Journal of Materials and Environmental Science
ISSN : 2028-2508
CODEN : JMESCN

J. Mater. Environ. Sci., 2020, Volume 11, Issue 7, Page 1033-1043

http://www.jmaterenvironsci.com

Total phenolic content, antioxidant and physicochemical properties of bioyogurt supplemented with Cactus extract and cream cheese made from Moroccan Oulmes bovine milk

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Received 15 May 2020,
Revised 08 June 2020,
Accepted 10 June 2020

Keywords
- ✓ yogurt,
- ✓ cream cheese,
- ✓ *Opuntia Ficus Indica*,
- ✓ antioxidant capacity,
- ✓ polyphenols.

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Abstract

The antioxidant potential of dairy products is therefore close to that of different food groups or plants, in the present study yogurt fermented with *Lactococcus lactis* and *Enterococcus mundtii* was made, then we prepared yogurt with *Opuntia Ficus Indica* cladode and fruit extracts, all yogurts were refrigerated up to 28 days. pH of cactus cladode yogurt was lower 3.93± 0.03 than that made with cactus fruit extract 4.01±0.04, whereas titratable acidity (TA) was higher than plain yogurt during storage. Highest DPPH inhibitions were shown by both yogurts (made with cladode and fruit extract) on days 7 and 15 with extended storage to 28 days. Refrigerated yogurt made with cactus cladode and fruit extract, and yogurt made with starter culture showed transient reduction in TPC by day 14 (30.12± 3.1 and 41.75± 7.2, and 40.87±4.3 µgGAE/ml respectively), but increased to highest values 44.18± 2.5; 46.18±6.2 and 44.68±3.7 µgGAE/ml, respectively; ($p< 0.05$) by day 28. In the other hand, the total phenolic content (TPC) and antioxidant capacity of cream cheese increased during storage (15days in4°C). Cheese made with *Lactococcus lactis* had highest TPC (41.87±5.0 µgGAE/ml; $p<0.05$) and highest DPPH inhibition (37.64± 4.1%; $p <0.05$) compared to cheese made with *Enterococcus mundtii* (23.75± 5.1µgGAE/ml and 36.18±4.0%). It has also been reported that the development of phenolic flavor defects in cheese is due to p-cresol, produced by an atypical salt resistant strain of *Lactococcus*.

1. Introduction

Dairy products, when consumed regularly in reasonable quantities, are nevertheless associated with a significantly lower prevalence of obesity, diabetes type 2, metabolic syndrome, cardiovascular disease and certain cancers [1]. In this protective effect, it is surprising that their antioxidant potential has rarely been promoted, whereas this is systematically the case for plant products. It is therefore particularly interesting to examine the scientific research on antioxidant power of dairy products, such as milk, yogurt and cheese [2]. The results show clearly that this potential is not negligible and must probably be one of the protective mechanisms, among others, of dairy products in relation to certain chronic diseases. It should also be noted that traditional yoghurts made at home would have a higher antioxidant activity than commercial yogurts [3].

Bacterial fermentation influences the compositional quality of yogurt, in particular its composition as a protein fraction, and consequently its antioxidant activity [4-5]. Galleher [6] during the manufacturing of yogurt, the heat treatment of milk has an effect on the denaturation of proteins, which also induces a change in the antioxidant activity. Moreover, the specific characteristics of LAB strains have a strong influence on antioxidant activity [4-7-8-9-10]. The scientific literature shows clearly that dairy products are a significant source of antioxidants, in particular because of their protein fraction. Overall, the cheeses exhibit a very great diversity of composition and structure, which can modulate their antioxidant potential. Thus, the results of two studies using the same antioxidant test show that dairy products have an antioxidant capacity of the same order of magnitude as cereals, legumes and fruit juices [11-12].
Therefore, all parts of the cactus plant (*Opuntia Ficus Indica*) are rich in members of the polyphenol family such as various flavonoids and phenolic acids. the cactus fruit is known for its health benefits, such as against hypoglycemia, hypolipidemia and its antioxidant properties, this is due in particular to its composition of ascorbic acid, vitamin E, fibers, amino acids , and carotenoids, and antioxidant compounds [13-14]. Several studies tell us that the cactus cladodes contain phytochemicals, dietary fiber, minerals, and antioxidants compounds such as flavonoids and quercetin [15-16]. Moreover, the cactus cladodes is known by its mucilage composition that has a significant antioxidant power [17]. The current study focuses on using additive such as cactus cladodes and cactus fruit extract, to improve the antioxidant and the viability of lactic acid bacteria in cow milk yogurts during refrigerated storage. So the aim of the present work was, firstly, evaluate the physicochemical and antioxidant properties, and the total phenolics components of cream cheese made from Oulmes cow milk, then those of yogurt made from Oulmes cow milk and fortified by cactus cladodes and fruit extract.

