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The Effect of Xanthan Gum and Flaxseed Mucilage as Edible Coatings in Cheddar Cheese during Ripening

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Abstract: The object of this study was to investigate the possibility of using xanthan gum and flaxseed mucilage as edible coatings for Cheddar cheese during ripening for 90 days. Five samples of Cheddar cheese blocks were coated with different coating materials in triplicate as follows: Coated with polyvinyl acetate as control (C), coated with 0.5% xanthan gum (XG), coated with 0.75% flaxseed mucilage (FM1), coated with 1% flaxseed mucilage (FM2), and coated with 1.25% flaxseed mucilage (FM3). All samples were kept at 8 ± 2 °C in a cold room for 90 days. The statistical analysis of the results showed that the moisture content of the samples decreased and the protein content increased during the ripening period ($P < 0.01$). The pH, acidity, fat in dry matter, and TCA-SN/TN of samples were significantly affected by xanthan gum and flaxseed mucilage treatment ($P < 0.01$). The free fatty acid composition of samples was significantly affected by edible coatings. Edible coatings affected the growth of non-starter lactic acid bacteria and the total mesophilic aerobic bacteria in a non-significant manner ($P > 0.01$). The growth of starter bacteria was significantly altered under the effect of edible coating materials ($P < 0.05$). Tyrosine and tryptophan contents as an index of proteolysis, lipolysis, and sensory evaluation of samples were not significantly different.

Keywords: Cheddar cheese; edible coatings; xanthan gum; flaxseed mucilage

1. Introduction

Packaging is the technique of using suitable components to preserve food from production to consumption. Plastic packaging is technically degradable, but in natural conditions it takes much longer than for bio-materials [1]. The major environmental problem with plastics is their low degradability and the stability of macro- and micro-plastics in both soil and water [2].

Therefore, manufacturers try to reduce the application of plastic materials for packaging and develop flexible and biodegradable films and edible coatings for food packaging [3]. Recent works suggest plant-based biomaterials and composites as food packaging for all types of products [2].

Environmentally friendly coatings are basically made of hydrocolloid, lipid, and composite. Hydrocolloid coatings are composed of proteins, cellulose derivatives, pectin, and other polysaccharides (carbohydrates and gums); lipid coatings consist of waxes, acylglycerols, and fatty acids, while composite coatings generally contain both lipid and hydrocolloid components [4,5]. The main

advantage of films and edible coatings to traditional synthetic coatings is that they can be consumed along with the food [6].

Over the past few decades, a variety of polysaccharides have been investigated and introduced as new compounds for films and edible coatings for food packaging [7].

Xanthan gum (XG) is a natural, high-molecular-weight exopolysaccharide and is known as an important industrial biopolymer [8]. Xanthan gum is synthesized by *Xanthomonas campestris* under unfavorable conditions [9]. This gum possesses a major cellulosic chain with a primary structure. The biopolymer has been used in different foods for a variety of reasons, including stabilizing, viscosifying, emulsifying, thickening, and temperature stability [10]. The gum could also be used as an edible coating for foods and thus extends their shelf life. Most recently, the effect of santhan gum coating has been studied in fresh-cut pears [9], minimally processed prickly pears, pumpkins [11], papayas [12], and fresh-cut apples [13].

Flaxseed (*Linum usitatissimum* L.) is one of the oldest crops; it is flat and ovate and belongs to the family linaceae(s). This seed has been effectively used to prevent diseases such as hypotension and hypocholesterolemia because it contains alpha-linolenic acid, lignans, and polysaccharides (other than starch) [14]. Its hull consists of four layers; the outer layer contains soluble fiber. This soluble fiber is also known as mucilage. Mucilage creates a gel-like layer when dissolved in water [15]. Therefore, it is a suitable compound for coating or film formation [16]. Flaxseed mucilage (FM) coating has been applied to potato chips [14] and fresh-cut cucumber [17].

Cheddar cheese is one of the most important varieties of cheese [18]. This cheese is susceptible to microbial and chemical spoilage during storage under refrigeration. Therefore, the application of an edible coating may solve spoilage issues to some extent and increase the shelf-life of the cheese. Thus, this study was carried out to investigate the possibility of using xanthan gum and flaxseed mucilage as coating bio-materials for Cheddar cheese packaging during ripening and study the effect of these compounds on the cheese's overall quality.

2. Methods and Materials

2.1. Raw Materials

The edible coating solutions were prepared with: Xanthan gum (Sigma Chemistry, Germany), flaxseed (*Linum usitatissimum*) purchased from a traditional market in Urmia, Iran in 2017 and its mucilage was extracted as described by Tabibloghmani et al. [14]. Commercial coating (polyvinyl acetate) was obtained from Kalleh Dairy Co. (Amol, Iran). We also used glycerol 87% (Panreac, Spain) and ethanol 96% (Bidastan Co., Gazvin, Iran). All ingredients used were food grade. Fresh Cheddar cheese (protein 24%, fat 29.5%, dry matter 61.6%, salt 2% and pH 5.25), was purchased from Kalleh Dairy Co. (Amol, Iran).

2.2. Preparation of Coating Solutions

The optimal concentration for xanthan gum coating solution was 0.5% [9], whereas 0.75%, 1%, and 1.25% concentrations were used as optimal concentrations for a flaxseed mucilage coating solution [14]. All the solutions contained 50% glycerol as a plasticizer. All solutions were sterilized using UV light for 1 h.

