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Effect of Using Buffalo's Milk with Cow's Milk and Selected Bacterial Strains on the Properties and Safety of Gouda Cheese

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THE present study evaluated the chemical and rheological changes in Gouda cheese as affected by the partial use of buffalo's milk and different types of probiotic bacteria, as well as biogenic amines (BAs) content was evaluated to assess the safety of cheese. Results revealed that free amino acids content increased progressively during the ripening period. The partial replacement of cow's milk by 25% of buffalo's milk in cheese [T1 (25% buffalo's milk + 75% cow's milk, T2 (25% buffalo's milk + 75% cow's milk + *L. helveticus* CH5) and T3 (25% buffalo's milk + 75% cow's milk + *L. plantarum* ATCC14917)] increased the cheese's hardness and adhesiveness at zero time. However, at the end of ripening period, no significant differences were recorded between the control (100% cow's milk) and T3 which suggesting more improving impact due to the used probiotic bacteria (*L. plantarum*). Additionally, the detected BAs levels in all samples were located within the safe limits recommended by the FDA. However, T3 had the lowest BAs content because the addition of probiotic bacteria decreased BAs formation in cheese.

Keywords: Gouda cheese, Chemical Composition, Probiotics, Quality properties, Biogenic amines.

Introduction

Gouda and Edam cheeses constitute the 2 main types of Dutch cheeses and internationally differ in their requirements for the milk fat content used to produce the cheese; partial skim milk is used for Edam cheese, and whole milk is used for Gouda cheese (Codex Alimentarius, 2013). Gouda cheese is well-known in the world as a semi- hard cheese of near white or ivory through to light yellow or yellow colour and a firm texture (Codex Alimentarius, 2013). Traditionally, Gouda cheese is produced from bovine milk and brined before ripening, depending on required product characteristics, for 1 to 20 months (Hoorde et al., 2008). Gouda cheese has been widely spread in many countries between 2010 and 2014. Gouda cheese production in the United States increased from 19 to 48 million pounds per year (USDA, 2014) but several challenges have highlighted the possible effect of the cheese production environment and the influence of technological factors of cheese manufacturing.

Recently, the demand for this cheese type has been increased in Egypt and some local dairy factories manufactured it in a commercial scale (El-Nagar et al., 2010). The economic advantages of rapid development of more intense cheese flavour in shorter periods of time would be substantial (Jo et al., 2018). Acceleration of cheese ripening can also be a mean for increasing the production of cheese in developing countries where investment in storage facilities. Development of the required characteristics can occur partially by bacteria and additive enzymes through different metabolic pathways which affect the texture and body of the cheese matrix

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as well as flavours (Hynes et al., 2003). Adding lactic acid bacteria to cheese is an effective way to accelerate cheese ripening.

On the other hand, it is well-known that cow's milk is mostly used for making many semi-hard and hard cheese varieties. However, type of milk is considered as an effective quality factor for cheese quality. In this respect, cow's milk or buffalo's milk or their mixtures and products obtained from these milk types are recommended as raw materials for making Gouda cheese (Codex, 2013). Cow's milk was partially replaced with buffalo's milk and the impact of such replacement on composition and quality of the resultant Gouda cheese was studied (El-Nagar et al., 2010). Owing to importance of starter culture on quality and ripening of cheese, El-Soda et al. (2000) investigated the role of the traditional starter culture with and without *Lactobacillus helveticus* CH5 and *Lactobacillus plantarum* ATCC14917 on the composition and quality of Gouda cheese. Such qualities besides the safety of the resultant cheese are quite important from the nutrition and health benefits point of view.

However, the production of semi- hard and hard cheese is often associated with the formation of biogenic amines (BAs) (Silla-Santos, 1996). These BAs are mainly produced by the decarboxylation of amino acids by microorganisms. These microorganisms with the ability of amino acids decarboxylation, such as *lactobacilli*, *micrococci*, *enterococci* and *enterobacteriacae*, pose an essential factor for BAs formation (Suzzi and Gardini, 2003). In general, BAs production in cheese is also affected by many other factors and several symptoms for the adverse effects of BAs were reported, when consumed with high levels in foods. The reported symptoms are headache, sweating, respiratory disorders, nausea, oral burning, skin rashes, heart palpitation and hypo or hypertension (Stratton et al., 1991). Recent trends in food quality and safety promote an increasing search for trace compounds that can affect human health. Biogenic amines (BAs) belong to this group of substances. The ability of probiotic bacteria, such as lactic acid bacteria (like *L. Lactis*, *L. Plantarum*, *L. parabuchneri*, *S. thermophilus*, *Ln. mesenteroides*, *Lc. lactis*, and *Ln. lactis*), to decrease the formation of BAs has been reported in the recent literature (Fadda et al., 2001; Callejón et al., 2014; Guarcello et al., 2016).

In the current study, we hypothesized that the partial replacement of cow's milk by buffalo's milk in Gouda cheese could provide a positive impact on the feasibility of Gouda cheese production; particularly in the countries where buffalo's milk is more available than cow's milk like in Egypt. As well, it was hypothesized that the addition of probiotic bacteria could maintain the original properties of Gouda cheese against the few changes due to the partial addition of buffalo's milk. Furthermore, the addition of probiotic bacteria to the traditional starter could reduce the formation of BAs in Gouda cheese.