2. Materiel and methods

2.1. Milk samples

Fresh milk was obtained from cows inhabiting the Oulmes region. Oulmes is a rural place located 150 km northwest of Rabat and is in the high mountains of the Middle Atlas and middle hills situated in the west in the Amazigh area. Of the main Moroccan local breeds, Oulmes-Zaer dates to 1912 and was recognized by ministerial decree in 1982. It is an original Moroccan race reared for two purposes, milk and meat production, and it is well adapted to harsh environments [18]. This region is also well known as a natural reserve of aromatic and medicinal plants [19].

2.2. Starter culture

The starter culture used in this study for making yogurt, are the *Lactococcus lactis and Enterococcus mundtii*, the strain was isolated from goat milk sampled from Oulmes goat cattle identified and selected as starter in previous study [20].
The strain was maintained on M17-agar at 4–6 °C. The starter culture was obtained by overnight incubation at 37 °C in M17-broth, then another overnight in skim milk at 37°C. A mixture of yogurt bacteria consisting of *Lactococcus lactis* and *Enterococcus mundtii* were mixed thoroughly with the preheated milk prior to an overnight incubation at 41°C. The yogurt formed was refrigerated (4°C) and used as starter culture within 7 days [21].

2.3. Opuntia Ficus Indica water extracts preparation

In this study, the cladodes and the fruits of *Opuntia ficus indica* (variety without thorns) were collected in the region of Rhamna (Morocco), the cladodes are less than 1 year old, and the fruits are mature with a brix of 14 °. The cladode and the fruit (10 g) were soaked in 100 ml of distilled water for 12 h in a water bath (70°C) with shaking, followed by centrifugation (2000 rpm, 15 min at 4°C) and the

supernatant was harvested [22]. The clear solution obtained was refrigerated (4°C) and used within 3 days as *O. Ficus Indica* water extract in the preparation of bioyogurt.

2.4. Preparation of bioyogurt

Opuntia Ficus Indica water extract (10 ml) was added into pre-warmed (41°C) pasteurized full cream milk (85 ml) which has been previously mixed with starter culture (5 ml) [23]. The mixture was thoroughly mixed and aliquoted (50 ml) into disposable glass containers. Yogurts were incubated (41°C) for 6 h and stored in a refrigerator (4°C) for 2 h (fresh yogurt or 0-day storage) up to 22 days. Control yogurts were prepared using a commercial yogurt in place of starter culture used and without the herbal water extract.

2.5. Cheese making procedure

The cheese making procedure is described by [24], the whole bovine raw milk was pasteurized (72 °C, 10 min), then cooled to 37°C and the starter culture was added (3%) [25]. The milk was shacked for 30 min, coagulation took place over 30–45 min after the addition of *Silybum marianum*. Then we put the container in 37°C for 18h, the next day we pass to molding and draining over 24h.

2.6. pH and titratable acidity (TA) determination of bioyogurt

Yogurt was homogenized in distilled water (1:9) and the pH was read using a digital pH meter. The titratable acidity (TA) of homogenized yogurt was determined by titrating 10-ml samples with 0.1 N NaOH, phenolphthalein was used as indicator. TA was expresses as °D (degrees Dornic) [26].

