International Journal of
Dairy Science

ISSN 1811-9743

Academic
Journals Inc.

www.academicjournals.com

OPEN ACCESS International Journal of Dairy Science

ISSN 1811-9743
DOI: 10.3923/ijds.2024.46.56

Research Article
Producing Egyptian Hard Cheese as an Alternative to Cheddar in Processed Cheese

Enas Shehata, Morsi El Soda, Mohsen Nawar and Sameh Awad

Department of Dairy Science and Technology, Faculty of Agriculture, Alexandria University, Egypt

Abstract

Background and Objective: Cheddar cheese is an essential ingredient used in processed cheese manufacturing in Egypt. About 15 to 20% of aged imported Cheddar is used in processed cheese making, while Ras cheese is the main traditional hard cheese in Egypt. Ras cheese is not used in the making of processed cheese due to several problems, such as a high salt content, contamination with high levels of molds, yeasts and a hard rind. This research aimed to produce a new natural hard cheese as a cheese base for processed cheese making to replace the Cheddar cheese. **Materials and Methods:** The new hard cheese trials were made using pasteurized milk and two cultures (mesophilic or thermophilic), reducing the addition of salt without the cheddaring step. A protective culture against molds and yeasts was used and the cheese was packaged under vacuum. The obtained cheese samples were analyzed for physicochemical properties, hardness, microbiology, free amino acids and sensory evaluation during ripening. The obtained data were subjected to statistical analysis. **Results:** The aged new hard cheese had good physicochemical and microbiological properties compared to commercial Ras and the new hard cheese received a high level of acceptability from panelists. The cheese made with thermophilic culture received high scores compared with cheese made with mesophilic culture after 3 months of ripening. **Conclusion:** The new hard cheese could be used as an alternative to imported Cheddar, especially in processed cheese manufacturing. This would reduce the importing of raw materials and production costs, as well as improve Egyptian products to pass and meet international standards.

Key words: Cheddar cheese, Ras cheese, cheese making, low-salt cheese, protective culture, package under vacuum, exopolysaccharide

Citation: Shehata, E., M. El Soda, M. Nawar and S. Awad, 2024. Producing Egyptian hard cheese as an alternative to Cheddar in processed cheese. Int. J. Dairy Sci., 19: 46-56.

Corresponding Author: Sameh Awad, Department of Dairy Science and Technology, Faculty of Agriculture, Alexandria University, Egypt

Competing Interest: The authors have declared that no competing interest exists.

Data Availability: All relevant data are within the paper and its supporting information files.

INTRODUCTION

Egyptian dairy industries need local hard cheese of good quality to replace Cheddar and Ras in making processed cheese. Natural cheeses are combined with other ingredients, such as emulsifying salts, to produce processed cheese. The texture, functional, microstructural and sensory features of processed cheese are largely controlled by the kind of cheese characteristics and age of the natural cheese, the type and quantity of emulsifying salt and manufacturing conditions[1].

Cheddar is a key ingredient in processed cheese made in Egypt and is expensively purchased in "foreign currency". Its local alternative is Ras cheese, the main traditional hard cheese in Egypt, but Ras cheese had some serious problems that prevented it from being used in processed cheese, such as: (1) Mold at the cheese surface, which led to a decrease in cheese yield, a decrease in cheese consumer appeal and increased the health risks, (2) Hard rind, (3) High salt content, (4) Presence of aflatoxin[2] and (5) Presence of anaerobic bacteria *Clostridium* spp.[3]. Besides, most Ras cheese is produced on a small scale without stability to specifications.

Many studies have modified the technological criteria of low-fat Cheddar to improve cheese characteristics, such as reducing cooking time and temperature, using a high pH range at milling and washing cheese curd with cold water[4]. The properties of reduced-fat Cheddar were enhanced by EPS-producing lactic cultures[4]. Reduced-fat processed cheese's flavor and texture are further enhanced by these changes in the functional and textural characteristics of young cheeses produced using EPS-producing cultures while combined with enzyme-modified cheese (EMC)[5].

Bacteriocins (LAB) have gained attention as a component of hurdle technology to meet customer demands for naturally preserved foods[6]. *Propionibacterium freudenreichii* subsp. *shermanii* JS and *Lactobacillus rhamnosus* LC705 or *Lactobacillus paracasei* SM20 were the two LAB strains used as antifungals, which are sold under the brand name HoldbacTM[7]. Testing of these cultures on the surface of hard cheese and in yogurt to hinder yeasts and molds from spoiling has proven beneficial[7]. The applications of protective cultures for controlling undesirable microorganisms were reviewed by Bintsis and Papademas[8].

So, the new technology should be recommended for Egyptian-modified hard cheese production. This includes inhibiting the fungus on the cheese surface using protective culture, reducing the cheese salt, applying EPS-producing culture to enhance the cheese texture and packaging under vacuum. So, this study aimed to produce a new hard cheese as an alternative to imported Cheddar, especially in processed cheese manufacturing. The effect of using EPS-producing and protective cultures on chemical, microbiological and sensory characteristics was investigated in this research.

MATERIALS AND METHODS

Study area: The study was carried out at the Laboratory of Dairy Microorganisms and Cheese Research, Department of Dairy Science and Technology, Faculty of Agriculture, Alexandria University, Egypt from October, 2022 to June, 2023.