Therefore, the present investigation aimed to: i) evaluate the impact of the partial substitution of cow's milk by buffalo's milk on the chemical and rheological properties of Gouda cheese, ii) to study the impact of the addition of probiotic bacteria to the traditional starter on the levels of formed BAs in Gouda cheese.

Materials and Methods

Fresh cow's (TS: 12.25%, Fat: 3.5%, Protein: 3.2%) and buffalo's milk (TS: 14.25%, Fat: 6.0%, Protein: 3.5%) were obtained from the herds of Faculty of Agriculture, Cairo University, Egypt. Commercial starter of mesophilic culture FD-DVS R-704 (consisting of *Lactococcus lactis subsp. lactis* and *Lactococcus lactis subsp. cremoris*) was obtained from Chr. Hansen's Lab., Denmark and used as the control starter. Probiotic bacteria of *Lactobacillus helveticus* CH5 and *Lactobacillus plantarum* ATCC14917 were obtained from the Egyptian Microbial Culture Collection (EMCC) belonging to Cairo Microbial Resources Center (MIRCEN), Faculty of Agriculture, Ain Shams University, Egypt. Hansen's powder rennet and Annatto were obtained from Chr. Hansen's Lab., Copenhagen, Denmark. Yellow wax coating material imported from Germany was obtained from AWA for food additives company, Alexandria, Egypt.

Preparation of Gouda cheese

Gouda cheese was preparation as described By Scott (1998), using standardized fresh cow's milk (3.0% fat) and buffalo's milk (6.0% fat). Milk of all treatments was heat treated at 73°C/20 sec., cooled to 32°C and 0.02% $CaCl_2$ was added directly before coagulation. Four batches of Gouda cheese were made as follows :

- Control cheese was made from fresh cow´s milk and commercial starter of mesophilic culture.

- T1 made from mixed cow's milk (75%) and buffalo's milk (25%) with commercial starter.
- T2 made from mixed cow's milk (75%) and buffalo's milk (25%) with commercial starter and probiotic bacteria of *Lactobacillus helveticus* CH5 (1:1).
-T3 made from mixed cow's milk (75%) and buffalo's milk (25%) with commercial starter and probiotic bacteria of *Lactobacillus plantarum* ATCC14917 (1:1).

All cheese treatments were then carefully coated with yellow wax JM0043 (Made in EU) and kept in the ripening room at 10-12ºC and 85-95% relative humidity for 3 months. Then all treatments included fresh (zero time), 1, 2 and 3 months of ripening were analyzed. The experimental work was done in 3 replicates.

Chemical analysis of Gouda cheese

The moisture of cheese was determined according to British Standards Institution (BSI, 1989). Titratable acidity (TA) and fat content of cheese were determined by the methods described by AOAC (2005). Total nitrogen (TN) was determined (using micro-Kjeldahal) by the method of International Dairy Federation (IDF, 1991). The total volatile fatty acids (TVFA) of cheese were determined by the distillation method described by Kosikowski (1982); values were expressed as mL (0.1N) NaOH/100g cheese. The pH values were measured according to the methodology of BSI (1989) using a glass electrode of digital pH meter, type (Orion Research model SA720) USA.

Determination of free amino acids

The analysis was carried out using the Pico-Tag method after deproteinization and precipitation with 5-sulfosalicylic acid followed by centrifugation to remove the precipitated protein. The supernatants were taken for free amino acids analysis using HPLC-Pico-Tag method according to Heinrikson & Meredith (1984) and Cohen et al. (1989). Phenylisothiocyanate (PITC, or Edman's reagent) was used for pre-column derivatization, while reversed-phase gradient elution high-performance liquid chromatography (HPLC) separates the phenylthiocarbamy (PTC) derivatives which were detected by their UV absorbance.

Textural properties determination

Texture profile analysis (TPA) such as hardness, springiness, cohesiveness, gumminess, adhesiveness and chewiness of cheese were measured with an Instron Universal Testing Machine (Model 4302, Instron Corporation, Canton M.A, England) according to the procedure of Bourne (1978).

Determination of biogenic amines (BAs)

Reagents for biogenic amines determination; a) Dansyl chloride solution: 500mg of dansyl chloride (5- {Dimethylamino} naphtalene -1-sulfonyl chloride) were dissolved in 100 mL acetone. b) Standard solutions: Stock standard solutions of the tested amines: 25mg of each standard were dissolved in 25 mL distilled water individually.

Nine BAs including; Treptamine, β-phenyl-ethyl-amine, Putrescine, Cadaverine, Histamine, Serotonin, Tyramine, Spermidine and Spermine were extracted and determined using HPLC-UV according to the method of Krause et al. (1995). The HPLC system was an Ultimate 3000 Thermo Fisher system (Germany) equipped with auto sampler, pump, UV detector set at 254 nm wavelength. Agilent Poroshell 120EC-C18 4um (4.6 mm × 150 mm) column was used for biogenic amines separation. Data were integrated and recorded using Chromeleon Software program.

Statistical analysis

Statistical analysis of the obtained data was performed according to SAS Institute (1990) using liner Model (GLM). Duncan's multiple rang was used to separate among means of three replicates of the data.