2.7. Preparation of water extracts of control and fortified yogurt

Yogurt samples (10g) were homogenized with 2,5ml of distilled water, the homogenates were acidified to pH 4.0 with HCl (0.1 M) followed by heating (water bath; 45°C, 10 min) and centrifugation (5000g, 10 min, 4°C). The pH of supernatants was brought to 7.0 using NaOH (0.1 M) and re-centrifuged (5000g, 10 min 4°C) for further precipitation of proteins and salts [27]. The supernatants were harvested and kept in the refrigerator (4°C) and used within 12 h of preparation to measure the antioxidant activity.

2.8. Chemical analysis of cheese

The pH of cheese samples was measured by direct insertion of a pH probe [28]. The titratable acidity (TA) of homogenized cheese was determined by titrating 10-ml samples with 0.1 N NaOH, phenolphthalein was used as indicator. TA was expresses as °D (degrees Dornic) [26].

2.9. The preparation of cheese extracts

For total antioxidant activity, about 2g of each cheese sample was homogenized with about 10ml of distilled water. The homogenate was transferred quantitatively to a test tube containing 5 ml of distilled water and was boiled for 5 minutes and then cooled. The resultant solution was filtered through a cheese-cloth and the volume was adjusted to 20 ml with distilled water. These sample solutions were used to measure the total antioxidant activity [29].

To measure the total phenolic content in cheese samples, we followed the protocol of [29] with some modifications; about 2g of cheese sample was homogenized and extracted for 30 minutes with 100ml of 80% methanol containing 1% HC1 then placed in shaker at 200rpm. The mixture was cooled, filtered and the residue was washed with the same solvent.

2.10. Total phenolic content assay

Yogurt water extract, or cheese extract (1.0 ml) was mixed with ethanol (1.0 ml, 95% v/v) and 5 ml dH_2O [30]. Folin–Ciocalteu reagent (0.5 ml; 50% v/v) was added to each sample and after a thorough mixing the solutions were allowed to stand for 5 min. at room temperature. Na_2CO_3 (1.0 ml, 5% g/100 ml) was then added, after 60min of reaction at room temperature, the absorbance was measured at 760 nm.

2.11. Antioxidant activity by 1,1-diphenyl-2-picrylhydrazyl radical (DPPH) inhibition assay

The DPPH inhibition was determined by measuring the free radical scavenging ability of bioyogurt water extracts [30]. Briefly, 3ml of DPPH (60 mmol/L in ethanol) were mixed with 250 µl yogurt extracts or cheese extract, we use 250 µl of water as a control. The mixture was shaken thoroughly and allowed to stand at room temperature. The constant absorbance readings at 517 nm were recorded after 5 min and the inhibition of DPPH oxidation (%) was calculated as follows [31]:

DPPH scavenging activity (%) = [(A blank –A sample) / A blank] × 100

where A is the absorbance.

DPPH free radical scavenging activity tests were all carried out in triplicate.

3. Statistical analysis

Results are expressed as means ± standard deviation (SD). Significant differences between means were tested by ANOVA on arc transformed data followed by Tukey's studentized range test at $P<0.05$.

4. Results and discussion

4.1. pH and TA in yogurt

The pH at the end of fermentation, as shown in Figure 1, was lower for yogurt made with *O. Ficus Indica* cladode and fruit extract than that for yogurt made with starter culture (*Lactococcus lactis* and *Enterococcus mundtii*), 3.93± 0.03; 4.01±0.04, and 4.09±0.03 respectively. During yogurt storage, a common characteristic such as post-acidification associated with microbial metabolic activity is measurable even if it is small [32].