Milk and cultures: Raw cow's milk (60 kg for each treatment; fat 3.4%, total salts 12.25%, acidity of 0.16%, pH of 6.73) was obtained from the Faculty of Agriculture dairy unit at Alexandria University. The freeze-dried yogurt starter culture (DVS YF-L 904, thermophilic YoFlex®, extra-high viscosity culture from Christian Hansen Lab, Denmark) is composed of *Lactobacillus delbrueckii* subsp. *bulgaricus* and *Streptococcus thermophilus*. Besides using mesophilic homofermentative lactic cultures (DVS, MO-30, Chr. Hansen Lab), it consisted of *Lactococcus lactis* ssp. *cremoris* and *lactis* for Cheddar. In addition to using a protective culture consisting of *Lactobacillus rhamnosus*, which produces bacteriocins that inhibit unwanted bacteria, yeasts and molds (Gruppo, Clerici-Sacco).

Cheese making: New hard cheeses were manufactured in duplicate from pasteurized cow's milk (heated to 72°C for 15 sec and cooled to 31°C). Cheese milk (45 kg) was assigned using computer-controlled cheese equipment (INRA, Poligny, France), equipped with four 11 L vats. The following cheese treatments were used: (1) ThermoN: New hard cheese made with EPS-producing thermophilic starter cultures (DVS YF-L 904, WI; 0.02% wt/wt), (2) MesN: New hard cheese made with mesophilic cultures (DVS MO-30, WI; 0.02% wt/wt) and a protective culture consisting of *Lactobacillus rhamnosus* (Lyofast LRB, SACCO and Italy). Cultures were added to milk (31°C), which was ripened for 1-2 hrs until the pH dropped by about 0.1. Calcium chloride (0.02%) was then added. To coagulate milk in 30 min, a commercial rennet was added. The coagulum was cut within 30 min, cooked to 39-40°C (at a rate of 1°C per 5 min) and held at this temperature for 45 min. Most of the whey was drained when the pH reached 6.2. Curd was salted (commercially pure, fine-food-grade salt) at 5% in three equal

applications over 15 min. The cheese was shaped and pressed into 1 kg circular blocks. Before packaging, the moisture was determined to avoid the pasty texture of the cheese blocks. While the moisture was below 41% (it takes about 7-10 days), the blocks were brushed with a natamycin solution at 1% (w/v). Polyethylene-polyamide bags were used in packaging under vacuum and stored at 8-10°C with a relative humidity of about 80±5% for up to 6 months until used in processed cheese making. The cheese yield was calculated as the mass ratio between the curds obtained after the pressing stage and the weight of the milk. Cheeses were sampled in duplicate, fresh and at 1, 3 and 6 months of the ripening period for some physicochemical, hardness, microbiological and sensory characteristics.

Commercial mild-ripened Ras cheese: Good-quality one-month-old Ras cheese, with an average chemical composition of 64.8% DM, 32.3% fat and pH 5.9 was obtained from the local market in Alexandria, Egypt. The commercial Ras cheese is stored for ripening for up to 6 months as a control for traditional Ras cheeses.

Chemical composition of new hard cheese: Total protein was measured by the micro-Kjeldahl according to AOAC[9] and fat content by the Gerber method according to AOAC[9]. The salt content was determined according to the direct method[9], while fat in dry weight (FDM%) was calculated. The titratable acidity and the moisture content were also determined[9]. The pH was measured using a glass electrode (Adwa model AD 1030). The mass ratio of the curd formed after the pressing step to the weight of milk was used to express the cheese yield.

Hardness: Objective tests for hard cheese samples were carried out using a Texture Analyzer (TA1000, CNS-Farnell, England) with the TA 17 conical probe (30-25 mm diameter) at a speed of 0.5 mL/sec and a distance of 10 mm in cheese cubes (20×20×20 mm). The hardness is measured (1 g) by the value of the peak force of the initial compression (one bite). The analyses were carried out 3 times.

Proteolysis assessments

Water-soluble nitrogen: Water-soluble nitrogen (WSN) was extracted by milling 10 g of mixed cheese samples and 100 mL of distilled water at 40°C was added. The mixtures were shaken for 2 hrs and centrifuged at 15000 g for 30 min (CM-6MT ELMI, Skyline centrifuge). The 100 mL of supernatant (WSN) was adjusted to pH 4.6 and the solution was made up to 125 mL with distilled water and centrifuged at 15.000 g for 30 min before filtering. The supernatant was used to measure the soluble nitrogen at pH 4.6 by the Kjeldahl method according to AOAC[9].

Free amino acid profiles: The profile of free amino acids in the extract of the new hard cheeses was separated using the Sykam system 7000 Amino Acid Analyzer, Gewerbering, Eresing, Germany (Na high-performance 25 cm column). Free amino acids were extracted by the method of Lemieux *et al.*[10], as follows: 1 g of each sample was extracted by boiling under reflux with 80 mL of 50% ethanol 3 times (each time for 3 hrs). The combined ethanolic solutions were filtrated and treated with a trichloroacetic acid solution (10%) for clarification. Under lower pressure, the supernatant fluid was concentrated to 5 mL. Distilled water was used for washing the residue. The volume of the filtrate was adjusted to 100 mL using distilled water. Following a vacuum-dried process at 70°C, 5 mL of the diluted sample were dried under vacuum at 70°C and then dissolved in 5 mL of loading buffer (0.2 N sodium citrate buffer, pH 2). The sample was filtered through a 0.45 micropore filter and injected at a volume of 50 µL into the amino acid analyzer. The flow rate was 1 mL/min. The visible light detector (Sykam, Gewerbering, Eresing, Germany) was used to monitor the analyses. Retention time and separated area were obtained using the Helwett Packard 3390 recording integrator and expressed as mg/g.