Results and Discussion

Chemical composition of cheese

The chemical composition of Gouda cheese is presented in Table 1. The results revealed that the moisture content of cheese samples ranged from 39.19 to 42.39 % during the ripening period. As well, the moisture content of all cheese samples significantly decreased during ripening period that may be due to the biochemical changes and lactic acid development, which cause curd contraction and expulsion of the aqueous phase of cheese as well as evaporation of some moisture during ripening. These results are in agreement with those obtained by Van den Berg et al. (2004) and El-Nagar et al. (2010). The Protein/DM and Fat/DM content of different treatments markedly ($p \leq 0.05$) increased in all treatments (T1, T2 and T3) in comparison with control and significantly increased up to the end of ripening period. These results could be due to the partial use of 25%

buffalo's milk (with high protein and fat content) in T1, T2, and T3. While the gradual increases in protein and fat contents of treatments could be attributed to the evaporation of water during ripening. Similar results were obtained by Ismail et al. (2004) and Jo et al. (2018).

The changes in total volatile fatty acids (TVFA) contents of Gouda cheese due to the applied treatments and advancing ripening period are shown in Table 1. It could be observed that the fresh and ripened cheese treated with *Lb. plantarum* (T3) had the highest TVFA, while the use of buffalo's milk with the commercial starter (T1) significantly decreased TVFA content in fresh and cheese of different ripening degree. The increase of TVFA with advancing ripening period was significant ($P \leq 0.05$) in all samples. These results confirmed those obtained by El-Soda et al. (2000) and Ismail et al. (2004). Gomez et al. (1996) reported that *L. plantarum* ESI 144 cultures attenuated by heat or freeze-shocking might be required to reduce acidifying activity, maintaining a favorable impact on sensory attributes like flavour quality or intensity because of the increase in the de-bittering activity present in cheese. The significant increase in TVFA contents with advancing the ripening could be attributed to fat hydrolysis and the formation of volatile fatty acid compounds.

TABLE 1. Chemical composition of Gouda cheese during the ripening period.

Constituent	Ripening period (days)	Treatments				LSD (0.05)
		C	T1	T2	T3	
Moisture (%)	Fresh	41.73 ± 0.252^{Ba}	$42.390.168 \pm {}^{Aa}$	41.42 ± 0.147^{Ba}	42.28 ± 0.170^{Aa}	0.3638
	30	40.81 ± 0.181^{Bb}	41.63 ± 0.256^{Ab}	40.62 ± 0.350^{Bb}	41.97 ± 0.127^{Aa}	0.4587
	60	39.80 ± 0.150^{Cc}	40.68 ± 0.247^{Bc}	39.83 ± 0.103^{Cc}	41.23 ± 0.176^{Ab}	0.3333
	90	39.22 ± 0.166^{Bd}	39.53 ± 0.350^{Bd}	39.19 ± 0.281^{Bd}	40.52 ± 0.245^{Ac}	0.5076
	LSD	0.3614	0.5019	0.4559	0.3480	
Protein / DM (%)	Fresh	41.32 ± 0.100^{Dc}	41.70 ± 0.065^{Cd}	41.85 ± 0.064^{Bd}	42.07 ± 0.045^{Ad}	0.1351
	30	41.82 ± 0.036^{Cb}	41.88 ± 0.040^{Cc}	42.06 ± 0.050^{Bc}	42.52 ± 0.046^{Ac}	0.0820
	60	42.32 ± 0.045^{Da}	42.44 ± 0.061^{Cb}	42.78 ± 0.055^{Bb}	42.91 ± 0.060^{Ab}	0.1050
	90	42.43 ± 0.060^{Da}	42.84 ± 0.070^{Ca}	43.13 ± 0.055^{Ba}	43.42 ± 0.056^{Aa}	0.1143
	LSD	0.1230	0.1137	0.1068	0.0982	
Fat / DM (%)	Fresh	51.36 ± 0.141^{Cb}	53.67 ± 0.125^{Ac}	52.81 ± 0.127^{Bc}	52.83 ± 0.111^{Bc}	0.2390
	30	51.56 ± 0.090^{Cab}	53.86 ± 0.060^{Ab}	53.12 ± 0.097^{Bb}	53.27 ± 0.127^{Bb}	0.1819
	60	51.67 ± 0.125^{Da}	54.02 ± 0.050^{Aa}	53.21 ± 0.050^{Ca}	53.46 ± 0.146^{Bb}	0.1937
	90	51.88 ± 0.190^{Db}	54.15 ± 0.062^{Aa}	53.36 ± 0.093^{Ca}	53.73 ± 0.144^{Ba}	0.2482
	LSD	0.2663	0.1514	0.1809	0.2505	
T.V.F.A (mL 0.1 N NaOH $100g^{-1}$ cheese)	Fresh	12.83 ± 0.777^{BCd}	11.87 ± 0.451^{Cd}	13.30 ± 0.600^{Bd}	14.87 ± 0.603^{Ad}	1.1666
	30	18.73 ± 0.321^{Ac}	13.77 ± 0.764^{Bc}	18.07 ± 0.451^{Ac}	17.57 ± 0.907^{Ac}	1.2345
	60	21.00 ± 0.656^{Bb}	17.67 ± 0.666^{Cb}	21.33 ± 0.513^{Bb}	23.67 ± 0.666^{Ab}	1.1854
	90	28.33 ± 0.777^{Aa}	22.27 ± 0.503^{Ca}	24.13 ± 0.551^{B}	29.03 ± 0.702^{Aa}	1.2126
	LSD	1.2439	1.1486	1.0025	1.3742	

C: control-100% Cow's milk + Cheese starter. **T1:** 75% Cows + 25% Buffaloes milk + Cheese starter.