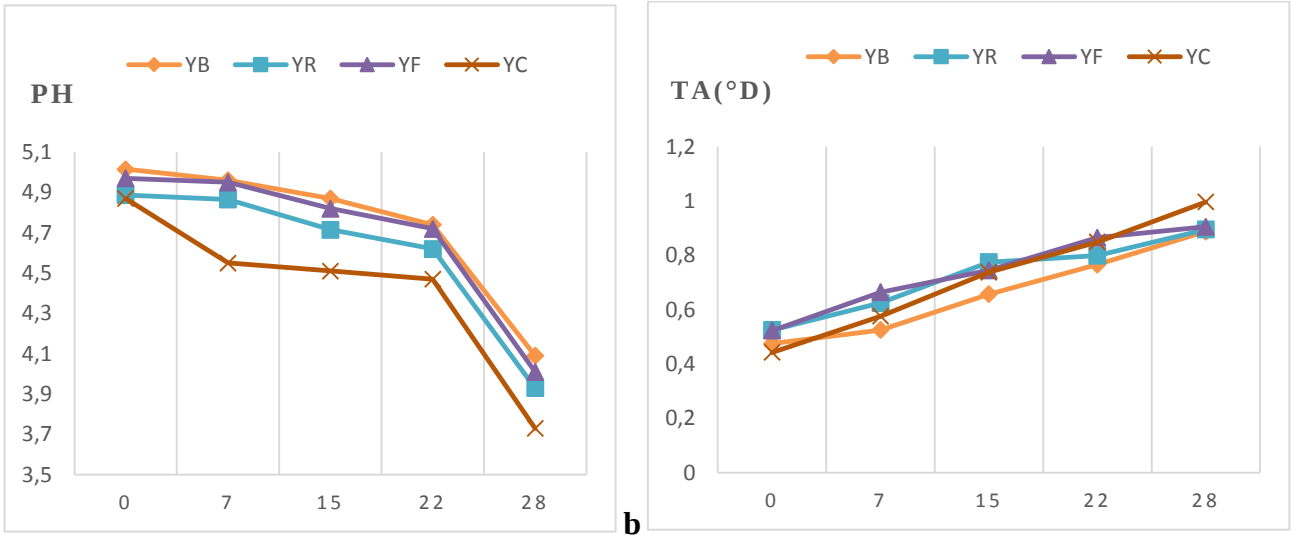


Figure 1: Changes in **a**) pH and **b**) titratable acidity (TA) during refrigerated (4°C) storage in days (**28days**). **YB**: Yogurt with starter culture (*Lactococcus lactis* and *Enterococcus mundtii*), **YR**: Yogurt with Cactus racket extract, **YF**: Yogurt with Cactus fruit extract, **YC**: Yogurt made with commercial yogurt. Values are presented as mean ± SEM (n = 3).

Relative changes in organic acid formation and alkaline nature of milk protein breakdown products induce pH fluctuations during storage [33-34]. The measurement of pH indicates free H+ concentration, generated through the production of organic acids by lactic acid bacteria (LAB). Probably, the decreasing of pH resulted in accumulation of acetic acid [35], citric acid, butyric acid, acetaldehyde, formic acid and lactic acid [36] by the breakdown of sugar (e.g. lactose) and protein products [34-37]. Early study reported that organic acids were linearly related to accumulation of TA [38]. However, TA, reflects the total amount of hydrogen ions present in the fermented milk sample with the exception of those bound to alkaline ions, nevertheless, TA was suggested more relevant in the evaluation of fermentation capacity of microbes [39]. Thus, the addition of *O. Ficus Indica* extracts appeared not to affect microbial fermentation of cow milk.

4.2. Inhibition of 1,1-diphenyl-2-picrylhydrazyl radical (DPPH) by bioyogurts

DPPH inhibition of yogurt in day 0 in the presence of Cactus cladode extract and Cactus fruit extract was higher than yogurt made with starter culture (*Lactococcus lactis* and *Enterococcus mundtii*), 27.96± 5.1%; 28.01±5.0 %, and 25.53±5.0 %, respectively, while the percentage of inhibition of yogurt made with a commercial yogurt was the lower with 22.43±5.1% (Figure 2). Which is in agree with Zekovic, et al., and Lucinia et al., [40,41] who describe that the antioxidant activity of yogurts can be reinforced by the presence of natural extracts.