Sensory evaluation: Cheese samples were prepared by cutting cubes and coding the samples using random numbers. A group of nine cheese graders, comprising employees, cheese producers and cheese consumers, performed the sensory analysis. The nine-point hedonic scale, which ranges from 1 (indicating extreme dislike) to 9 (indicating extreme like), was used to evaluate the samples. Cheeses were evaluated after manufacturing at 1, 3 and 6 months of age, assessing appearance, flavor, odor and texture.

Microbiological analyses of cheese: Cheese samples (10 g) were homogenized in a laboratory blender Stomacher (Seward 400 London, UK) with 90 mL of sterile 2% sodium citrate solution. The samples were then serially diluted using a sterile saline solution. Serial dilutions were plated on violet-red bile Agar (VRBA; Biolife, Italy) to enumerate presumptive coliform colonies according to Awad[11]. The cheese slurry was analyzed for its counts of lactic acid bacteria (LAB) using de Man Rogosa Sharpe Agar medium (MRS) as

described by Ghoddusi and Robinson[12] at 37°C for 48 hrs. The Oxytetracycline Glucose Yeast Extract Agar (O.G.Y.E. agar, LAB, United Kingdom) was used for the enumeration of yeasts and molds[13]. The plates were incubated at 25°C for 5 days. The procedure of a bacterial count on Sulfite Cycloserine Agar (SC, Biolife, Italy) to detect *Clostridium perfringens*[14]. The plates were incubated under anaerobic conditions at 37°C for 20 hrs. The presumed colonies were black. In general, results were calculated as a log of the mean colony-forming unit per gram (CFU/gm) of the three cheeses under study. Experimental hard cheese was analyzed after 1 week and after 1, 3 and 6 months of ripening periods.

Statistical analyses: Data were fed to the computer and analyzed using IBM SPSS software package version 20.0 (Armonk, NY: IBM Corp.). Quantitative data were described using the mean and standard deviation. The significance of the obtained results was judged at the 5% level. The tests used were the One-way Analysis of Variance (ANOVA) test for normally distributed quantitative variables to compare between more than 2 groups and the *post hoc* test (LSD) for pairwise comparisons.

RESULTS AND DISCUSSION

Cheese yield: The average cheese yield of ThermoN was 10.95%. While the average cheese yield of MesN was 10.65%, El Soda[15] found the yield of full-fat Cheddar to be 10.20%. The higher yield in this study may be attributable to the modification in manufacture due to the no cheddaring step. Exopolysaccharide-producing strains improved Cheddar yield by 0.28 to 1.19/100 kg as a result of increased moisture retention of 3.60 to 4.80% when compared to control cheese[4]. The presence of EPS was shown to significantly raise moisture levels[16]; this was thought to be the consequence of the EPS's ability to retain water through hydrogen bonding.

Physicochemical and hardness results: The cultures used in this study had some impact on the gross chemical composition (moisture, acidity, pH, fat, salt and total protein) of new hard cheeses and, consequently, the hardness values as shown in Table 1. The moisture mean values of fresh ThermoN were greater than those of MesN; the moisture was 41.22 and 39.97% in ThermoN and MesN, respectively. In general, Ras cheese had the lowest moisture content at any ripening period. The differences in moisture were obvious ($p \leq 0.05$) between ThermoN and MesN, except for 1 month of ripening. In agreement with this, the EPS-producing culture increased the moisture of Cheddar cheese. The increased water-holding ability of exopolysaccharides is thought to be the cause of ThermoN cheese's elevated moisture content, in addition to an increase in yield compared with MesN cheese. It is well known that an increase in moisture elevates the activity of LAB. A gradual decrease in moisture content was detected during the ripening period.

The pH values exhibited a reverse trend towards titratable acidity (TA) along the ripening period. The pH of all cheeses decreased as the cheese aged and the titratable acidity value increased gradually as well. Compared to MesN, during the rest of the ripening period, the pH of ThermoN cheese was significantly ($p \leq 0.05$) higher than that of MesN throughout the ripening period, but it remained similar at 5.19 and 5.15, respectively, after 3 and 6 months of ripening. It was observed that there are no differences in pH among cheeses with EPS and non-EPS hard cheeses at the same age of 1 day or 1 month[17].

The acidity percentages increase in all types of cheeses as the duration of curing is prolonged. Since all ThermoN with added lactobacilli exhibited significantly ($p \leq 0.05$) higher acidity than the other cheeses, the acidities of ThermoN were 0.89, 1.38 and 1.54% after 1, 3 and 6 months of ripening, respectively. This result could be explained by the continued production of lactic acid by live lactobacilli cells that could survive a much longer period in cheese than lactococci[8,18]. Dabour *et al.*[19] observed the same behaviour, which they attributed to the higher moisture content or the acidification capacity of this EPS-producing strain. This increases lactose retention and bacterial activity, which in turn causes more acid accumulation in cheese[4]. According to Zhang *et al.*[20], the bacterial cells exhibited a high degree of salt tolerance due to the protective impact of EPS, which decreased the pH further during the ripening process. Besides, the effect of soluble and nitrogenous compounds present in cheese slurry on the growth of LAB. The difference in the change in acidity from pH is due to the formation of alkaline-nitrogenous compounds; besides, deamination produces ammonia during protein breakdown[21], as discussed below in Table 2-4.