2.3. Cheese Coating Method

The cheese blocks (about 500 g) were exposed to UV light for sterilization for 1 h. The method introduced by Penna-Serna et al. [19] was applied to separately coat the cheese with determined concentrations of xanthan gum and flaxseed mucilage. Coating was performed in three layers with gentle brushing. For this purpose, the first layer of coating solution was brushed on the surface of the blocks and left to dry for 1 h. The second layer was applied and dried, similarly to the first step. Finally, the third layer was applied onto the blocks and dried for 4 h at 24 °C and a relative humidity

of 50%. Thus, five trials of Cheddar cheese were prepared with different coatings, including: control (C, coated Cheddar cheese with polyvinyl acetate as commercial coating), xanthan gum (XG, coated Cheddar cheese with 0.5% xanthan gum as edible coating), and flaxseed mucilage (FM1, FM2, and FM3 coated Cheddar cheese with 0.75%, 1%, and 1.25% flaxseed mucilage as edible coating, respectively. The coated samples were kept at 8 ± 2 °C in a cold room for 90 days.

2.4. Microbial Analysis

Ten grams of each sample were transferred into the sterile stomacher bag under aseptic conditions and diluted 1:10 (w/v) with sterile trisodium citrate (2 g/100 mL), followed by a 2-min homogenization using a stomacher (Seward Laboratory, London, UK). The dilution series were prepared by adding 1 mL of each concentration to 9 mL of sterile peptone water (0.1% w/v, Sigma-Aldrich, Darmstadt, Germany). In order to count non-starter lactic acid bacteria (NSLAB), 1 mL of each dilution was transferred to the MRS agar medium using the pour plate method. The cultured bacteria were incubated under anaerobic conditions at 37 °C for 72 h using a gas-pack system (Merck, Darmstadt, Germany). The starter bacteria (SB) were cultured on M17 agar medium under aerobic conditions at 37 °C for 72 h [20]. Total mesophilic aerobic bacteria (TMAB) numbers were counted on plate count agar incubated under an aerobic condition at 30 °C for 72 h [21].

2.5. Evaluation of Proteolysis

Trichloroacetic acid 12%-soluble nitrogen as percent of total nitrogen (TCA-SN/TN) was extracted using the method introduced by Gripon et al. [22], which was modified by Bergamini et al. [23]. The extracted nitrogen content of each solution was determined using the Kjeldahl method.

Furthermore, the amounts of aromatic amino acids (free tyrosine and tryptophan amino acids) were evaluated in 12% trichloroacetic acid using the method described by Khosrowshahi et al. [24] on days 30, 60, and 90 of ripening. In order to determine the amounts of aromatic amino acids, the absorbance of extracted solutions was measured by the addition of a folin–phenol reagent at a wavelength of 650 nm. The amounts of the amino acids were then determined using a standard curve of tyrosine–tryptophan prepared at concentrations of 0, 5, 10, and 50 µg/mL in a trichloroacetic acid 12% solution.

2.6. Evaluation of Lipolysis

Evaluation of lipolysis was performed on days 30, 60, and 90 of ripening by titration of free fatty acids. Fat was extracted from the cheese samples using diethyl ether and the fatty acid index (meq/100g fat) was determined by titration of potassium hydroxide [25].

2.7. Free Fatty Acid Composition Analysis

The method introduced by Tarakci et al. [26] was used to determine the composition of free fatty acids. Briefly, 10-g specimens were obtained from each cheese sample for fat extraction using a chloroform/methanol solution (2:1 v/v). The mixture was then subjected to homogenization using an Ultra Turrax homogenizer (Cat X120, ProfiLab24 GmbH, Berlin, Germany). Fifteen milliliters of 1 mM $CaCI_2$ were added to the homogenized solution, which was then shaken for 30 s. The obtained mixture was centrifuged at 2000 rpm for 15 min. The chloroform phase was transferred to the funnel vacuum evaporator in order to dry out as well as to determine the composition of the free fatty acids. The components of the free fatty acids (FFA) were evaluated after methylation [27] by applying a gas chromatography apparatus YL (Model 6500, GC system, YL Instrument Co., Ltd., Anyang, Korea) using a capillary column (60 m × 0.25 mm ID, 0.25 µm) and nitrogen carrier gas. The injected volume was 1 µL. The temperature of the injector and detector apparatus was maintained at 270 °C and 280 °C, respectively. Comparison of the retention times with valid standards (Supelco 37 Components, FAME Mixture, Cat. No. 18919-1AMP) was used to detect the fatty acids. The results obtained were calculated on the basis of mg/100 g of free fatty acids. All experiments were carried out in triplicate.

2.8. Compositional Analysis

Compositional analysis was performed on the cheese samples at days 1, 30, 60, and 90 of ripening. The amount of protein was determined using the micro-Kjeldahl procedure [28], while fat content was evaluated by the Gerber volumetric method, titratable acidity, and moisture by oven-drying at 102 °C [29]. pH values of the cheese samples were measured using a digital pH meter (Model 691, Metrohm, Herisau, Switzerland) after calibration with fresh pH 4.0 and 7.0 standard buffers at +20 °C.

