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Novel flaxseed mucilage-based probiotic film: Application to sheep Sicilian cheese preservation

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ABSTRACT. Edible probiotic films incorporated by probiotics can be used to keep away from undesirable microbes on the surface of the cheese to improve its quality and promote beneficial effects on consumer health. In this study, flaxseed, *Lactobacillus rhamnosus* and *Lactobacillus acidophilus* as probiotics were used to produce active edible packaging and evaluate its application in the sheep Sicilian cheese surfaces stored 10 days at 4°C. Microbiological, physicochemical, sensorial quality, antibacterial and antioxidant effects of cheeses were studied. The flaxseed mucilage-probiotic film improved cheese preservation by reducing the weight loss, preserving the pH and colour. Advantageously, flaxseed coating allowed improving probiotic viability throughout storage period. Native microbiota counts remained under safe levels. Probiotic coating cheeses exhibited 12.8±0.25 mm of maximum diameter inhibition against *Listeria monocytogenes* and enhanced oxidation (maximum DPPH inhibition: 51±0.33%) with acceptable sensorial properties for consumers. Hence, flaxseed mucilage probiotic film has proved to be suitable for quality preservation of functional foods.

Keywords: Flaxseed mucilage; Probiotic film; sheep cheese; functional food.

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Introduction

The dynamic changes in current consumer interest have directed more and more attention to the development of edible films for food packaging applications (Puligundla & Lim, 2022). Their properties emphatically influence both the packaged product and the environment as they are biocompatible and biodegradable (Pavli, Tassou, Nychas, & Chorianopoulos, 2018). Edible films are thin layers of food grade materials that are applied to food products and that can improve food quality and shelf life by reducing the mass transfer rate of water vapor, gases and volatile compounds between the food and the environment (Yang et al., 2023). Edible films and coatings might be heterogeneous regarding their composition. These protective layers are formed by blend of polysaccharides, proteins and/or lipids (Bourtoom, 2008) and a plasticizer, usually glycerol, is also added to improve the flexibility of the films. Flaxseed mucilage is useful in producing transparent, odorless, and flexible edible films and coatings (Lu et al., 2021). Flaxseed mucilage predominantly occurs health benefits such as delayed gastric emptying, reduced serum cholesterol, and improved glycemic control. Flaxseed exhibits good water-binding capacity and rheological properties (Puligundla & Lim, 2022).

The addition of bioactive agents such as antioxidants, antimicrobials, vitamins, etc gives edible films the ability to actively preserve the food product and potentially add them nutritional and functional value (Coimbra, Alarico, Empadinhas, Braga, & Gaspar, 2023). In this context, the incorporation of probiotics in edible films has been investigated (Lopes & Brandelli, 2018). The bioactive packaging may contain bioactive agents that are eventually released into the food (Espitia, Batista, Azeredo, & Otoni, 2016). In the edible coatings containing probiotics, the release is not even required, since the coating itself is supposed to be eaten with the food.

Generally, cheeses have been part of the human diet and their nutritional value have been documented extensively. The self-life of cheeses, especially of soft and spread cheeses, can be affected by many types of spoilage bacteria, yeasts, and molds that may occur on the cheese surface during storage. Thus, currently, active cheese packaging has been utilized to avoid damage and spoilage. Active packaging, due to its antioxidant and antibacterial properties, can be utilized to keep away from undesirable microbes on the surface of cheese as well as prolong its shelf life and improve its quality (Kontogianni et al., 2022; Benbettaïeb, Debeaufort, & Karbowiak, 2018). The edible coating can be used to manage the taste, colour, and cheese nutritional value; can be consumed with cheese and avoid the generation of waste (Costa, Maciel, Teixeira, Vicente, & Cerqueira, 2018). Furthermore, sensorial and nutritional properties of cheese could be boosted, either because of the coating structure and composition itself or by enclosing natural products with functional properties within its matrix (El-Sayed, El-Sayed, Mabrouk, Nawwar, &Youssef, 2021).