The fat content in all the studied cheeses increased with the cheese's ripening. In general, ratios of fat and FDM% were found to be significantly ($p \leq 0.05$) higher in ThermoN than MesN cheese during ripening (Table 1). The fat in the dry matter percentage were 51.21% after 1 week of manufacture and 50.69, 50.66 and 51.16% during the 1, 3 and 6 months of ripening, respectively, in ThermoN samples.

Table 1: Physicochemical characteristics and hardness of hard cheese samples during ripening

Parameter	Ripening period	ThermoN (n = 6)	MesN (n = 6)	Ras (n = 3)
Moisture (%)	1 week	41.22^{aA}	39.97^{aB}	N.D
	1 month	38.94^{bA}	38.65^{bA}	35.24^{aB}
	3 months	37.87^{cA}	36.17^{cB}	31.92^{bC}
	6 months	35.54^{dA}	34.49^{dB}	26.92^{cC}
pH	1 week	5.46^{aA}	5.21^{aB}	N.D
	1 month	5.26^{bB}	5.09^{bC}	5.90^{aA}
	3 months	5.19^{cB}	5.07^{bC}	5.30^{bA}
	6 months	5.15^{cB}	4.92^{cC}	5.31^{bA}
Acidity (%)	1 week	0.53^{dB}	0.57^{dA}	N.D
	1 month	0.89^{cA}	0.72^{cB}	0.28^{cC}
	3 months	1.38^{bA}	0.76^{bC}	1.05^{bB}
	6 months	1.54^{aA}	1.07^{aC}	1.11^{aB}
Salt (%)	1 week	1.42^{cB}	1.71^{dA}	N.D
	1 month	1.60^{bC}	1.86^{cB}	2.56^{cA}
	3 months	1.63^{bC}	1.95^{bB}	2.96^{bA}
	6 months	1.82^{aC}	2.16^{aB}	3.72^{aA}
Salt/moisture (SM%)	1 week	3.43^{dB}	4.27^{dA}	N.D
	1 month	4.10^{cC}	4.81^{cB}	7.26^{cA}
	3 months	4.31^{bC}	5.39^{bB}	9.26^{bA}
	6 months	5.11^{aC}	6.27^{aB}	13.82^{aA}
Fat (%)	1 week	30.10^{cA}	26.53^{cB}	N.D
	1 month	30.95^{bB}	27.40^{cC}	32.25^{cA}
	3 months	31.48^{bB}	28.75^{bC}	33.25^{bA}
	6 months	32.98^{aB}	30.35^{aC}	39.75^{aA}
Dry matter (DM%)	1 week	58.78^{dB}	60.03^{dA}	N.D
	1 month	61.06^{cB}	61.35^{cB}	64.76^{cA}
	3 months	62.13^{bC}	63.84^{bB}	68.09^{bA}
	6 months	64.46^{aC}	65.52^{aB}	73.08 ± 0.08^{aA}
Fat/dry matter (FDM %)	1 week	51.21^{aA}	44.18^{bB}	N.D
	1 month	50.69^{aA}	44.66^{bB}	49.80^{bA}
	3 months	50.66^{aA}	45.04^{bC}	48.84^{bB}
	6 months	51.16^{aB}	46.33^{aC}	54.39^{aA}
Protein (%)	1 week	21.55^{dB}	22.73^{dA}	N.D
	1 month	22.31^{cB}	23.43^{cA}	23.76^{bA}
	3 months	23.41^{bC}	24.04^{bB}	24.50^{bA}
	6 months	24.01^{aC}	24.56^{aB}	27.69^{aA}
Protein /DM (%)	1 week	36.65^{bB}	37.86^{aA}	N.D
	1 month	36.54^{bB}	38.19^{aA}	36.69^{bB}
	3 months	37.67^{aA}	37.66^{aA}	35.98^{bB}
	6 months	37.25^{abA}	37.49^{aA}	37.88^{aA}
Hardness (g)	1 week	180.0^{dB}	191.2^{dA}	N.D
	1 month	234.8^{cC}	450.5^{cB}	734.7^{cA}
	3 months	327.0^{bC}	537.0^{bB}	830.0^{bA}
	6 months	418.5^{aC}	837.3^{aB}	1035.0^{aA}

Data was expressed using Mean±SD, SD: Standard deviation and N.D: Not determined, Means within the same column with no common superscript, $^{a-d}$Differ ($p \leq 0.05$) for comparing ripening time, means within the same raw with no common capital superscript and $^{A-C}$Differ ($p \leq 0.05$) for comparing studied cheeses

Table 2: Soluble nitrogen percentage of hard cheese samples during ripening

Ripening time	ThermoN	MesN	Ras
1 week	0.52^{cA}	0.27^{cB}	N.D
1 month	0.82^{bA}	0.30^{cB}	0.20^{bC}
3 months	0.84^{bB}	0.96^{aA}	0.43^{aC}
6 months	1.02^{aA}	0.83^{bB}	0.11^{cC}

Data was expressed using Mean±SD for 6 replicas for each cheese type, ND: Not determined, Means within the same column with no common superscript, $^{a-d}$Differ ($p \leq 0.05$) for comparing ripening time, means within the same raw with no common capital superscript and $^{A-C}$Differ ($p \leq 0.05$) for comparing studied cheeses