2.9. Sensory Analysis

At the end of ripening (day 90), the cheese samples were subjected to sensory evaluation by a consumer panel of 15 individuals consisting of staff from the Agricultural Research Center of West Azerbaijan (Urmia, Iran). The cheese samples were cut into standard bite-sized pieces of about 1 cm^3. The samples were served on plates together with a consumer sensory evaluation questionnaire on a blind-labeled basis. The consumers were asked to evaluate sensory characteristics such as texture, flavor, color, and cutting [30], on a hedonic scale of 1 (extreme dislike) to 5 (strongly positive response).

2.10. Statistical Analysis

A factorial method in the form of a complete randomized design was used for the statistical design of the present study. The results were analyzed using Minitab 16 software.

3. Results and Discussion

3.1. Cheese Composition

The moisture, protein, FDM, and acidity contents as well as pH of the examined cheese samples during ripening period of 90 days are illustrated in Table 1. The results showed the pH values of the cheese samples were significantly changed under the effects of different coating materials (edible and commercial coatings) and ripening times ($P < 0.01$).

The pH of the cheese samples increased until day 30 (except for the FM3 sample) and then gradually decreased until day 90 of ripening. The reduction in pH values during ripening is due to the metabolism of the remaining lactose to lactic acid by NSLAB [31]. Higher levels of dissolved CO_2 in the cheese atmosphere produced buffering effects due to the lower CO_2 permeability of 1.25%. Flaxseed coating may explain the stable pH value of FM3 sample until day 30 of ripening [32]. Changes in the pH values of the cheese samples may be the consequence of alkaline compound formation due to proteolytic degradation during the ripening period [33]. At the end of the ripening period, the highest and lowest pH rate were detected in FM1 and control samples (5.42 versus 5.21), respectively ($P < 0.01$), which contributed to the increase in NSLAB bioactivity after 90 days in the control sample and the increment in lactic acid production.

The titratable acidity of the cheese samples increased during ripening time ($P < 0.01$). According to the results of the microbial analysis, the NSLAB population increased during the 90 days of ripening, which could increase the production of lactic acid and subsequently increase the titratable acidity [34]. The greatest number of NSLAB counts on day 90 of ripening was detected in the XG sample, which could confirm the higher titratable acidity of this sample compared to the other samples.

The results showed that the moisture content of the cheese samples decreased significantly during the ripening period ($P < 0.01$). These results were consistent with those reported by Buriti et al. and Kasimoglu et al. [35,36], who have shown that the moisture content was reduced during ripening in all the cheese samples examined in their study. It is expected that the reduced moisture may be due to synergism and osmotic flow during the ripening period. The results showed that the types of coatings used to coat Cheddar cheese had no significant effect ($P > 0.01$) on the moisture content of the samples, and all the samples, with the exception of the control, reached the same levels of moisture content on day 90 of ripening. At the end of the ripening period, the highest and lowest rate of moisture were detected in the control and FM3 samples, respectively. The higher hydrophilic properties of 1.25%

flaxseed mucilage coating increases water absorption, which leads to a further decrease in the moisture rate in the FM3 sample.

Table 1. Chemical composition of the cheeses throughout 90 days of storage.

Properties	Treatment	Ripening (Day)		
		30	60	90
pH SEM = 0.01	C	5.50 ^{b}	5.46 ^{c}	5.21 ^{e}
	XG	5.51 ^{b}	5.41 ^{c}	5.29 ^{d}
	FM1	5.61 ^{a}	5.58 ^{a}	5.42 ^{c}
	FM2	5.49 ^{bc}	5.47 ^{c}	5.31 ^{d}
	FM3	5.52 ^{b}	5.45 ^{c}	5.31 ^{d}
Acidity (%) SEM = 0.055	C	0.46 ^{f}	0.97 ^{cd}	1.30 ^{b}
	XG	0.68 ^{dc}	1.00 ^{c}	1.50 ^{a}
	FM1	0.45 ^{f}	0.85 ^{d}	1.05 ^{c}
	FM2	0.78 ^{d}	1.11 ^{bc}	1.21 ^{b}
	FM3	0.58 ^{e}	0.98 ^{cd}	1.27 ^{b}
Moisture (%) SEM = 0.54	C	32.55 ^{d}	33.20 ^{c}	31.61 ^{e}
	XG	35.54 ^{a}	33.22 ^{c}	29.86 ^{g}
	FM1	34.20 ^{b}	32.51 ^{d}	28.93 ^{gh}
	FM2	34.70 ^{ab}	31.69 ^{e}	28.10 ^{h}
	FM3	34.22 ^{b}	30.86 ^{ef}	27.28 ^{i}
FDM (%) (Fat in Dry Matter) SEM = 0.814	C	46.47 ^{g}	52.39 ^{ab}	53.75 ^{a}
	XG	49.45 ^{cd}	49.04 ^{d}	47.75 ^{ef}
	FM1	45.22 ^{h}	44.74 ^{gh}	46.43 ^{g}
	FM2	44.76 ^{gh}	50.66 ^{c}	48.68 ^{de}
	FM3	47.08 ^{f}	48.10 ^{e}	47.09 ^{f}
Protein (%) SEM = 0.755	C	35.11 ^{b}	34.59 ^{bc}	35.35 ^{ab}
	XG	32.62 ^{cd}	34.68 ^{bc}	36.34 ^{a}
	FM1	32.46 ^{cd}	35.07 ^{b}	36.00 ^{a}
	FM2	34.77 ^{bc}	34.89 ^{bc}	36.38 ^{a}
	FM3	32.19 ^{d}	35.09 ^{b}	33.99 ^{c}

Notes: C, control, coated with polyvinyl acetate; XG, coated with 0.5% xanthan gum; FM1, FM2, and FM3 coated with 0.75%, 1%, and 1.25% flaxseed mucilage, respectively. Superscript letters (a–h) beside mean values in columns and rows show the difference in Duncan's multiple range test ($P < 0.01$). SEM: Standard Error Mean.