T2: 75% Cows + 25% Buffaloes milk + {Cheese starter + *L.helveticus* (1:1)}.

T3: 75% Cows + 25% Buffaloes milk + {Cheese starter + *L.plantarum* (1:1)}.

A, B, C & D and **a, b, c & d:** means with the same letter among treatments and the storage period respectively are not significantly different ($p<0.05$).

pH values and titratable acidity

The changes in the pH values and titratable acidity (%) of cheese as affected by using *Lb. helveticus* CH5 and *Lb. plantarum* ATCC14917 culture and buffalo's milk are given in Fig. 1. Data showed that the lowest pH values were observed in fresh cheese treated with *Lb. plantarum* (T3) and cheese treated with *Lb. helveticus* (T2). This agrees with Gomez et al. (1996) who found that cheese pH was significantly ($P \leq 0.001$) lower in cheese from milk with added *L. plantarum* ESI 144.

In addition, it could be noticed that the pH values of all experimental cheeses gradually decreased during the ripening period. Also, during early cheese making (0-12 hr) (Gomez et al., 1996), pH values in cheese with added *lactobacilli* were 0.05-0.10 units lower than in cheese without added *lactobacilli*, indicating the adjunct metabolism of lactose and the production of lactic acid. The decrease in pH values during ripening could be related to the hydrolysis occurred in lactose and protein contents.

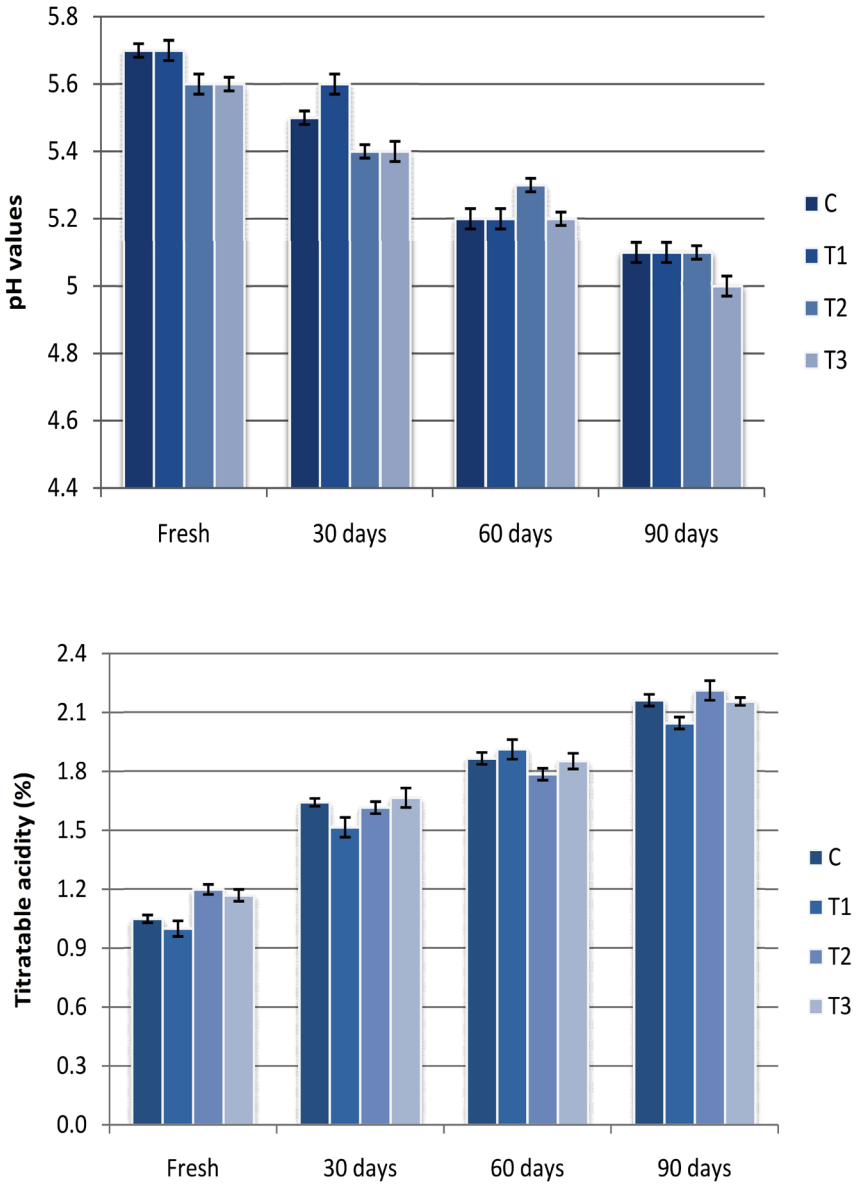

Fig. 1. Changes in the pH values and titratable acidity (TA%) of cheese during ripening period (see legend of Table (1) for treatments).