Variations in the moisture and fat contents were presumably attributed to EPS production. To the best of our knowledge, very little research on adding EPS culture has been done with full-fat cheese. As the ropy strain *Streptococcus thermophilus* was added to Mexican Manchego-type cheese[22] a dense network was formed, which allowed the product to retain

Table 3: Soluble nitrogen/total nitrogen percentage of hard cheese samples during ripening

Ripening time	ThermoN	MesN	Ras
1 week	14.83^{cA}	8.00^{cB}	N.D
1 month	23.50^{bA}	8.17^{cB}	5.0^{bC}
3 months	23.50^{bB}	25.00^{aA}	10.03^{aC}
6 months	26.50^{aA}	21.00^{bB}	3.0^{cC}

Data was expressed using Mean±SD for 6 replicas for each cheese type, N.D: Not determined, Means within the same column with no common superscript, $^{a-d}$Differ ($p\leq0.05$) for comparing ripening time, means within the same raw with no common capital superscript and $^{A-C}$Differ ($p\leq0.05$) for comparing studied cheeses

Table 4: Free amino acid composition (mg/g protein) in hard cheese samples during ripening

	1 month		3 months		6 months	
Ripening periods/amino acids	ThermoN	MesN	ThermoN	MesN	ThermoN	MesN
Aspartic acid	0.2	0.19	0.25	0.28	0.54	0.13
Threonine	0.11	0.27	0.15	0.44	0.43	0.22
Serine	0.3	0.03	0.65	0.04	1.01	0.03
Glutamic acid	0.58	0.03	1.19	0.06	0.77	0.01
Proline	0.62	0.08	0.37	0.08	0.85	0.09
Glycine	0.09	0.03	0.16	0.06	0.27	0.03
Alanine	0.05	0.11	0.18	0.04	0.40	0.24
Cystine	0.03	0.06	0.04	0.08	1.00	0.09
Valine	0.38	0.15	0.66	0.17	1.56	0.13
Methionine	0.15	0.02	0.23	0.11	0.66	0.02
Isoleucine	0.07	0.02	0.15	0.03	0.44	0.02
Leucine	0.67	0.44	1.37	0.63	2.45	0.38
Tyrosine	0.04	0.06	0.04	0.06	0.11	0.04
Phenylalanine	0.60	0.24	1.14	0.31	1.60	0.16
Histidine	0.75	0.30	0.31	0.21	0.74	0.21
Lysine	0.28	0.04	0.19	0.06	0.44	0.02
Ammonia	1.00	0.51	0.71	0.78	1.13	0.57
Arginine	0.11	0.00	0.00	0.00	0.00	0.00

more water and fat and had a more open structure, resulting in it being softer. It could be demonstrated that the rise in viscosity during milk fermentation is due to the matrix formed by caseins and EPS[1,23], this matrix could keep more water and fat, as the cheese's chemical study showed in Table 1. The opposite was observed in the fat content of Cheddar made with EPS-producing or EPS-non-producing cultures[4,19]. It is well established that decreasing the moisture content and increasing the salt level until the end of the ripening period of Ras cheese were also reported in other study by Awad[24], as were observed in all types of studied cheeses. The significant differences ($p\leq0.05$) in salt and salt in the moisture percentage were relatively lower in ThermoN cheese than in MesN cheese due to the variations in moisture. Ras cheese has a greater salt content than other cheeses, which reached 2.96 and 3.72% after 3 and 6 months of ripening, respectively. Additionally, SM% rose to 9.26 and 13.82%, respectively; these findings are close to full-fat Ras cheese[24].

Changes in the total protein content of Ras and fresh cheeses made with specific strains were presented in Table 1, which increased throughout the ripening period. The protein content of new hard cheeses ranged from 21.55 to 24.56%. But ThermoN cheese with EPS-producing strains generally contained a lower ($p\leq0.05$) protein content than MesN, so it could be understood that the water and fat contents are higher in the EPS-producing cheese, resulting in a decrease in the total protein content 4.19. As a consequence, the protein in the dry matter content was lower in ThermoN than MesN cheese in the 1st month of curing, whereas no variation was detected after 3 and 6 months of curing between the two new hard cheeses.

The hardness of all treatments and Ras cheese increased widely as the ripening period progressed, reaching maximum values at the end of the ripening period (Table 1), depending on the moisture loss. The values of hardness in the new treatments were 418.50 and 837.30 g after 6 months of ripening and the hardness of Ras increased very widely to 1035.00 g. This finding fits with research that looked at the microstructure of full-fat cheese and found that as the cheese aged, less expressible serum caused fat and protein to interact more[4]. As well as FDM%, moisture in non-fat substances (M/NFS), pH and SM%, were considered important factors responsible for cheese texture. It is an interesting observation that ThermoN cheese was found to have the lowest values of hardness along the ripening period, followed by MesN cheese

and Ras, in descending order except for the fresh ones. Adding microorganisms that produce EPS resulted in a decrease in hardness as a result of the increase in moisture content[4]. These elements might have been the main cause of a softer cheese because protein-water adsorption lessens the hydrophobic interactions that are essential for preserving the casein matrix's structure[25]. The hardness of Ras was widely higher than the hardness of new cheeses along the cheese's ripening and this result matched with the results of Ayad *et al.*[26], who found that Ras cheese, whether mild or mature, had higher firmness and salt/moisture content (SM%) and was also lower in moisture content than imported cheese varieties such as Cheddar.