The fat in dry matter (FDM) values of the cheese samples increased significantly during the ripening period ($P < 0.01$). This may be the result of an increase in the fat content due to the moisture loss caused by the hydrophilic nature of the coating materials, accompanied by a decrease in lipid levels due to lipolysis. All of these changes kept the amount of fat at its initial level [19,37]. In the control sample, the amount of FDM was higher than those coated with edible coatings at the end of ripening ($P < 0.01$). This may result from the substitution of fat by moisture in the protein matrix of cheese because of the hydrophilic nature of the commercial coating, which is higher compared to those of the other edible coatings, which can accordingly result in an increase in the amount of FDM [37] in this sample.

The protein content increased in all samples throughout the ripening period ($P < 0.01$). However, there was no significant difference between the protein content of the cheese samples coated with different bio-materials ($P > 0.01$). It is expected that the nature of the coating materials does not affect the protein composition of the cheese. The results of the present study are consistent with those of Henriques et al. [38].

3.2. Lipolysis

Hydrolysis of milk fat during cheese production and ripening is attributed to the activity of natural lipases of the milk, the lipolytic enzymes of the starter and non-starter lactic acid bacteria, as well as the lipases of the psychrotrophic bacteria [39]. The amounts of free fatty acids (FFA) were

used as indicators of lipolysis in the cheese samples. The results of this study showed that the amount of FFA increased significantly in all specimens during ripening ($P < 0.01$) (Figure 1).

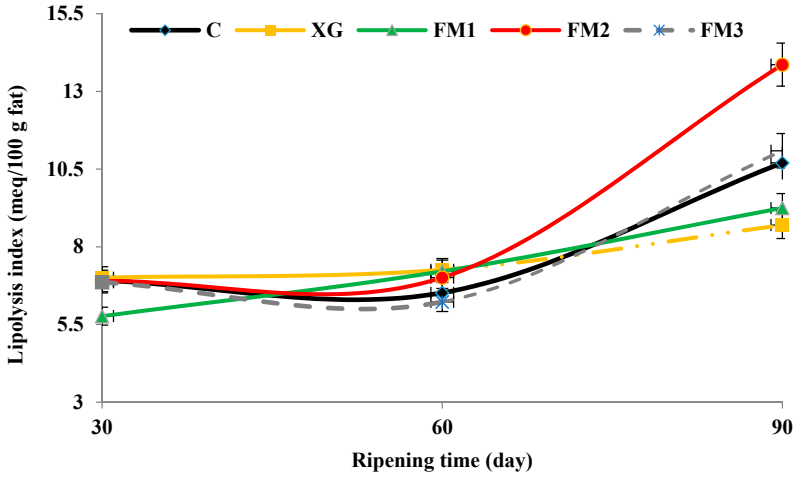

Figure 1. Changes in lipolysis index of coated Cheddar cheeses during ripening. C, control, coated with polyvinyl acetate; XG, coated with 0.5% xanthan gum; FM1, FM2, and FM3 coated with 0.75%, 1%, and 1.25% flaxseed mucilage, respectively. Each dot represents the mean of the experimental data with an error bar of three replications.

These findings are consistent with the results of another study [40]. The highest increase in FFA on day 90 of ripening was observed in the FM2 sample, which may be due to an increment in the lipolytic activity of the starter producer of the exopolysaccharide.

3.3. Proteolysis

Proteolysis rate is an indicator of the ripening degree of the cheese, which reflects the development of the texture, aroma, and flavor of the cheese [41]. The TCA-SN/TN rate showed an increasing trend in the coated cheese during the ripening period ($P < 0.01$) (Figure 2). The results of this study are consistent with those reported by Yilmaz and Dagdemir [34] and EL-Sisi, Mohamed Gapr & Kamaly [42]. The changes in proteolysis rate during ripening were lowest in the control sample. Furthermore, there was a significant difference in the TCA-SN/TN rate between different samples ($P < 0.01$). The lowest and highest levels of TCA-SN/TN were detected in FM1 and FM2 samples, respectively. The increased levels of TCA-SN/TN over the 90 days of ripening can be associated with an increase in the NSLAB population and, consequently, an increase in the protease enzymes, which led to a higher proteolysis rate [43,44].

The amount of tyrosine and tryptophan amino acids decreased in all the specimens during day 30 to 60 of ripening and then showed an increase until the end of the ripening period (Figure 3). In the present study, the highest amounts of tyrosine and tryptophan amino acids, followed by an increased level of proteolysis, were found in the XG sample, which was consistent with the microbial analysis of this specimen and indicated the presence of the largest NSLAB population in the XG sample on day 90 of ripening. An increase in the activity of SB and an increment in the degree of proteolysis due to the activity of these bacteria in XG were seen after 30 days of ripening [45].