In this context, the objective of the current research was to evaluate the flaxseed-edible coating incorporated with *L. rhamnosus* LGG, *L. acidophilus* probiotic strains on the microbiological, physicochemical, sensory quality, antibacterial and antioxidant activities of sheep cheese.

Materials and methods

Film preparation

Flaxseed mucilage extraction

Flaxseed powder was purchased from a local market in Tunis, Tunisia and used for coating formulation. To prepare the coating solutions, flaxseeds (30 g) were mixed with 900 mL of distilled water. The mixture was stirred at 1000 rpm and 80 to 100°C for at least 3 h to produce an optimal mucilage solution. Then, the mucilage solution was cooled to room temperature at 25°C. To separate the mucilage solution from the flaxseeds, the mixture was harvested by centrifugation at 3900 rpm for 15 min. Then, the flaxseeds were further filtered with a cheese cloth (Tee, Wong, Tan, & Talib, 2016).

Probiotic film formation

The freeze-dried *Lactobacillus acidophilus* (LA-5®) and *Lactobacillus rahmnosus* (LGG®) (CHR HANSEN, France) were activated in MRS broth at 37°C for 24h. Bacterial cells were then harvested by centrifugation at 6000 rpm for15 min at 4°C. To formulate flaxseed mucilage-probiotic coatings, flaxseed solution was mixed aseptically with the biomass of probiotic strains at 5% (w/w) and glycerol at 15% as a plasticizer. The solutions were then stirred for 30 min without heating to produce a homogenous solution and obtain a suspensions of about 10^9 CFU mL^{-1}. After that, 20 g of each coating were put onto a Petri dish and maintained at 37°C for 24 to 48h. A film was formed upon evaporation of the cast mixture in a convection oven at 35°C for 20h. After that, the films were kept in a desiccator at 25°C and 52% relative humidity. Finally, the films were stored at 4°C for at least two days prior to subsequent use (Tee et al., 2016).

Cheese coating with flaxseed mucilage probiotic film

The "Béja Sicilian cheese" (fresh sheep cheese) (4 kg) was purchased from a small manufacturer in the region of Beja (North of Tunisia) on the day of analysis. The cheese samples were cut into uniform cubes (80 g), sizing 25 mm^3, divided in four groups according to the treatment: uncoated control cheeses (Control), samples coated with Flaxseed solution only (FCC), samples coated with Flaxseed solution containing *Lactobacillus rhamnosus* (LGG) (FCCLR) and samples coated with Flaxseed mucilage film containing *Lactobacillus acidophilus* (LC) (FCCLA). Cheese samples were stored at 4°C in transparent polypropylene plastic boxes. The cheeses were sampled at 1, 4, 7 and 10 days of storage.

Evaluation of weight loss

The weight loss of the cheese samples was determined by the following equation:

$$\text{Weight loss (\%)} = \frac{(W_0 - W_t)}{W_0} \times 100$$

Where: W_0 is the initial weight of sample and Wt is the weight of the same sample after 1, 4, 7 and 10 days of storage at 4°C.

Evaluation of water activity, pH and colour

The pH and aw were measured by a microprocessor pH-meter BT-500 (Boeco, Hamburg, Germany) and a NOVASINA aw Sprint TH-500 apparatus (Novasina, Switzerland), respectively. The colorimetric parameters

L^* (lightness), a^* (redness) and b^* (yellowness) of cheeses were determined using a colorimeter (Minolta Chroma Meter CR-300, Tokyo, Japan).

Microbiological analysis

The microbiological analysis was determined according to the approach of Kontogianni et al. (2022) with minor modifications. Total mesophilic bacteria counts were enumerated by the pour plate method using Plate Count Agar PCA (Biokar Diagnostics, France) and incubated for 48h at 37°C. Lactic acid bacteria counts were performed by surface seeding method using Man Rogosa and Sharpe agar plates (Biokar Diagnostics, France) after incubation for 48h at 37°C. Yeasts and molds were enumerated on Sabouraud Dextrose Agar (Biokar, Beauvais, France) at 30°C for 5 days.