Proteolysis analysis

Water-soluble nitrogen: The water-soluble nitrogen (pH4.6-SN) was determined after 1 week and at three ripening periods (1, 3 and 6 months) in all types of the studied cheeses (Table 2-3). The WSN signifies proteins (excluding most casein), all peptides, amino acids and smaller N compounds such as amines, urea and ammonium. The WSN% and WSN%TN had a significant ($p \leq 0.05$) variation between Ras and the new hard cheeses (ThermoN and MesN). In all cheeses, WSN% and WSN%TN values increased gradually ($p \leq 0.05$) during ripening until the 3rd month; at 6 months of ripening, the WSN of ThermoN increased significantly, but the WSN of MesN and Ras decreased; the reduction could be related to the reduction of free amino acids after 6 months of MesN ripening, as observed in Table 4. It is well known that the levels of WSN percentage increased in cheese during ripening[24]. Except for the 3rd month of ripening, the highest WSN% and WSN%TN values were found in ThermoN; this may be explained by the effect of EPS and the higher levels of proteolysis, as opposed to the lowest WSN% and WSN%TN values in commercial Ras. These results resemble those observed by Awad *et al.*[4], who found that the high WSN in cheese manufactured by the ropy strain *Lactococcus lactis* ssp. *cremoris* JFR1 was associated with high moisture and residual coagulant activity levels (RCA), which induce proteolysis.

Comparison of free amino acid content (FAA) of hard cheese samples during ripening: Table 4 demonstrated the difference between ThermoN and MesN cheeses at 1, 3 and 6 months of ripening. As precursors to the production of cheese flavour components, free amino acids and free fatty acids are known to contribute to cheese flavour either directly or indirectly. It was noted a notable correlation between the FAA content and the ripening time[4,24]. It was found that the concentrations of FAA tend to rise with the ripening time, as expected, because during proteolysis, these compounds are released by the proteolytic agents, mainly by microbial enzymes.

Table 4 shows the analysis of the free amino acids of new hard cheese produced by thermophilic cultures after storage periods of 1, 3 and 6 months. Histidine, leucine and proline were found after 1 month of ripening in high concentrations (0.75, 0.67 and 0.62 mg/g, respectively), as well as leucine, glutamic acid and phenylalanine (in that descending order) as follows: 1.37, 1.19 and 1.14 mg/g, respectively, after 3 months of ripening. After 6 months of ripening, the main free amino acids were leucine, phenylalanine, valine, serine and cystine (in descending order)and their concentration increased to above 1.00 mg/g, while ammonia concentration increased by ripening for 6 months to 1.13 mg/g. Most free amino acids reached their maximum concentration after 6 months of ripening, except glutamic acid, which decreased after reaching its maximum after 3 months of ripening as a result of secondary decomposition, which occurs in the later stage of cheese maturation.

Table 4 shows that seventeen free amino acids have been demonstrated in 1-, 3- and 6-month-old cheese and arginine was absent in all MesN results. In the early ripening period, the main free amino acids were leucine, histidine, threonine and phenylalanine (in descending order) and were present within the ranges of 0.24 to 0.44 mg/g. All remaining free amino acids were present at low levels (lower than 0.20 mg/g). Whereas the main free amino acids at values between 0.63 and 0.21 mg/g after 3 months of maturing were leucine, threonine, phenylalanine, aspartic acid and histidine, in descending order. Generally, after 6 months of ripening, all free amino acids and ammonia decreased except for a very slight increase in proline and cysteine, while the alanine content increased greatly from 0.04 to 0.24 mg/g. Table 4 summarizes the variation in amino acid concentration as follows: Within the first 3 months of ripening, threonine, leucine, phenylalanine and histidine were generally in higher proportions during the ripening period, along with aspartic acid after 3 months and alanine after 6 months of ripening.

Amino acids in cheese appear to be catabolized by one of two major pathways[27], initiated by the action of an aminotransferase or a lyase, although other catabolic pathways (e.g., deamination or decarboxylation) also occur and produce many flavor compounds. These results explain the shift between pH and acidity caused by the process of breaking down FAA to produce NH_3. Secondary

decomposition takes place during the later stages of cheese ripening, so most free amino acids decrease after reaching their maximum, giving 6-month-old MesN a higher score on flavour tests in comparison to ThermoN 6-mo. Also, Bove *et al.*[28] found that the strains of *L. rhamnosus* isolated during late cheese ripening showed an increased capacity for FAA use. This could explain the sensory results, which showed the best flavor in 6-month-old MesN cheese, as well as the decrease in FAA after 6 months of ripening.

The findings indicate that this EPS-producing culture may have the potential to be a useful culture, increasing the moisture retention of cheese during the early ripening period without significantly affecting the characteristic flavor and aroma properties of new hard cheese until 3 months of ripening. The highest values of WSN% and FAA were found in cheeses containing EPS-producing cultures after 6 months.

Concerning ThermoN cheese, when comparing, the water-soluble nitrogen (%) (pH 4.6-SN) and the total FAA (mg/g protein) had the same trend during different ripening periods of 1, 3 and 6 months. It was found that the WSN and total FAA were 0.82% and 6.03 mg/g at the 1st month of ripening, then increased slightly to 0.84% and 7.79 mg/g at the 3rd month of ripening and then increased widely to 1.02% and 14.40 mg/g at the 6th month of ripening, respectively. Additionally, in MesN cheese, it was found that the water-soluble nitrogen (%) (pH4.6-SN) and the total FAA (mg/g) had similar patterns during ripening periods of 1, 3 and 6 months. The WSN and total FAA were at 0.30% and 2.58 mg/g at the 1st month; these values elevated to 0.96% and 3.44 mg/g at the 3rd month of ripening; these values then decreased significantly to 0.83% and 2.39 mg/g at the end of the ripening period.