On the other hand, Aljewicz et al. (2014) found that the increase in pH was observed in control cheese and cheese with *L. acidophilus* NCFM between the 6th and 10th week of ripening. Variations in pH values were significantly ($p<0.05$) correlated with changes in *Lactobacillus* counts (R=–0.807) and the content of SN/TN (R=0.775) fraction produced during proteolysis.

The titratable acidity of the resultant cheese from all treatments increased with the decrease of pH values and advancing the ripening period. This increase in the titratable acidity could be explained by the development of lactose fermentation and milk constitutes by lactic acid and probiotic bacteria. El-Nagar et al. (2010) found that the cheese treated with whole viable cells of *Lb. helveticus* had the highest titratable acidity throughout all stages of ripening ($P\leq0.01$) compared to the other treatments.

Free amino acids (FAA) content

Obtained data in Table 2 showed that significant differences were observed in FAA values among treatments, T3 and T2 displayed the majority of the highest values in the 90 days old cheese. Data also showed that the highest level of glutamic acid was found in T3 followed by T2 then T1 and the control after 90 day of ripening in agreement with Fenelon et al. (2000) who found high content of glutamic acid in bovine Cheddar cheese. The glutamic acid presence in cheeses is responsible for their pleasant flavour (Ardo, 2006). Also, the highest level of serine (90 days old cheese) was found in T2 followed by T3. Similar results were obtained by Liu et al. (2003) who also found that degradation of serine resulted in the production of strong aromatic compounds. Quantitatively, free amino acids markedly varied between different Gouda cheese treatments ranging from 1.63 to 427.6 (mg $100g^{-1}$ sample) during the tested ripening period.

All of the free amino acids (FAA) increased during the ripening process except for arginine, proline and cysteine which decreased as confirmed by El-Abd et al. (2010). The proteolytic role of the used probiotic bacteria was given in details in the literature. Accumulation of the free amino acids due to the use of *Lactobacillus helveticus* (as in T2 in the present study) or *Lactobacillus plantarum* (as in our T3) was given for different cheese by El-Soda et al. (2000), Madkor et al. (2000), Kenny et al. (2006), El-Nagar et al. (2010) and Duan et al. (2019).

Generally, the values of total free amino acids were 365.02, 561.69, 1183.29 and 592.97 (mg $100g^{-1}$) of 60 days old in the control, T1, T2 and T3 cheese respectively, whereas at 90 days of ripening the corresponding values were 797.26, 878.54, 2018.83 and 1728.31 mg $100g^{-1}$ in order. This suggests that T2 and T3 were the best in this respect. These obtained results were in accordance with those of Milesi et al. (2008 and 2009) who added *Lb. plantarum* to cheese and observed an increase in the value of total FAA as compared to the control. In this concern, McSweeney (2004) stated that increasing the levels of FAA in cheeses, due to the use of probiotic bacteria, could be helpful in the development of cheese flavor; where FAA could contribute as precursors to the cheese's taste and aroma.

Texture profile analysis (TPA)

Table 3 shows the rheological properties of Gouda cheese as affected by the applied treatments. The control fresh cheese had the lowest significant hardness value (16.3 N), whereas using buffalo's milk (T1) significantly increased the cheese hardness (22.3 N). Quantity and type of caseinate in buffalo's milk as well as the high calcium content seem to be responsible for such increase in hardness. Some improvement was noticed in both T2 and T3. Such impact was also observed during ripening. However at the end of ripening period, significant differences were observed between the control and cheese from T2 and T3 suggesting more improving impact due to using of probiotic bacteria. The impact of T2 as well as the decrease in hardness with advancing ripening period agreed with the given trend for Gouda cheese by El-Naggar et al. (2010). However, Mehanna & Pasztor-Huszar (2012) attributed the increase in hardness during cheese ripening to the corresponding decrease in moisture content which acts as plasticizer in the protein matrix of cheese. Moisture content of T1 cheese was always higher than moisture of control cheese at any ripening time as previously shown in Table 1.

TABLE 2. Free amino acids content (mg 100 g^{-1}) in the 60 and 90 days old Gouda cheese.