Antioxidant activity

Water-soluble extracts (WSE) of cheese were prepared according to Mahmoudi, Telmoudi, Chouaibi, and Hassouna (2021).

The DPPH (1,1-diphenyl-2-picrylhydrazyl) radical-scavenging ability of chesses was assessed according to Al-dhaheri et al. (2017). For this, 1mL of the extracts at different concentrations was mixed with 1 mL of the DPPH. After stirring, the tubes were placed in the dark for 30 min; the absorbance was then measured at 517 nm

The DPPH radical scavenging capacity was determined by the following formula:

$$\text{DPPHradical scavenging activity (\%)} = \frac{(AB0 - AB1)}{AB0} \times 100$$

Where AB_0 was the absorbance of the blank and AB_1 was the absorbance of the test sample (coated or uncoated cheese).

Antibacterial activity

The antibacterial activity of cheese samples against pathogens such as *Salmonella Typhimurium* (ATCC 25922), *Staphylococcus aureus* (ATCC 6810), *Listeria monocytogenes* (ATCC 070 101 121) and *Esherichia coli* (ATCC 25922) was assessed using agar disc diffusion method previously described by Mahmoudi et al. (2021). Briefly, 0.1 mL (10^6 CFU mL^{-1}) of each overnight pathogen was spread on nutrient agar (Biokar Diagnostics, France). From each extract, 100 µL was put on sterile discs, which already placed onto the agar surface, and plates were then incubated for 24h at 37°C. Diameter of clear zone around discs was measured in millimeters (disc diameter included) as antibacterial activity.

Sensory analysis

The sensory evaluation of cheeses was inspired from Mudgil, Barak, and Darji (2018). Cheese samples were served on day 10 of storage, under normal light conditions in paper plates marked with two-digit randomized codes. Colour, flavour, odor, texture and overall acceptability were evaluated by a panel of 30 trained members using five-point hedonic scale; 1 (Not acceptable), 2 (Fair acceptable), 3 (Acceptable), 4 (Very acceptable), 5 (Highly acceptable).

Statistical analysis

Statistical analyses were performed by SPSS version 22.0. The mean differences between samples were compared using the one way analysis of variance (ANOVA). Differences were considered significant at P<0.05.

Results and discussion

pH, aw and weight loss

The pH, water activity and weight loss were evaluated during 10 days of storage (Table 1). The pH decreased in all samples (P<0.05) at the end of storage. Similar behaviour was observed by Leandro et al. (2021) for coated coalho fresh cheese. This reduction (6.31-5.84 at day 1-10) is due to the production of lactic acid by lactic and probiotic bacteria leading to the cheese acidification (Ksoudaet al., 2019; Mahmoudi et al., 2021).

Water activity is defined as the amount of free water present in a product and available for bacterial growth. After 10 days of storage, the aw increased in all samples (P<0.05) due to whey exudation. Since the coating represents a barrier against moisture loss, the aw of the coated cheese was significantly (P<0.05) lower compared to the control.

Table 1. Changes in pH, aw, weight loss and colour in cheeses.