When compared to MesN, ThermoN had the highest levels of total FAA (mg/g) and water-soluble nitrogen (%) after 6 months of ripening. These results matched those of Hassan *et al.*[23], who reported that thermophilic starter cheeses have higher FAA concentrations and proteolysis levels. Additionally, this is consistent with research that found supplemental lactobacilli-containing cheeses to have higher FAA levels[24]. It is well emphasized that as cheese ages, fats and proteins break down through lipolysis and proteolysis into smaller units, increasing the flavor intensity of the cheese. The MesN cheese, after 6 months of ripening, where the proteolytic activity began and is desired since FAA are precursors for flavor and aroma compounds in cheese, decreased besides WSN%[4].

Some microbial counts in hard cheese samples during ripening: Adding a protective culture, along with *Lactococcus* spp., improved the safety of MesN cheese. The coliform group count was significantly ($p \leq 0.05$) lower in MesN than ThermoN, which were log10 2.03 and log10 3.48 CFU/g, respectively, at 1 week old. After a month, the microbial count continued to decrease in both treatments, but the lowest rate and count were in MesN (log101.87 CFU/gm). However, the colonies on VRB agar disappeared in both treatments after 3 months of ripening. Yeast count was also influenced by the protective cultures used in the production of MesN cheese; even if the yeast counts initially appeared higher, they fell dramatically. The number of yeast cells per gram in MesN dropped from log10 3.20 to log101.66 CFU/gm during the 6 months of ripening. Additionally, as shown in Table 5, there was a wide difference in yeast count between MesN and ThermoN during the whole ripening phase. After 1 and 3 months of ripening, the whole

Table 5: Some microbial counts in hard cheese samples during ripening

Microbial analyses	Ripening periods	ThermoN (n = 6)	MesN (n = 6)	Ras (n = 3)
Log_{10} CFU/g on MRS agar	1 week	4.72^{cB}	6.54^{dA}	N.D
	1 month	8.23^{aB}	7.59^{bC}	8.71^{aA}
	3 months	8.17^{aA}	8.08^{aA}	7.92^{bA}
	6 months	7.43^{bA}	7.04^{cA}	7.14^{cA}
Log_{10} CFU/g on VRB agar	1 week	3.48^{aA}	2.03^{aB}	N.D
	1 month	3.20^{bA}	1.87^{bB}	3.22^{aA}
	3 months	≤ 10	≤ 10	3.09^{b}
	6 months	≤ 10	≤ 10	≤ 10
Yeast count Log_{10} CFU/g	1 week	2.62^{cB}	3.20^{bA}	N.D
	1 month	4.76^{aB}	3.74^{aC}	5.82^{aA}
	3 months	3.28^{bB}	2.38^{cC}	4.65^{bA}
	6 months	2.48^{cA}	1.66^{dB}	≤ 10
Mold count Log_{10} CFU/g	1 week	≤ 10	≤ 10	N.D
	1 month	1.21^{A}	≤ 10	1.47^{aA}
	3 months	≤ 10	1.32^{A}	1.14^{aA}
	6 months	≤ 10	≤ 10	N.D

Data was expressed using Mean±SD, SD: Standard deviation, N.D: Not determined, Means within the same column with no common superscript, $^{a-d}$Differ ($p \leq 0.05$) for comparing ripening time, Means within the same raw with no common capital superscript and $^{A-C}$Differ ($p \leq 0.05$) for comparing studied cheeses

Table 6: Sensory characteristics of hard cheese samples during ripening

Sensory parameters	Periods ripening	ThermoN (n = 9)	MesN (n = 9)
Appearance	1 week	7.67 ± 0.87^{aA}	8.00 ± 0.87^{aA}
	1 month	7.67 ± 1.12^{aA}	7.00 ± 1.12^{bA}
	3 months	8.33 ± 0.71^{aA}	7.89 ± 0.60^{aA}
	6 months	7.89 ± 0.60^{aB}	8.56 ± 0.53^{aA}
Flavor	1 week	7.11 ± 1.05^{bA}	7.56 ± 1.13^{abA}
	1 month	6.78 ± 1.48^{bA}	6.78 ± 1.20^{bA}
	3 months	8.33 ± 0.71^{aA}	6.89 ± 0.78^{bB}
	6 months	7.11 ± 0.60^{bB}	8.00 ± 0.71^{aA}
Odour	1 week	8.00 ± 0.0^{aA}	7.44 ± 1.01^{bA}
	1 month	8.00 ± 0.87^{aA}	6.89 ± 1.05^{bB}
	3 months	8.44 ± 0.73^{aA}	6.89 ± 0.78^{bB}
	6 months	7.89 ± 1.27^{aA}	8.33 ± 0.50^{aA}
Texture	1 week	7.44 ± 0.73^{aA}	7.22 ± 1.09^{bA}
	1 month	7.00 ± 1.22^{aA}	6.11 ± 1.36^{cA}
	3 months	8.00 ± 0.71^{aA}	7.44 ± 0.88^{abA}
	6 months	7.11 ± 0.78^{aB}	8.33 ± 0.50^{aA}