The increased levels of tyrosine and tryptophan amino acids were related to decomposition of proteins into amino acids as a result of proteolysis during the ripening period. The highest amounts of tyrosine and tryptophan amino acids were detected in sample XG and the lowest value was measured in the FM1 sample. Lawrence and Gills [45] have concluded that the increased level of accessible water enhanced the activity of microorganisms, enzymes, and proteolysis grade.

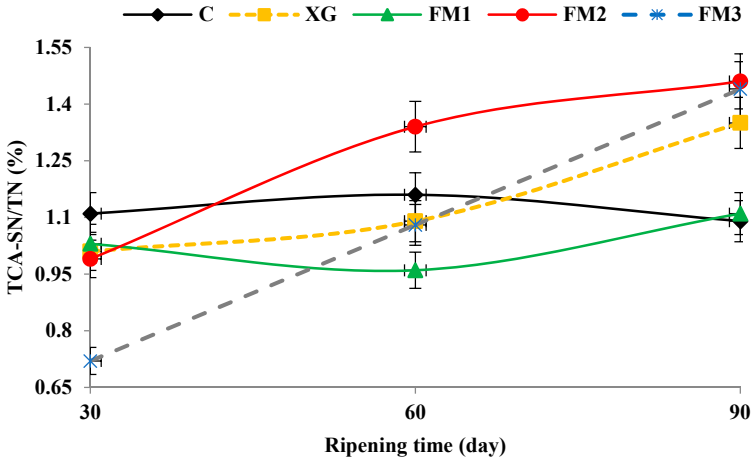

Figure 2. Changes in trichloroacetic acid 12%-soluble nitrogen as a percent of total nitrogen (TCA-SN/TN) of coated Cheddar cheeses during ripening. C, control, coated with polyvinyl acetate; XG, coated with 0.5% xanthan gum; FM1, FM2, and FM3 coated with 0.75%, 1%, and 1.25% flaxseed mucilage, respectively. Each dot represents the mean of the experimental data with an error bar of three replications.

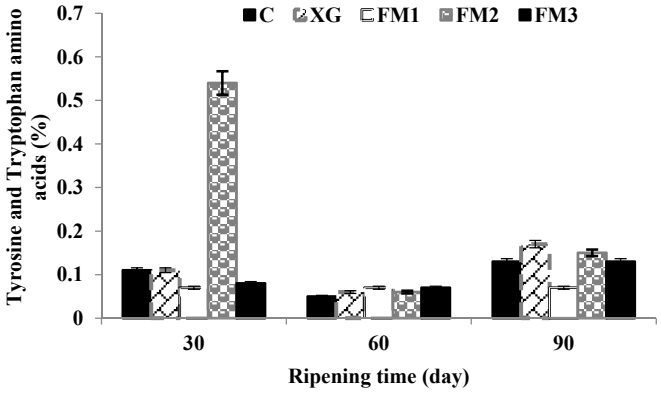

Figure 3. Changes in the amount of free tyrosine and tryptophan amino acids of coated Cheddar cheeses during ripening. C, control, coated with polyvinyl acetate; XG, coated with 0.5% xanthan gum; FM1, FM2, and FM3 coated with 0.75%, 1%, and 1.25% flaxseed mucilage, respectively. Each dot represents the mean of the experimental data with an error bar of three replications.

3.4. Free Fatty Acid Composition

The odor and flavor of cheese are directly affected by the free fatty acids (FFA) released during lipolysis, along with other volatile components and compounds derived from the proteolysis process [46]. In Cheddar cheese, lipase is derived from various sources such as milk, starter, NSLAB, and rennet [47].

The components of FFA in the coated Cheddar cheese are presented in Table 2. A reduction in the C4:0, C6:0, C14:0, C14:1, and C18:0 ($P < 0.01$), C10:0 and C20:0 ($P < 0.05$), and C18:1 and C18:2 ($P > 0.01$) fatty acids was observed during the ripening period. This may be contributed to the hydrolyzation of fatty acids to other compounds, such as ketones, alcohols, lactones, aldehydes, etc. [48].

Table 2. Changes in free fatty acids (mg/100 g) during ripening of cheese trials.