Storage time (days)	Cheeses			
	Control	FCC	FCCLR	FCCLA
pH				
1	6.31 ± 0.1 (a. A)	6.3 ± 0.1 (a,A)	6.29 ± 0.1 (a. A)	6.28 ± 0.1 (a. A)
4	6.00 ± 0.1 (a. A)	6.12 ± 0.1 (b,AB)	6.19 ± 0.1 (b. B)	6.18 ± 0.1 (b. A)
7	5.92 ± 0.1 (a. B)	6.1 ± 0.1 (b, BC)	6.08 ± 0.1 (b. B)	6.09 ± 0.1 (b. B)
10	5.84 ± 0.1 (a. C)	5.95 ± 0.1 (b,C)	5.92 ± 0.1 (b. C)	5.93 ± 0.1 (b. C)
aw				
1	0.9 ± 0.1 (a. A)	0.91 ± 0.1 (a. A)	0.9 ± 0.1 (a. A)	0.9 ± 0.1 (a. A)
4	0.93 ± 0.1 (a. B)	0.91 ± 0.1 (a. A)	0.91 ± 0.1 (a. A)	0.91 ± 0.1 (a. A)
7	0.955 ± 0.1 (a. C)	0.915 ± 0.1 (b. B)	0.918 ± 0.1 (b. B)	0.917 ± 0.1 (b. B)
10	0.987 ± 0.1 (a. D)	0.914 ± 0.1 (b. C)	0.918 ± 0.1 (b. B)	0.918 ± 0.1 (b. B)
Weight loss (%)				
1	0 ± 0.0	0 ± 0.0	0 ± 0.0	0 ± 0.0
4	10 ± 0.2 (a. A)	6.5 ± 0.14 (b.A)	6.7 ± 0.3 (c.A)	6 ± 0.11 (d.,A)
7	17 ± 0.15 (a.B)	6.9 ± 0.1 (b.AB)	6.8 ± 0.2 (c.A)	6.2 ± 0.18 (d.A)
10	22 ± 0.3 (a. B)	7.1 ± 0.1 (b.B)	6.9 ± 0.15 (c.A)	7.1 ± 0.16 (d.A)
L*				
1	83.20 ± 0.1 (a.A)	87.78 ± 0.1 (b.A)	87.88 ± 0.1 (b.A)	88.88 ± 0.1 (b.A)
4	82.55 ± 0.1 (a.B)	86.81 ± 0.1 (b.B)	86.63 ± 0.1 (b.B)	86.80 ± 0.1 (b.B)
7	82.58 ± 0.1 (a.B)	86.5 ± 0.1 (b.B)	86.7 ± 0.1 (b.B)	86.5 ± 0.1 (b.B)
10	79 ± 0.1 (a.,C)	82.3 ± 0.22 (b.C)	82.33 ± 0.2 (b.C)	82.22 ± 0.2 (b.C)
a*				
1	0.37 ± 0.1 (a.,A)	0.16 ± 0.1 (b.A)	0.16 ± 0.1 (b.A)	0.17 ± 0.1 (b.A)
4	0.55 ± 0.1 (a.B)	0.48 ± 0.1 (b. B)	0.48 ± 0.1 (b.B)	0.45 ± 0.1 (b.B)
7	0.94 ± 0.1 (a,C)	0.85 ± 0.1 (b. C)	0.82 ± 0.1 (b.C)	0.83 ± 0.1 (b.C)
10	1.27 ± 0.2 (a.D)	0.88 ± 0.2 (b.C)	0.91 ± 0.2 (b.D)	0.92 ± 0.2 (b.D)
b*				
1	16.22 ± 0.1 (a.A)	24.14 ± 0.1 (b. A)	24.94 ± 0.1 (b. A)	24.35 ± 0.01 (b.A)
4	17.36 ± 0.1 (a.B)	25.66 ± 0.11 (b. B)	25.71 ± 0.11 (b. AB)	25.68 ± 0.1 (b.B)
7	18.27 ± 0.1 (a.,C)	26.9 ± 0.1 (b.C)	26.92 ± 0.1 (b. B)	26.91 ± 0.1 (b.C)
10	19.41 ± 0.2 (a.D)	28.22 ± 0.12 (b.D)	28.28 ± 0.18 (b. C)	28.27 ± 0.42 (b.B)

*a. A Mean values ± SD (n=3) within a row with different superscript letters are significantly different (P<0.05). Lower-case letters Different letters indicate significant differences between samples in the same storage day (p < 0.05) and upper-case letters indicate significant differences for the same sample within different days of storage (p < 0.05). Control: Uncoated cheese; FCC: Flaxseed Coated Cheese; FCCLR: Flaxseed Coated Cheese inoculated with probiotic strain *L.rhamnosus*; FCCLA: Flaxseed Coated Cheese inoculated with probiotic strain *L.acidophilus*.