Amino Acids (mg 100 g^{-1})	60 day old					90 day old				
	C	T1	T2	T3	LSD	C	T1	T2	T3	LSD
Aspartic Acid	10.9±1.069^{C}	13.23±1.262^{C}	37.62±1.026^{A}	24.22±1.663^{B}	4.1745	30.40±1.143^{C}	26.55±0.923^{C}	43.44±1.590^{B}	112.0±3.197^{A}	6.2965
Glutamic Acid	12.7±1.358^{C}	21.7±0.957^{B}	40.0±2.369^{A}	12.3±0.926^{C}	4.9539	15.11±1.488^{D}	50.7±2.026^{C}	102.58±2.058^{B}	131.83±1.761^{A}	6.0253
Serine	3.38± 0.280^{D}	17.59±0.915^{C}	86.19±2.378^{A}	26.03±1.703^{B}	5.018	30.43±1.419^{C}	22.30±1.807^{D}	88.85±1.787^{A}	39.43±2.102^{B}	5.8546
Glycine	1.63± 0.186^{C}	8.86±0.712^{B}	17.6±0.473^{A}	2.87±0.203^{C}	1.4637	11.99±1.412^{C}	10.84±0.728^{C}	35.58±1.198^{A}	26.8±2.610^{B}	5.3517
Histidine	16.6±0.935^{C}	17.0±0.930^{C}	101.87±5.172^{A}	31.57±1.271^{B}	8.9461	119.85±1.822^{B}	58.13±1.093^{D}	157.9±2.589^{A}	83.4±1.484^{C}	5.9735
Arginine	48.01±1.377^{B}	32.60±1.559^{C}	137.6±3.553^{A}	39.61±1.543^{C}	7.169	23.5±1.917^{B}	19.2±2.101^{B}	109.7±3.464^{A}	7.00±0.323^{C}	7.3271
Threonine	5.2±0.225^{B}	3.0±0.104^{B}	39.83±2.258^{A}	6.7±0.866^{B}	3.9638	13.21±1.111^{C}	5.6±0.346^{D}	114.40±2.833^{A}	25.5±1.442^{B}	5.5198
Alanine	10.53±1.099^{B}	26.10±1.380^{A}	9.27±0.436^{B}	23.40±1.725^{A}	4.0858	27.63±1.844^{D}	51.43±1.547^{C}	92.70±1.547^{A}	56.83±0.697^{B}	4.8027
Proline	86.2±1.853^{A}	16.8±1.443^{D}	22.33±1.277^{C}	40.3±1.200^{B}	4.779	40.10±1.415^{A}	11.43±1.001^{C}	19.13±0.817^{B}	10.10±0.814^{C}	3.3945
Tyrosine	8.3± 0.745^{C}	16.6±2.020^{B}	192.0±2.136^{A}	14.10±0.768^{B}	5.1022	25.0±0.930^{C}	29.53±1.776^{C}	241.67±2.689^{A}	69.5±1.905^{B}	6.29
Valine	15.9±1.998^{D}	24.57±1.392^{C}	45.10±2.023^{B}	51.9±2.464^{A}	6.5415	71. 43±1.364^{A}	45.8±1.426^{B}	50.8±1.934^{B}	74.16±1.524^{A}	5.1452
Methionine	11.43±0.353^{D}	26.57±2.505^{B}	17.83±1.214^{C}	35.0±1.511^{A}	5.1975	48.23±1.373^{C}	52.60±1.461^{C}	164.6±2.245^{A}	68.41±1.231^{B}	5.3017
Cysteine	13.18±1.925^{B}	22.7±0.473^{A}	11.9±1.039^{BC}	8.6±0.346^{C}	3.6938	2.9±0.225^{C}	13.9±1.030^{A}	5.4±0.283^{B}	4.55±0.343^{BC}	1.8659
Isoleucine	4.2±0.237^{D}	85.79±2.381^{A}	33.6±1.732^{B}	10.20±0.866^{C}	5.0186	15.02±0.972^{D}	99.40±2.523^{C}	196.5±3.255^{A}	159.10±1.970^{B}	7.6103
Leucine	27.29±1.242^{C}	17.20±0.814^{D}	170.6±1.447^{A}	61.20±1.291^{B}	3.9831	84.03±1.359^{D}	128.58±1.320^{C}	264.6±2.255^{A}	155.33±2.607^{B}	6.4131
Phenylalanine	26.17±1.561^{D}	51.35±1.370^{B}	111.4±2.138^{A}	43.0±1.506^{C}	5.4454	61.09±2.358^{D}	70.37±1.444^{C}	177.78±1.862^{B}	276.77±3.091^{A}	7.4116
Lysine	73.71±1.462^{C}	160.03±4.710^{A}	124.53±1.601^{B}	161.97±2.183^{A}	9.1735	177.43±2.239^{B}	182.18±1.481^{B}	153.2±1.790^{C}	427.6±6.016^{A}	11.131
Total	**365.02**	**561.69**	**1183.29**	**592.97**		**797.26**	**878.54**	**2018.83**	**1728.31**	

C: control-100% Cow's milk + Cheese starter. **T1:** 75% Cows + 25% Buffaloes milk + Cheese starter. **T2:** 75% Cows + 25% Buffaloes milk + {Cheese starter + *L.helveticus* (1:1)}.
T3: 75% Cows + 25% Buffaloes milk + {Cheese starter + *L.plantarum* (1:1)}. **A, B, C & D:** Averages with the same letter among the free fatty acid are not significantly different ($p<0.05$).

Adhesiveness (Table 3) of fresh cheese or during the first 60 days of ripening followed the same trend of hardness. Thus, cheese from T1 had the highest value (P≤ 0.05) but the differences of this property between T2, T3 and the control were insignificant. No definite trend for impact of the applied treatments was noticed during ripening. However, all adhesiveness values gradually increased (P≤0.05) during the ripening period, however the differences between T3 and the control were insignificant. This property is quite important since it means the necessary work to overcome the force between cheese and teeth during the eating (Mehanna & Pasztor-Huszar, 2012).

Cohesiveness values of all treated cheese were lower (P≤0.05) than those of the control while a significant decrease was recorded during ripening.

TABLE 3. Textural properties of Gouda cheese during the ripening period.