Fatty Acid	Treatment	Ripening Time (Day)	
		1	90
Butyric acid C4:0 SEM = 0.04	C	2.55 ^{a}	2.35 ^{b}
	XG	2.55 ^{a}	1.56 ^{d}
	FM1	2.55 ^{a}	1.88 ^{c}
	FM2	2.55 ^{a}	1.16 ^{e}
	FM3	2.55 ^{a}	1.74 ^{cd}
Caproic acid C6:0 SEM = 0.02	C	2.15 ^{a}	1.94 ^{b}
	XG	2.15 ^{a}	1.51 ^{d}
	FM1	2.15 ^{a}	1.76 ^{c}
	FM2	2.15 ^{a}	1.23 ^{e}
	FM3	2.15 ^{a}	1.44 ^{de}
Caprylic acid C8:0 SEM = 0.01	C	0.19 ^{d}	0.51 ^{c}
	XG	0.19 ^{d}	0.50 ^{c}
	FM1	0.19 ^{d}	0.89 ^{a}
	FM2	0.19 ^{d}	0.60 ^{b}
	FM3	0.19 ^{d}	0.59 ^{b}
Capric acid C10:0 * SEM = 0.07	C	3.30 ^{a}	3.31 ^{a}
	XG	3.30 ^{a}	3.18 ^{ab}
	FM1	3.30 ^{a}	3.05 ^{bc}
	FM2	3.30 ^{a}	2.83 ^{d}
	FM3	3.30 ^{a}	2.87 ^{d}
Lauric acid C12:0 SEM = 0.01	C	0.93 ^{d}	3.70 ^{a}
	XG	0.93 ^{d}	3.73 ^{a}
	FM1	0.93 ^{d}	3.57 ^{b}
	FM2	0.93 ^{d}	3.41 ^{c}
	FM3	0.93 ^{d}	3.37 ^{c}
Myristic acid C14:0 SEM = 0.01	C	35.55 ^{a}	12.15 ^{d}
	XG	35.55 ^{a}	12.35 ^{b}
	FM1	35.55 ^{a}	12.25 ^{c}
	FM2	35.55 ^{a}	11.38 ^{f}
	FM3	35.55 ^{a}	11.82 ^{e}
Myristoleic acid C14:1 SEM = 0.01	C	11.77 ^{a}	1.37 ^{d}
	XG	11.77 ^{a}	1.47 ^{c}
	FM1	11.77 ^{a}	1.26 ^{ef}
	FM2	11.77 ^{a}	1.30 ^{de}
	FM3	11.77 ^{a}	1.55 ^{b}
Palmitic acid C16:0 SEM = 0.01	C	3.65 ^{d}	35.85 ^{c}
	XG	3.65 ^{d}	36.32 ^{b}
	FM1	3.65 ^{d}	36.35 ^{b}
	FM2	3.65 ^{d}	36.65 ^{a}
	FM3	3.65 ^{d}	36.64 ^{a}
Palmitoleic acid C16:1 SEM = 0.01	C	1.15 ^{d}	1.55 ^{c}
	XG	1.15 ^{d}	1.67 ^{b}
	FM1	1.15 ^{d}	1.67 ^{b}
	FM2	1.15 ^{d}	1.72 ^{a}
	FM3	1.15 ^{d}	1.66 ^{b}
Stearic acid C18:0 SEM = 0.01	C	10.06 ^{b}	9.74 ^{c}
	XG	10.06 ^{b}	10.13 ^{a}
	FM1	10.06 ^{b}	9.57 ^{d}
	FM2	10.06 ^{b}	10.16 ^{a}
	FM3	10.06 ^{b}	9.74 ^{c}
Oleic acid C18:1 SEM = 6.471	C	21.04 ^{a}	11.80 ^{b}
	XG	21.04 ^{a}	11.80 ^{b}
	FM1	21.04 ^{a}	11.87 ^{b}
	FM2	21.04 ^{a}	12.73 ^{b}
	FM3	21.04 ^{a}	12.34 ^{b}
Linoleic acid C18:2 SEM = 1.03	C	2.97 ^{a}	1.53 ^{b}
	XG	2.97 ^{a}	1.48 ^{b}
	FM1	2.97 ^{a}	1.56 ^{b}
	FM2	2.97 ^{a}	1.59 ^{b}
	FM3	2.97 ^{a}	1.53 ^{b}
Arashidic acid C20:0 * SEM = 0.01	C	0.12 ^{a}	0.07 ^{b}
	XG	0.12 ^{a}	0.05 ^{b}
	FM1	0.12 ^{a}	0.11 ^{a}
	FM2	0.12 ^{a}	0.05 ^{b}
	FM3	0.12 ^{a}	0.05 ^{b}

Notes: C, control, coated with polyvinyl acetate; XG, coated with 0.5% xanthan gum; FM1, FM2, and FM3 coated with 0.75%, 1%, and 1.25% flaxseed mucilage, respectively. Superscript letters (a,b) beside mean values in columns and rows show the difference in Duncan's multiple range test ($P < 0.01$). * Significance declared at the level ($P < 0.05$). SEM: Standard Error Mean.

The amount of C4:0 fatty acid significantly decreased during ripening ($P < 0.01$), while the amounts of C8:0, C12:0, C16:0, and C16:1 fatty acids significantly increased during this period ($P < 0.01$). The amount of C4:0 fatty acid obtained from cheese was higher than that reported by Katsiari et al. [49] (0.85 mg/100 g). It has been shown that high levels of C4:0 fatty acid in cheese imply selective lipolytic activity [48]. The lowest and highest amounts of C4:0 fatty acid on day 90 of the ripening period were found in the FM2 and control samples, respectively. A smaller amount of C4:0 fatty acid meant a less rancid flavor, which led to higher protection of cheese by 1% flaxseed mucilage coating against fat oxidation [50]. This fatty acid plays an important role in the organoleptic properties of cheese and improves the flavor [51]. The same trend was detected for C6:0 and C10:0 fatty acids, which reduced from 2.15 mg/100 g to 3.30 mg/100 g on the first day of the experiment to 1.57 mg/100 g and 3.04 mg/100 g on day 90, respectively.

The lowest and highest relative levels of both C6:0 and C10:0 fatty acids were observed in FM2 and the control samples, respectively. These findings are in agreement with previous studies reported in herby pickled cheese [26].

The concentrations of C8:0 and C20:0 fatty acids in FM1 cheese were significantly higher than those of the control and other samples coated with edible coatings. In contrast, the concentrations of C8:0 and C20:0 fatty acids of XG cheese were significantly lower than those of the control and other samples coated with edible coatings. The lower levels of C8:0 and C20:0 fatty acids in the XG sample may be due to a lower lipolysis rate in this sample (Figure 1).