As expected, the weight loss increased during storage in all cheese samples (Table 1). This is possibly due to the phenomena of whey exudation during storage leading to continuous moisture migration from cheese to surrounding environment. In fact, the uncoated control sample weight loss reached 10 ± 0.2 to 22 ± 0.3% (P<0.05). However, weight loss of coated cheeses were less than 8%. This difference may be explained by the water vapor barrier effect exerted by the flaxseed probiotic coating. This phenomena was reported by Ju et al. (2018).

Colour

Table 1 shows the colour coordinates (L*, a* and b*) for cheese samples during storage. Coated cheeses (FCC, FCCLR and FCCLA) presented higher L* (88.88 ± 0.1), i.e., the films are bright and higher b* values (28.28 ± 0.18) (P<0.05). However, a* values reduced (1.27 ± 0.2 to 0.92 ± 0.2) which explain the yellowish tendency on their color. These results were similar to those reported by Oliveira et al. (2019) for coated coalho cheese. Based on the above results, this pattern can be positive for a greater acceptance by consumers (Leandro et al., 2021).

Microbial quality

The addition of probiotic bacteria significantly affects the dynamic populations of mesophilic, lactic and yeasts and molds microorganisms (Table 2). The mesophilic counts observed in samples inoculated with *L.rhamnosus* and *L.acidophilus* (FCCLR and FCCLA) were about 1.5 orders log higher with respect to uncoated cheese. These differences could be explained by the growth of the probiotic even in PCA medium. In accordance with our results, Bambace, Alvarez, and Moreira (2019) reported no significant differences on total counts in coated probiotic blueberries samples. In addition, the lactic bacteria count was 8.3 ± 0.34 log_{10} CFU g^{-1} in flaxseed coated probiotic cheese (FCCLR) due to the viability of probiotics and the presence of flaxseed which enhance the probiotic growth in edible coat during cheese storage. Moreover, Pavli, Tassou, Nychas, and Chorianopoulos (2018) demonstrated that the inclusion of probiotics into edible film and coating may contribute to health benefits to the consumers.

Table 2. Evolution of microbial population in cheeses.

Storage time (days)	Cheeses			
	Control	FCC	FCCLR	FCCLA
	Mesophilic bacteria (log_{10} FCU g^{-1})			
1	3.26 ± 0.5 $^{(a. A)*}$	3.23 ± 0.14 $^{(a. A)}$	4.21 ± 0.22 $^{(b. A)}$	4.2 ± 0.13 $^{(b. A)}$
4	4.16 ± 0.31 $^{(a. B)}$	3.92 ± 0.18 $^{(a. A)}$	4.77 ± 0.18 $^{(b. B)}$	4.5 ± 0.18 $^{(b. A)}$
7	4.94 ± 0.22 $^{(a. B)}$	4.3 ± 0.26 $^{(a. AB)}$	5.81 ± 0.61 $^{(b. B)}$	5.81 ± 0.11 $^{(b. A)}$
10	5.69 ± 0.48 $^{(a. C)}$	5.94 ± 0.27 $^{(a. B)}$	7.25 ± 0.19 $^{(b. B)}$	7.25 ± 0.81 $^{(b. B)}$
	Lactic bacteria (log_{10} FCU g^{-1})			
1	3.66 ± 0.22 $^{(a. A)}$	3.50 ± 0.34 $^{(a. A)}$	7.11 ± 0.22 $^{(b. A)}$	7.10 ± 0.22 $^{(b. A)}$
4	4.25 ± 0.54 $^{(a. B)}$	4.10 ± 0.21 $^{(a. B)}$	7.52 ± 0.37 $^{(b. A)}$	7.60 ± 0.36 $^{(b. A)}$
7	4.62 ± 0.33 $^{(a. B)}$	4.18 ± 0.28 $^{(a. B)}$	8.2 ± 0.26 $^{(b. B)}$	8.27 ± 0.77 $^{(b. B)}$
10	4.85 ± 0.51 $^{(a. B)}$	4.30 ± 0.44 $^{(a. B)}$	8.3 ± 0.34 $^{(b. B)}$	8.32 ± 0.2 $^{(b. B)}$
	Yeasts and molds(log_{10} FCU g^{-1})			
1	1.3± 0.1 $^{(a. A)}$	1.31± 0.17 $^{(a. A)}$	1.29± 0.1 $^{(a. A)}$	1.28± 0.1 $^{(a. A)}$
4	1.4± 0.1 $^{(a. A)}$	1.34± 0.1 $^{(b. A)}$	1.3± 0.22 $^{(b. A)}$	1.3± 0.16 $^{(b. A)}$
7	1.85 ± 0.1 $^{(a. B)}$	1.62 ± 0.1 $^{(b. B)}$	1.43 ± 0.1 $^{(b. B)}$	1.32 ± 0.21 $^{(b. A)}$
10	1.87 ± 0.1 $^{(a. B)}$	1.66 ± 0.33 $^{(b. B)}$	1.44 ± 0.16 $^{(b. B)}$	1.33 ± 0.13 $^{(b. A)}$