Property	Ripening period (days)	Treatments				LSD (0.05)
		C	T1	T2	T3	
Hardness (N)	Fresh	16.30 ± 0.110^{Dd}	$22.300.135 \pm {}^{Bd}$	$24.01 \pm 0.128\ ^{Ad}$	18.25 ± 0.143^{Cd}	0.2443
	30	21.73 ± 0.135^{Cc}	23.24 ± 0.135^{Bc}	26.22 ± 0.115^{Ac}	19.89 ± 0.101^{Dc}	0.2309
	60	27.44 ± 0.103^{Ab}	25.56 ± 0.274^{Bb}	27.46 ± 0.086^{Ab}	24.53 ± 0.181^{Cb}	0.3344
	90	27.80 ± 0.120^{Ba}	28.00 ± 0.227^{Aa}	28.18 ± 0.139^{Aa}	27.54 ± 0.315^{BCa}	0.4053
	LSD	0.2218	0.3808	0.2237	0.3804	
Adhesiveness (mJ)	Fresh	0.34 ± 0.066^{Bc}	0.69 ± 0.111^{Ac}	0.42 ± 0.066^{Bc}	0.37 ± 0.056^{Bd}	0.1459
	30	0.54 ± 0.125^{Bb}	0.93 ± 0.064^{Ab}	0.58 ± 0.078^{Bc}	0.59 ± 0.062^{Bc}	0.1625
	60	0.70 ± 0.107^{Bb}	1.05 ± 0.030^{Aab}	1.03 ± 0.161^{Ab}	0.81 ± 0.038^{Bb}	0.1877
	90	1.27 ± 0.101^{Ba}	1.13 ± 0.020^{Ba}	1.56 ± 0.225^{Aa}	1.21 ± 0.050^{Ba}	0.2380
	LSD	0.1922	0,1254	0.2778	0.0986	
Cohesivenees (Ratio)	Fresh	0.72 ± 0.050^{Aa}	$0.420.035 \pm {}^{Ca}$	$0.58 \pm 0.030\ ^{Ba}$	0.52 ± 0.015^{Ba}	0.0660
	30	0.49 ± 0.030^{Ab}	0.29 ± 0.025^{Cb}	0.44 ± 0.036^{Ab}	0.36 ± 0.035^{Bb}	0.0601
	60	0.28 ± 0.030^{Ac}	0.22 ± 0.021^{Bc}	0.27 ± 0.021^{ABc}	$0.25 \pm 0.030A^{Bc}$	0.0487
	90	0.22 ± 0.015^{Ac}	0.17 ± 0.020^{Bd}	0.23 ± 0.015^{Ac}	0.20 ± 0.010^{Ad}	0.0293
	LSD	0.0637	0.0490	0.0505	0.0468	
Springness (mm)	Fresh	9.92 ± 0.047^{Ba}	12.56 ± 0.150^{Aa}	5.48 ± 0.187^{Da}	8.80 ± 0.135^{Ca}	0.263
	30	8.28 ± 0.288^{Bb}	11.42 ± 0.111^{Ab}	4.82 ± 0.140^{Db}	$5.56 \pm 0.\ 200^{Cb}$	0.3714
	60	5.82 ± 0.198^{Ac}	5.75 ± 0.102^{Ac}	4.57 ± 0.021^{Cc}	4.83 ± 0.101^{Bc}	0.2309
	90	5.25 ± 0.198^{Ad}	5.15 ± 0.101^{Ad}	4.46 ± 0.015^{Bc}	3.74 ± 0.111^{Cd}	0.2341
	LSD	0.3810	0.2222	0.2216	0.2680	
Gummness (N)	Fresh	14.04 ± 0.181^{Ca}	20.52 ± 0.185^{Ba}	23.78 ± 0.187^{Aa}	13.83 ± 0.066^{Ca}	0.3072
	30	9.80 ± 0.145^{Ab}	7.37 ± 0.175^{Db}	8.12 ± 0.092^{Cb}	9.36 ± 0.1377^{Bb}	0.2644
	60	6.12 ± 0.101^{Bc}	6.13 ± 0.107^{Bc}	3.88 ± 0.093^{Cc}	7.42 ± 0.111^{Ac}	0.1946
	90	5.63 ± 0.102^{Bd}	2.63 ± 0.157^{Cd}	2.48 ± 0.182^{Cd}	6.02 ± 0.118^{Ad}	0.2703
	LSD	0.3808	0.2995	0.2752	0.2092	
Chowieness (mJ)	Fresh	79.45 ± 0.122^{Da}	160.05 ± 0.057^{Aa}	129.70 ± 0.191^{Ba}	122.82 ± 0.205^{Ca}	0.2937
	30	54.63 ± 0.145^{Db}	76.26 ± 0.116^{Cb}	84.41 ± 0.218^{Ab}	83.59 ± 0.180^{Bb}	0.3188
	60	11.04 ± 0.150^{Dc}	17.02 ± 0.250^{Ac}	11.51 ± 0.343^{Cc}	16.32 ± 0.204^{Bc}	0.4659
	90	8.97 ± 0.101^{Cd}	13.54 ± 0.106^{Ad}	9.15 ± 0.118^{Cd}	11.91 ± 0.083^{Bd}	0.1937
	LSD	0.2471	0.2837	0.4378	0.3310	

C: control-100% Cow's milk + Cheese starter. **T1:** 75% Cows + 25% Buffaloes milk + Cheese starter.

T2: 75% Cows + 25% Buffaloes milk + {Cheese starter + *L.helveticus* (1:1)}.

T3: 75% Cows + 25% Buffaloes milk + {Cheese starter + *L.plantarum* (1:1)}.