Unlike C14:0 and C14:1 fatty acids, the higher concentration of C12:0 after 90 days of ripening is in agreement with results reported by Voigt et al. [52] in Cheddar cheese ($P < 0.01$). The highest levels of C16:0, C16:1, and C18:0 ($P < 0.01$), C18:1 and C18:2 ($P > 0.01$) were detected in the FM2 sample. Therefore, the high level of long-chain fatty acids in FM2 cheese may be related to the lipolysis amount (Figure 1). We found that the highest level of fatty acids belonged to C16:0 and C18:1 fatty acids, in accordance with other studies on hard cheeses [53,54].

3.5. Microbial Analysis

The effect of different coating materials, on the population of the NSLAB, SB, and TMAB bacteria in coated Cheddar cheese during the ripening period is shown in Figure 4a–c.

As illustrated in Figure 4a, the population of the NSLAB after 60 days of ripening was the highest in sample C and the lowest in FM2. This difference may be due to the reduction of oxygen penetration by commercial coating and the reduction of the relative oxygen pressure and, consequently, increased availability of the microaerophilic NSLAB in the control sample [42,55].

The highest increase in NSLAB population at the end of the ripening period was attributed to the samples coated with xanthan gum and the lowest increase was detected in the samples coated with 1% flaxseed mucilage. It is expected that coating, due to limiting the air penetration and the reduction of the relative oxygen pressure inside the cheese, can result in an increase in the survival of microaerophilic NSLAB [42,55,56].

This property was higher in the samples coated with xanthan gum. As shown in Figure 4b, the decrease in the number of SB counts during the ripening period can be due to the autolysis of these bacteria that resulted from the releasing of intracellular enzymes and cellular compounds including nucleic acid and glucose in the cheese matrix.

These compounds increase the survival rate of NSLAB in cheese, in accordance with other reports [57]. The highest and lowest bioactivity of starter bacteria were detected in xanthan gum coating ($\log_{10}$ 7.98 CFU/g) and control ($\log_{10}$ 7.57 CFU/g) samples, respectively ($P < 0.05$).

It seems that a xanthan coating on Cheddar cheese increases the bioactivity rate of lactic acid bacteria (starter and non-starter) in comparison with other coatings. Figure 4c shows that the number of TMAB increased on day 90 compared to day 1 of ripening ($P < 0.01$). The highest number of TMAB was detected in samples coated with xanthan gum ($\log_{10}$ 7.42 CFU/g) at the end of the ripening period

($P > 0.01$). This may be due to the less effective non-permeability of the xanthan gum coating against bacterial growth.

