. DPPH-scavenging activity of fresh milk, laban, and a laban-*P. jaubertii* mixture

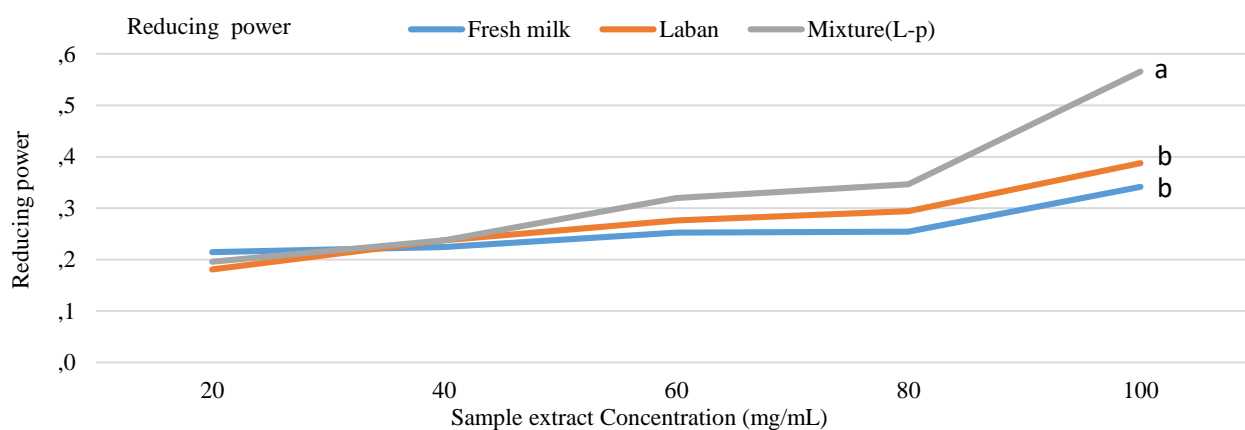


Figure 11. Reducing power of milk, laban and a laban-*P. jaubertii* mixture

This high scavenging ability of laban's proteins may be attributed to lactoferrin, which has been reported to be a key factor affecting scavenging activity (Shinmoto et al., 1992; Chiang and Chang, 2005). In addition, α -lactalbumin and β -lactoglobulin (Hernandez-Ledesma et al., 2005; Del Mar Contreras et al., 2011) may also contribute to the antioxidant activity of laban. Similar to this study, plain yoghurts have been found to have the highest FRAP values of products tested (1.4–2.4 mM Fe₂₊/dm³) (Najgebauer-Lejko, and Sady, 2015).

Conclusion

Fresh and fermented Yemeni milk contained a high fat content and considerable quantities of vitamin C, proteins, and sugar. Laban can be considered a promising functional food, because of its ability to reduce cholesterol, fats, proteins and sugar contents to produce polysaccharides, amino acids and peptides, fatty and organic acids, and vitamin C. Furthermore, through its traditional use history, it is stable and has favorable organoleptic properties. Fresh Yemeni milk and laban contained measurable polyphenol compounds and both products had good antioxidant activity. The fermentation process used to produce laban further enhances the antioxidant activity of the milk. *P. jaubertii* is known to have high polyphenol content and considerable antioxidant activity. Mixing laban and *P. jaubertii* gave a synergistic effect, resulting in greater antioxidant capacity. Therefore, it can be concluded that the traditional Yemeni fermented milk (Laban), possesses excellent therapeutic and nutritional potential.

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