Data was expressed using Mean±SD, SD: Standard deviation, Means within the same column with no common superscript, $^{a\text{-}d}$Differ ($p\leq0.05$) for comparing ripening time, means within the same raw with no common capital superscript and $^{A\text{-}D}$Differ ($p\leq0.05$) for comparing studied cheeses

ripening phase. After 1 and 3 months of ripening, Ras had the highest total yeast count, generally. The protective cultures applied in the manufacturing of MesN cheese had an impact on mold growth as well and there was no mold growth during ripening even though mold count was observed in samples of 3-month-ripened cheese, which may be due to post-contamination. The findings demonstrated the possible benefits of using protective microorganisms in the production of safe and healthy cheese. Proteolytic, psychrophilic and viable spore-producing bacterial populations were shown to drastically decline during Ras cheese's ripening process and the lowest yeast and mold counts were identified in Ras cheese, which included bifidobacteria during ripening[29].

Ras cheese has the worst microbial safety compared to both new treatments. Ras cheese exhibited heavy mold growth on its surface[30], while mold count was low apparent in the other examined cheeses. When compared to other types of cheese, Ras exhibited the highest mean values for yeast counts at 1 and 3 months of ripening, as well as the highest coliform counts, suggesting poor hygiene during production. Meanwhile, no growth of coliform, yeast, or mold was detected in 6-month-aged Ras cheese samples, indicating Ras cheese's increased safety as a result of its lengthy aging.

Concerning *Clostridium* perfringens colonies, no growth was detected in the new hard cheeses or Ras cheese samples. On the other hand, Eldenary *et al*.[3] identified *Clostridium* spp. in Ras cheese samples. The colony count on MRS peaked and fluctuated during new hard cheese maturing, but in Ras, MRS numbers decreased throughout ripening. Awad *et al*.[4] found that the count of LAB reached its maximum during or soon after Cheddar manufacture. Fresh MesN cheese had considerably more LAB colonies than ThermoN cheese, which makes sense given the presence of both mesophilic lactic acid and protective cultures in MesN cheese, while the latter contains only the thermophilic culture. At the first month of ripening, there was a significant ($p\leq0.05$) difference between MesN and ThermoN counts; in contrast, after 3 and 6 months of ripening, no considerable difference was observed between ThermoN and MesN counts within ripening in the LAB count on MRS.

Sensory characteristics of hard cheese samples during ripening: The sensory characteristics between ThermoN and MesN was demonstrated in Table 6. Concerning the appearance of ThermoN and MesN, no difference was observed among them in appearance, except after 6 months of ripening. Interestingly, the flavor and acceptability of all cheeses made with both mesophilic and EPS-producing cultures were in the acceptable range. No significant difference in flavor was detected between the two types of the new hard cheeses at the first month of ripening, whereas ThermoN has the best flavor value (8.33) at the 3rd month and MesN has the best flavor value at the 6th month of ripening (8.00). The odour values were significantly different between MesN and ThermoN throughout the ripening period. Concerning the texture score, little variation was detected by panelists between MesN and ThermoN, whereas MesN exhibited the highest scores at the 6th month of ripening compared to ThermoN, as the values were 8.33 and 7.11, respectively.

Moreover, the comments of the graders for MesN and ThermoN show that the texture becomes slightly doughy after

ripening for a month in ThermoN, while cheese made with mesophilic cultures has a harder texture but smells and tastes curdled. The taste was improved after 3 months of ripening for all samples, but ThermoN had the best taste and no presence of bitterness. While the MesN was the best in taste and texture and closest to the taste of mild Cheddar after 6 months of ripening, the ThermoN had a flat flavor and some graders noticed a slight bitterness in the ThermoN. These findings were in agreement with Awad *et al.*[4], as the EPS-positive strain lacked an appropriate peptidolytic system to further hydrolyze the bitter peptides into amino acids.

Overall, all panelists preferred the ThermoN 3 month and MesN 6 month cheeses, which had a smooth texture and flavor intensity similar to those of Cheddar. So, the positive results of this study support our hypothesis that the addition of EPS-producing cultures accelerates the rate of ripening and flavor development in the new hard cheese.

CONCLUSION

A new hard cheese was made by optimizing the manufacturing steps of Ras and Cheddar cheese to improve the texture and sensory acceptability of the final product to be used in making processed cheese. Two types of new hard cheese were produced using thermophilic (EPS) or mesophilic cultures with protective cultures. A higher cheese yield was detected in ThermoN. A positive relationship was found between water-soluble nitrogen (%) and total FAA (mg/g) during ripening periods of 1, 3 and 6 months in both new hard cheeses. The new cheeses received a high level of acceptability. Further research is planned to utilize such natural cheese in making processed spreadable cheese.

SIGNIFICANCE STATEMENT

The goal of this research was to produce a novel hard cheese that could replace imported Cheddar, particularly in the production of processed cheese. The new hard cheese has the potential characteristics: No growth mold at the cheese surface, good microbiological quality, no hard rind, low salt content, no health risk. These are good factors to recommend the new hard cheese for replacing imported Cheddar, particularly in the production of processed cheese. In addition to lowering production costs and the need for raw material imports, this would enhance Egyptian goods and enable them to pass and surpass international standards.

ACKNOWLEDGMENT

This paper is based upon work supported by the Science, Technology and Innovation Funding Authority (STDF) under grant number 44264.

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