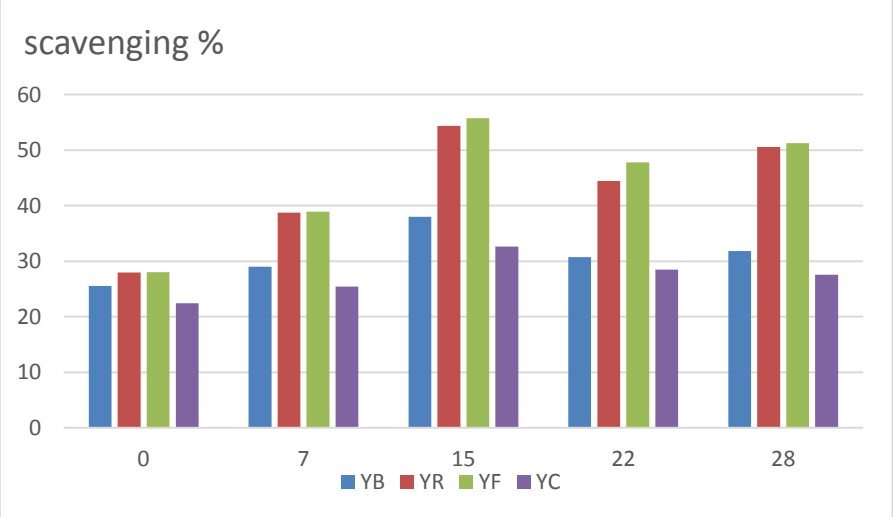


Figure 2: Changes in antioxidant activity (scavenging % of DPPH) of yogurt during refrigerated (4°C) storage. YB: Yogurt with starter culture (*Lactococcus lactis* and *Enterococcus mundtii*), **YR**: Yogurt with Cactus racket extract, **YF**: Yogurt with Cactus fruit extract, **YC**: Yogurt made with commercial yogurt. Values are presented as mean ± SEM (*n* = 3).

Yogurt with Cactus cladode and fruit extract showed consistently stronger effect than the other yogurts, while, refrigerated storage increased DPPH inhibition in all of them. Highest DPPH inhibitions were shown by both yogurts (made with cladode and fruit extract) on days 7 and 15 with extended storage to 28 days. *Opuntia Ficus Indica* has been previously shown to be rich in antioxidant compounds such as polyphenols, flavonoids, betaxanthin and betacyanin [42]. Hence, the various parts of cactus plant (pulp, peel, seeds and cladode) have revealed notable antioxidant activities [43-44-45]. In addition, numerous studies have demonstrated the beneficial effects of phenolics and antioxidants of *Opuntia* [13-46-47]. Yogurt itself has a large antioxidant capacity, related to the presence of different bioactive peptides from milk proteins through proteolysis by LAB because of fermentation and post-acidification during storage [4-5-7]. However, we observed that the type of Opuntia extract, cladode or fruit also affects the antioxidant activity of fortified yogurt [48]. Otherwise, higher yogurt antioxidant activity in the presence of those extracts, is beneficial in two respects, firstly to delay the oxidation process of lipids in yogurt

that are responsible for the formation of off-flavours and undesirable chemical compounds [49]. Secondly, to increase dietary antioxidants that are crucial in preventing the progressive impairment of pancreatic beta-cell function due to oxidative stress [50]. The presence of Opuntia in yogurts may thus be viewed advantageous in prolonging the shelf life of yogurt, and it may be indicated as a desirable characteristic that may enhance the therapeutic values of bioyogurt. Some last studies showed that the use of different additives or fruit in milk product, such as yogurt has a significant influence on those product qualities [51-52].

4.3. Total phenolic content of yogurt

The total phenolic contents estimated by the Folin–Ciocalteu reagent method of the different yogurts analyzed are presented in Figure 3. The content of which was higher in both yogurt fortified with cactus cladode extract and fruit extract ($p< 0.05$) than the yogurt made with starter culture (*Lactococcus lactis* and *Enterococcus mundtii*), 39.75± 5.1; 38.87±5.0, and 29.12±5.0 µg GAE/ml respectively. While the percentage of inhibition of yogurt made with a commercial yogurt was the lower with 26.5 ± 5.1 µg GAE/ml. Refrigerated yogurt made with cactus cladode and fruit extract, and yogurt made with starter culture showed transient reduction in TPC by day 14 (30.12 ± 3.1 and 41.75 ± 7.2, and 40.87 ± 4.3 µgGAE/ml respectively), but increased to highest values 44.18 ± 2.5; 46.18 ± 6.2 and 44.68 ± 3.7 µgGAE/ml, respectively; ($p < 0.05$) by day 28. The action of bacteria during storage to degrade polymeric phenolic compounds in the presence of Opuntia induces transient changes in TPS in the two yogurts. Also, the formation and degradation of polymeric phenolic compounds by lactic acid bacteria influences this difference in TPC in the two types of yogurts [53]. Further studies are required to characterize that some polyphenols are produced only by cladodes of *Opuntia* which may be responsible for the elevated TPC in yogurt made with cactus cladode extract during refrigerated storage [54,55].