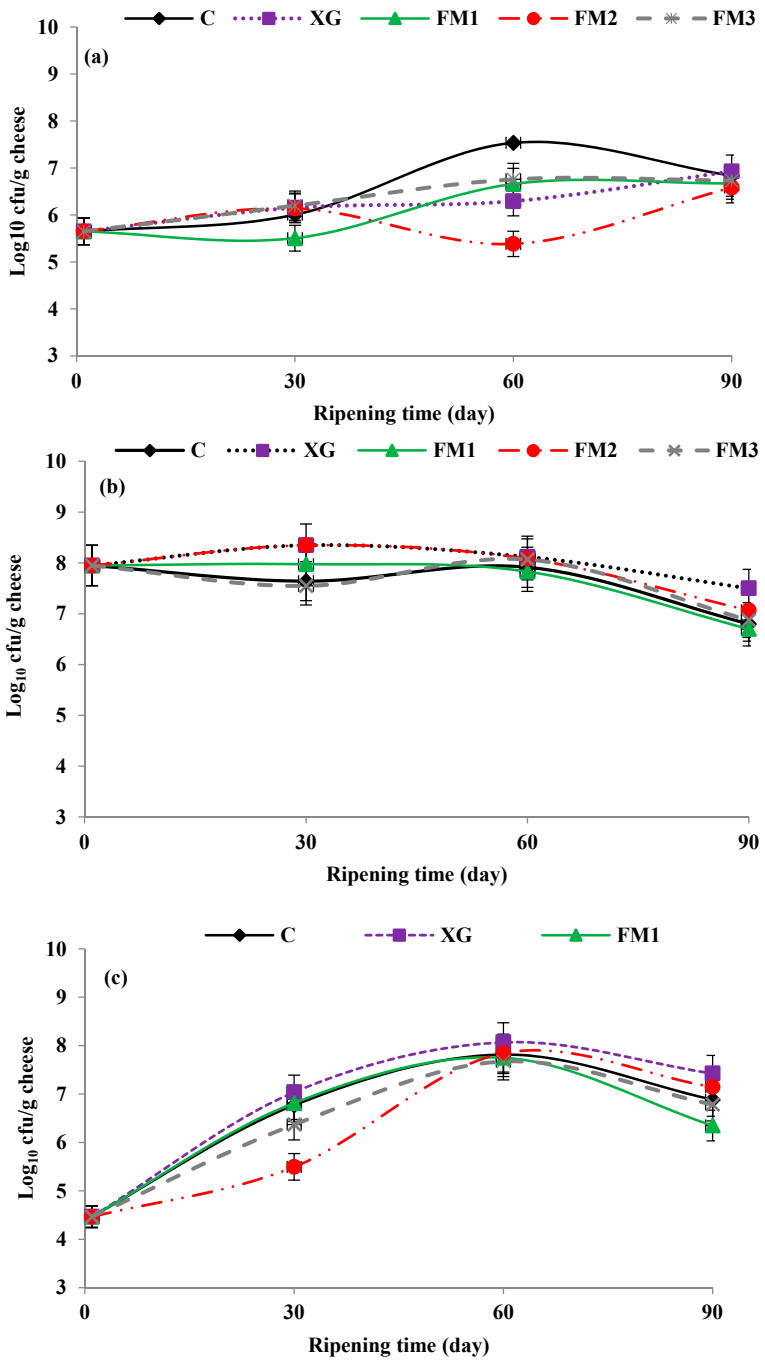

Figure 4. Variation in the counts of (**a**) NSLAB, non-starter lactic acid bacteria; (**b**) SB, starter bacteria; and (**c**) TMAB, total mesophilic aerobic bacteria in the coated Cheddar cheeses throughout 90 days of ripening. C, control, coated with polyvinyl acetate; XG, coated with 0.5% xanthan gum; FM1, FM2, and FM3 coated with 0.75%, 1%, and 1.25% flaxseed mucilage, respectively. Each dot represents the mean of the experimental data with an error bar of three replications.

3.6. Sensory Evaluation

Sensory evaluation was performed to investigate the flavor, texture, color, and cutting of the coated samples (Table 3). The flavor of cheese is directly affected by the free fatty acids (FFA) released

during lipolysis along with other volatile components and compounds derived from the proteolysis process [46]. The results showed no significant differences between the experimental specimens and control in terms of flavor, texture, color, and cutting. These results are concordant with those reported by Cui et al. [58], who reported that coating cheese with chitosan did not significantly affect the sensory properties of the cheese.

Table 3. Sensory scores in Cheddar cheese samples after 90 days of ripening.

Treatments	Flavor	Texture	Color	Cutting
C	3.00 ^{b}	3.33 ^{a}	3.44 ^{a}	3.44 ^{a}
X	3.44 ^{a}	3.44 ^{a}	3.77 ^{a}	3.66 ^{a}
F1	3.11 ^{ab}	3.00 ^{ab}	4.00 ^{a}	2.88 ^{ab}
F2	3.44 ^{a}	3.11 ^{ab}	3.55 ^{a}	3.11 ^{a}
F3	2.88 ^{b}	3.00 ^{ab}	3.22 ^{a}	3.33 ^{a}
SEM	1.04	1.04	0.83	1.22

Notes: C, control, coated with polyvinyl acetate; XG, coated with 0.5% xanthan gum; FM1, FM2, and FM3 coated with 0.75%, 1%, and 1.25% flaxseed mucilage, respectively. Superscript letters (a,b) beside mean values in columns show the difference in Duncan's multiple range test ($P < 0.05$). SEM: Standard Error Mean.

According to the sensory evaluation results, the use of edible coatings not only had no negative effect on the sensory properties of cheese samples, but also some of these coatings (XG, FM1, and FM2) improved the flavor of the cheese compared to the control sample.

Therefore, a coating on Cheddar cheese does not affect the amount of TMAB, which is consistent with the findings of Yilmaz and Dagdemir [33] and Sarioglu and Oner [57] in Kashar cheese.

4. Conclusions

The results showed that coating Cheddar cheese with bio-materials (xanthan gum and flaxseed mucilage) had no significant effects on the growth of TMAB and NSLAB in comparison with polyvinyl acetate, which is used as a commercial coating (control). In contrast, a xanthan gum coating significantly increased the bioactivity of SB. Coating the Cheddar cheese with xanthan gum and flaxseed mucilage showed significant effects on chemical properties such as acidity, pH, FDM, and moisture of cheese; whereas the control sample showed the highest FDM and moisture after 90 days of ripening, the highest pH and acidity rates were observed in the FM1 and XG samples. Although coating had no effect on the protein level of cheese, proteolysis occurred during 90 days of ripening in all samples. Consequently, the highest and lowest amounts of tyrosine and tryptophan amino acids as ripening index were observed in the XG and FM1 samples and soluble nitrogen in TCA was detected in the FM2 and C samples. During the ripening period, the rate of lipolysis increased significantly in different coated cheeses. On the other hand, the type of coating had no significant effect on the rate of lipolysis in different samples. However, on day 90, the highest rate of lipolysis was observed in the FM2 sample, though this was not significant. The levels of C4:0, C6:0, and C10:0 fatty acids were significantly higher in the control sample than in other samples at the end of ripening. Moreover, the lowest amounts of C14:1 and C18:0 and the highest amounts of C20:0 and C8:0 fatty acids were detected in the FM1 sample. The results showed the highest amounts of C12:0 and C14:0 fatty acids belonged to the XG sample. The highest amounts of C16:0, C16:1, C18:0, C18:1, and C18:2 fatty acids were observed in the FM2 sample. Sensory evaluation revealed that coated cheeses with edible and commercial coatings have no significant effect on sensory properties of Cheddar cheese such as flavor, texture, color, and cutting. However, the XG sample received the highest scores for flavor, texture, and cutting. The highest color score belonged to the FM1 sample with a coating of flaxseed mucilage (0.75%). A 1.25% flaxseed mucilage coating on the FM3 sample had an adverse effect on the color score.

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