*a. A Mean values ± SD (n=3) within a row with different superscript letters are significantly different (P<0.05). Lower-case letters Different letters indicate significant differences between samples in the same storage day (p < 0.05) and upper-case letters indicate significant differences for the same sample within different days of storage (p < 0.05). Control: Uncoated cheese; FCC: Flaxseed Coated Cheese; FCCLR: Flaxseed Coated Cheese inoculated with probiotic strain *L.rhamnosus*; FCCLA: Flaxseed Coated Cheese inoculated with probiotic strain *L.acidophilus*.

The yeast and molds numbers were different between coated cheese samples (P<0.05) These fundings were similars to those reported by Bambace, Alvarez, and Moreira (2019) who suggested that this fact could be attributed to the presence of maltodextrins used as cell viability protector of the probiotic strains. Nevertheless, the evolution of these population counts was similar during storage (P>0.05). Also, coating contains semi-permeable gas barrier, which result growth inhibition of this germs contributing to extend food stability (Kumar & Sethi, 2018).

Antibacterial and antioxidant activities

The inhibition abilities of the coated and uncoated cheese samples against pathogens are summarized in Table 3. Antibacterial activities of flaxseed probiotic coated cheeses were significantly higher than control (P<0.05) with a maximum inhibition diameter of 12.8 ± 0.25 mm.

Table 3. Evaluation of the antibacterial activity of cheeses.

Pathogen strains	Diameter of the inhibition zone ID (mm ± SD)			
	Cheeses			
	Control	FCC	FCCLR	FCCLA
Listeria monocytogenes	3.5 ± 0.2 ^{a}	10.2 ± 0.5 ^{b}	12.4 ± 0.7 ^{b}	12.8 ± 0.25 ^{b}
Staphylococcus aureus	3.8 ± 0.25 ^{a}	10.8 ± 0.1 ^{b}	12.5 ± 0.7 ^{b}	12.6 ± 0.36 ^{b}
Salmonella Thyphimurium	2.8 ± 0.2 ^{a}	7.9 ± 0.2 ^{b}	8.5 ± 0.15 ^{b}	8.7 ± 0.1 ^{b}
Escherchia coli	2.5 ± 0.14 ^{a}	7.2 ± 0.7 ^{b}	8.6 ± 0.29 ^{b}	8.61 ± 0.13 ^{b}