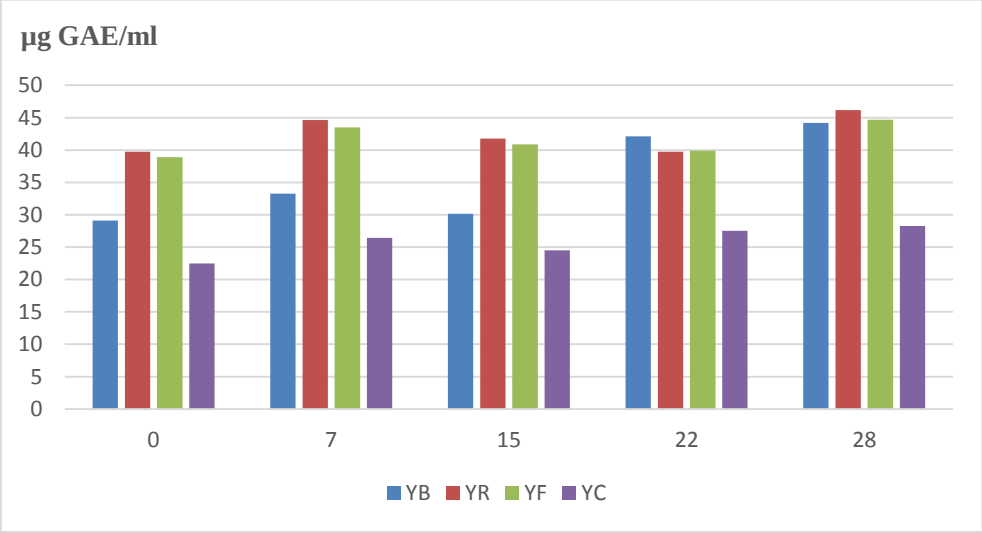
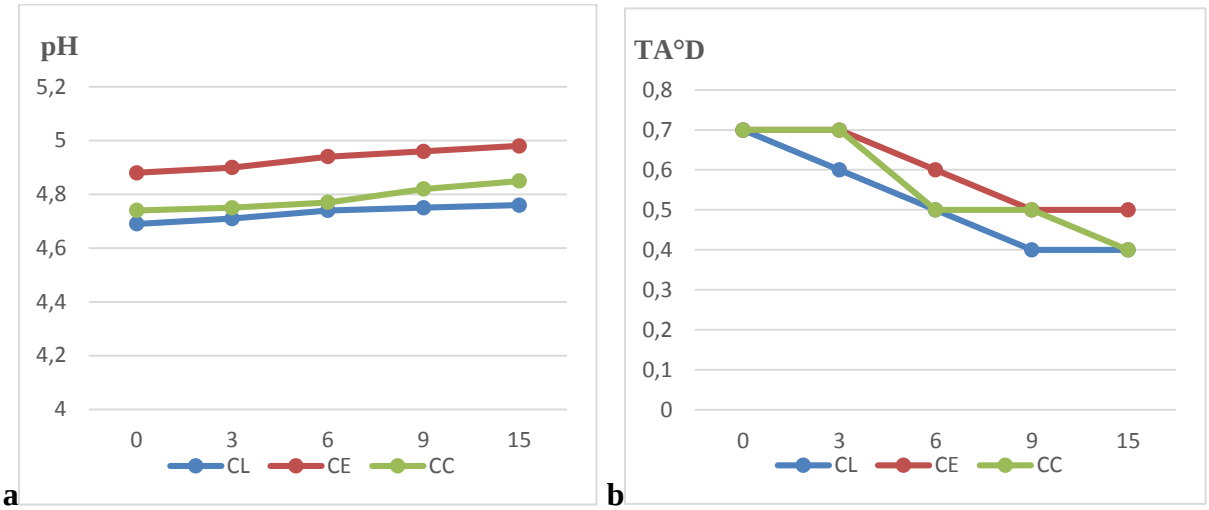


Figure 3: Changes in total phenolic content (TPC; µg GAE/ml) of yogurt during refrigerated (4°C) storage. YB: Yogurt with starter culture (*Lactococcus lactis* and *Enterococcus mundtii*), **YR**: Yogurt with Cactus racket extract, **YF**: Yogurt with Cactus fruit extract, **YC**: Yogurt made with commercial yogurt. Values are presented as mean ± SEM (n = 3).

4.4. pH and titratable acidity of cheese

The pH at the end of fermentation, as showed in Figure 4, was lower for cheese made with *Lactococcus lactis* than that made with *Enterococcus mundtii*, 4.69± 0.03; 4.88±0.04, respectively. In contrast, TA increased during refrigerated storage (Figure 4,b) which enhanced more ($p > 0.05$) in present of *Lactococcus lactis* compared to *Enterococcus mundtii*. However, TA showed a stability on day 9 of refrigerated storage.



a **b**

Figure 4: Changes in **a**) pH and **b**) titratable acidity (TA) during refrigerated (4°C) storage in days (**15 days**). **CL:** cheese made with *Lactococcus lactis*; **CE:** cheese made with *Enterococcus mundtii*; **CC:** cheese made with commercial dairy product**.** Values are presented as mean ± SEM (*n* = 3).

4.5. Inhibition of DPPH radical by cream cheese

DPPH inhibition of cheese made with *Lactococcus lactis* was higher than that made with *Enterococcus mundtii* over the storage period (15jrs in 4°C) as shown in figure 5, on days 15, highest DPPH inhibitions were shown by both cheese, 37.64± 4.1%; 36.18±4.0%, and 24.25±4.0% ($p > 0.05$) for that made with commercial dairy product.

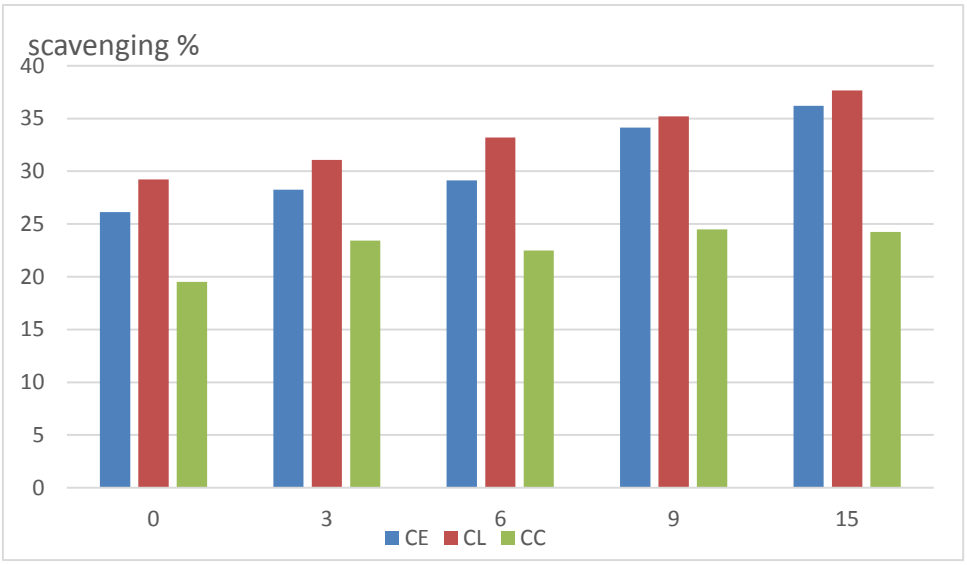


Figure 5: Changes in antioxidant activity (scavenging % of DPPH) of cheese during refrigerated (4°C) storage in days (**15 days**). **CL:** cheese made with *Lactococcus lactis*; **CE:** cheese made with *Enterococcus mundtii*; **CC:** cheese made with commercial dairy product**.** Values are presented as mean ± SEM (*n* = 3).