*a. A Mean values ± SD (n=3) within a row with different superscript letters are significantly different (P<0.05). Lower-case letters Different letters indicate significant differences between samples in the same storage day (p < 0.05). Control: Uncoated cheese; FCC: Flaxseed Coated Cheese; FCCLR: Flaxseed Coated Cheese inoculated with probiotic strain *L.rhamnosus*; FCCLA: Flaxseed Coated Cheese inoculated with probiotic strain *L.acidophilus*.

exhibited by coated cheese inoculated with *L.acidophilus* (FCCLA) against *Listeria monocytogenes*. This due to the antibacterial activity of edible coating which contain flaxseed as prebiotic and probiotic bacteria. At the same, Lu et al. (2021) reported that chitosan and flaxseed coated cheese exhibited stronger antibacterial activity against *E.coli.* Besides, Espitia, Batista, Azeredo, and Otoni (2016) approved that the incorporation of probiotics in the coating matrix results the reduction of the cheese spoilage through the protection of cheese surface against microbial contamination. Since edible coatings have the capacity to maintain high concentrations of the active substance on the cheese surface preventing its migration and the application efficiency increased. Also, Benbettaïeb, Debeaufort, and Karbowiak (2018) reported that in edible films, greater levels of antimicrobial agents, such as bacteriocins, organic acid, would remain in the film and at the surface of the food which prevents the microorganisms for a longer period.

The DPPH radical scavenging ability of different cheese samples were shown in Figure 1. DPPH inhibition increased during storage (P<0.05) in all cheeses. The values of flaxseed coated probiotic cheeses (FCCLR and FCCLA) were 50 ± 0.55 and 51 ± 0.33% respectively compared to control (21 ± 0.66%) at the end of storage (P<0.05). Our results are in agreement with Alvarez, Bambace, Quintana, Gomez-Zavaglia, and del Rosario (2020) who indicated a strong antioxidant activity of prebiotic coated of fresh apple. *L.rhamnosus* and *L. acidophilus* and flaxseed confered antioxidant activities in coated cheeses. For essential oil coating apllication, the results reported by Ksouda et al. (2019) confirm that the DPPH activity increased with essential oil coatings even to 59.47%.

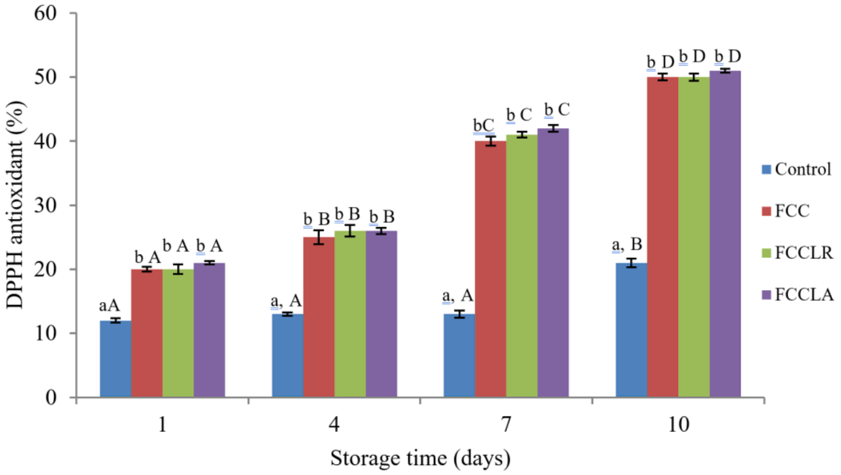

Figure 1. DPPH radical scavenging capacity of cheeses. *a. A Mean values ± SD (n=3) within a row with different superscript letters are significantly different (P<0.05). Lower-case letters Different letters indicate significant differences between samples in the same storage day (p < 0.05) and upper-case letters indicate significant differences for the same sample within different days of storage (p < 0.05). Control: Uncoated cheese; FCC: Flaxseed Coated Cheese; FCCLR: Flaxseed Coated Cheese inoculated with probiotic strain *L.rhamnosus*; FCCLA: Flaxseed Coated Cheese inoculated with probiotic strain *L.acidophilus*.