In a previous study on cheese, the total antioxidant capacity was significantly correlated with the seasonality (summer grazing versus winter) of production and maturation time. Significant correlations with retinol content, fat and protein content are also reported [55]. Overall, cheeses show a great diversity Composition and structure, fatty acids, β-carotene, vitamin E and xanthophylls, which can modulate their antioxidant potential. Also, the respective effects of milk composition and manufacturing processes have an effect on the antioxidant potential.

4.6. Total phenolic content of cheese

The total phenolic contents estimated by the Folin–Ciocalteu reagent method of the different cheese analyzed are presented in figure 6, the content of which was higher in both cheese made with

Lactococcus lactis and *Enterococcus mundtii*, than the cheese made with commercial dairy product, 23.75± 5.1; 41.87±5.0, and 15.12±5.0 µg GAE/ml respectively ($p < 0.05$).

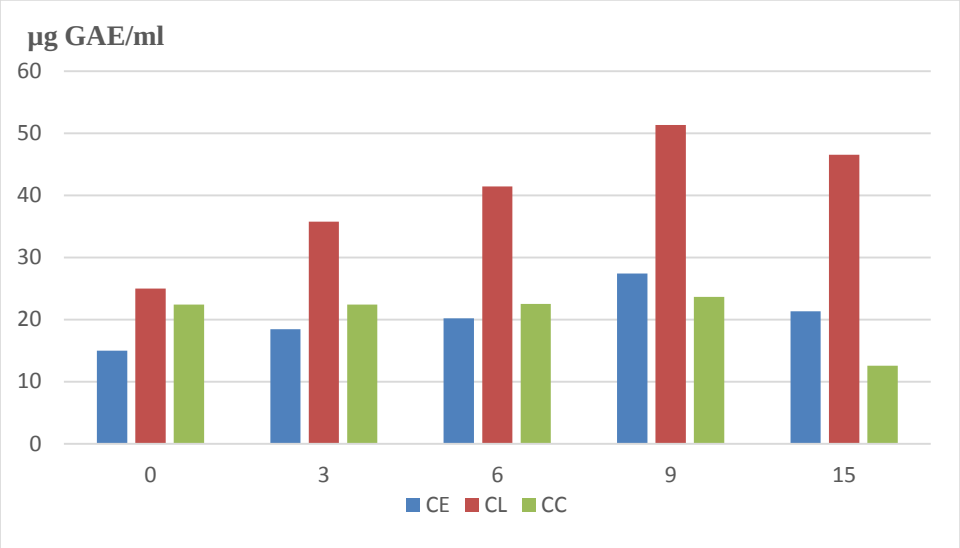


Figure 6: Changes in total phenolic content (TPC; µg GAE/ml) of cheese during refrigerated (4°C) storage in days (**15 days**). **CL:** cheese made with *Lactococcus lactis*; **CE:** cheese made with *Enterococcus mundtii*; **CC:** cheese made with commercial dairy product**.** Values are presented as mean ± SEM (n = 3).

The strain has a specific characteristic that influences the development of the radical scavenging activity, thus the production of antioxidant peptides in fermented milk [53-55-56]. It has also been reported [57] that an atypical salt resistant strain of *Lactobacillus* that came from rennet that had not been adequately filtered can lead to a development of phenolic flavor defects in cheese that is due to p- cresol.

Conclusion

Overall, highest DPPH inhibitions were shown by both yogurts (made with cladode and fruit extract); Refrigerated yogurt made with cactus cladode and fruit extract, and yogurt made with starter culture showed transient reduction in TPC, but increased to highest values by day 28 of storage. The antioxidant potential of dairy products is therefore close to that of different food groups or plants. In the other hand, of all dairy products, cheese could have the highest antioxidant potential, especially due to its higher protein content. the TPC and antioxidant capacity of cream cheese increased during storage. Some studies showed that an adequate intake of dairy products could decrease the oxidative stress in the metabolic syndrome and in obese subjects. Inclusion and augmentation of beneficial components in yogurt preparations should be pursued. Yogurt products with confirmed or novel health claims can become important components of a healthy lifestyle and can greatly benefit public health.

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