More specifically, Benbettaïeb, Debeaufort, and Karbowiak (2018) explained that antioxidant activities of cheeses would be exerted by natural organic acids and the incorporation of antioxidant agents in edible films forming preparations aims at increasing the product shelf life by protecting foods against oxidative rancidity, degradation and discoloration. Antioxidant activity can be achieved via different mechanisms, such as singlet oxygen deactivation, peroxide enzyme inhibition, chelation of transition metals (Gülçin, 2012).

Sensory assessment

Due to the fact that edible films are usually consumed with the coated products, they must have organoleptic properties that are as neutral as possible (Pavli et al., 2018). So, the characterization of sensorial quality of food is an important point when studying the feasibility of adding functional ingredients. As shown in Figure 2, results indicated the impact of coating on colour, odor, flavour and texture of cheese samples, after 10 days of storage (P<0.05). The FCC, FCCLR and FCCLA coated cheeses affected sensory quality and resulted in significantly higher scores (P<0.05) for all attributes compared to uncoated one. Finally, overall acceptability average scores ranged from 2.8 (control) to 3.8 (coated cheeses) in the 5-point Hedonic scale and the FCCLA was the most appreciated.

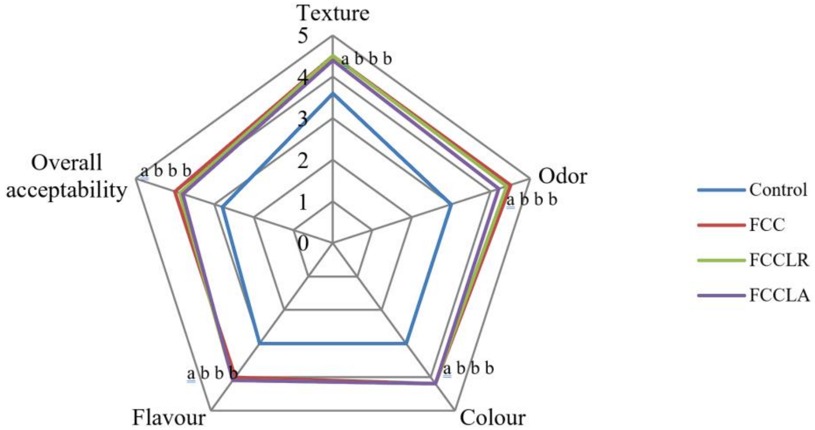

Figure 2. Sensory analysis of cheeses. *a. A Mean values ± SD (n=3) within a row with different superscript letters are significantly different (P<0.05). Lower-case letters Different letters indicate significant differences between samples in the same storage day (p < 0.05. Control: Uncoated cheese; FCC: Flaxseed Coated Cheese; FCCLR: Flaxseed Coated Cheese inoculated with probiotic strain *L.rhamnosus*; FCCLA: Flaxseed Coated Cheese inoculated with probiotic strain *L.acidophilus*.

Also, significant overall acceptability was detected by panelists in coated cheeses with scores of 4. These scores indicated that these samples were acceptable from a sensory point of view. García, Perez, Piccirilli, and Verdini (2016) found that whey and inulin edible coated cookies containing the probiotic *L.casei shirota* have no significant differences compared to the control and was highly accepted from the panelists.

Conclusion

L.rhamnosus and *L.acidophilus* are suitable probiotics to be added to flaxseed coated sheep cheeses during 10 days of storage. Furthermore, the flaxseed probiotic coatings were effective in reducing the weight loss, preserving pH and colour and improving the oxidative and antibacterial activities of the coated cheese. The sensory analysis showed high appreciation of flaxseed probiotic coated cheese.

For these reasons, flaxseed coated sheep cheese constitutes an effective carrier for probiotic lactobacilli and could be exploited for the development of innovative functional foods.

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