A, B, C & D and **a, b, c & d:** means with the same letter among treatments and the storage period respectively are not significantly different (p<0.05).

This trend was previously given by Mehanna and Pasztor-Huszar (2012) who attributed such decrease to proteolysis process during Ras cheese ripening which reduced the structural integrity of the protein. It may be interest to notice that cheese from T1 had always the lowest values. This was true at any ripening time suggesting the partial replacement of cow's milk with buffalo's milk decreased cohesiveness.

The highest springiness (12.56 mm) was recorded in fresh cheese made using 25% buffalo's milk (T1) instead of the same amount of cow's milk, whereas applying T2 and T3 as well as advancing ripening significantly decreased springiness. Different impact was given by El-Nagar et al. (2010). Gumminess of fresh cheese was the lowest (13.83 N) in T3, while cheese from T2 had the lowest value (2.48 N) at the end of ripening. El-Nagar et al. (2010) gave the same trend for adding *Lb. helveticus*, but all values gradually increased during ripening of Gouda cheese made by them. In our study, a significant decrease was recorded with advancing ripening of all cheese samples. Concerning cohesiveness, Table 3 shows that the control cheese at any ripening time had the lowest significant values. Ripening had more obvious impact than the applied treatments on decreasing cohesiveness. The decrease was always significant with advancing the ripening period of all cheese samples.

Generally, TPA parameters are useful from many points of view specially quality and sensorial texture attributes of cheese (Bara-Herczegh et al., 2001). The same authors concluded that the age and quality of Trappist cheese can be estimated from the texture parameters, while Adhikari et al. (2003) correlated the sensory and instrumental data and found that texture profile analysis gave hardness value positively correlated to sensory texture attribute such as dry, hardness and crumbly. This finding is quite important for accurate results concerning quality of cheese in general.

Biogenic amines (BAs) content

Based on the above studied parameters of the Gouda cheese properties, T2 and T3 showed the best characteristics as compared to the control sample and T1. However, in our published study (Elwahsh and El-Deeb, 2020), T3 represented the best scores of the sensory evaluation. So that, T3 was selected for further evaluation concerns the BAs content as compared to the control sample with the original characteristics. In this concern, the results in Table 4 revealed that cheese samples of both the control and T3 were free of treptamine and β-phenyl-ethyl-amine either at 60 or 90 days of ripening. As well, the control samples with 60 days of ripening were free of serotonine however at 90 days of ripening, only 0.28 mg kg^{-1} of serotonine was presented. T3 samples of 60 days old were free of serotonine, tyramine and spermidine, however, after 90 days of ripening, low quantities were appeared being 0.01, 0.3 and 0.16 mg kg^{-1}, respectively. All detected BAs in control and T3 cheeses were lower than 1 mg kg^{-1} except for putrescine, cadaverine and histamine. Cadaverine was the highest detected amine (~11.7 mg kg^{-1}) in the control samples but it was only 2.4 mg kg^{-1} in T3 samples. Putrescine scored 6.82 and 8.01 mg kg^{-1} for the control and only 0.17 mg kg^{-1} for the T3 samples of 60 and 90 days old, respectively.

Incidentally, histamine amine showed lower levels as 1.36 – 1.39 mg kg^{-1} for the control of the two ages and very low concentrations (0.01 mg kg^{-1}) in the corresponding T3 samples. Histamine is a very important amine from a toxicological point of view. Therefore, the obtained findings reflected the safety of Gouda cheese of the control and T3 samples, because all the detected concentrations of histamine were markedly lower than the permissible limits set by FDA (2019) in food in general (50 mg kg^{-1}). However, the high levels of putrescine and cadaverine, in the control sample, should also be taken into considerations because they can enhance the toxicity of histamine (Halász et al., 1994; Ruiz-Capillas & Jimenez-Colmenero, 2005). However, cheese from T3 had much lower values for both amines and this is quite important for the safety of cheese.

On the other hand, our results disagreed with Amine et al. (2007) and Ibrahim & Amer (2010) who gave very high levels of histamine, tyramine and cadaverine located in the ranges of 70.65-180.14, 90.65-100.29 and 40.84-100.9 mg kg^{-1}, respectively in Egyptian Gouda cheese. Also, Leuschner et al. (1998) found higher levels of histamine and tyramine in the German Gouda cheese than ours. Their levels of histamine and tyramine were 84.07-104.45 and 217.05-322.52, respectively (at 60 days of ripening) and were 178.9-237.94 and 337.32-636.19, respectively (at 90 days of ripening). While their recorded levels of putrescine (1.84-16.71 at 60 days and 3.95-41.57 at 90 days) were close to those levels of our study.

TABLE 4. Levels of biogenic amines (mg kg-1) in Gouda cheese samples

Biogenic amines	Control (60 days)	Control (90 days)	T3 (60 days)	T3 (90 days)	LSD (0.05)
Treptamine	ND	ND	ND	ND	-
β-phenyl-ethyl -amine	ND	ND	ND	ND	-
Putrescine	6.82±0.473^{B}	8.01±0.294^{A}	0.17±0.011^{C}	0.17±0.023^{C}	0.9095
Cadaverine	11.69±0.669^{A}	11.79±0.456^{A}	1.8±0.139^{B}	2.94±0.023^{B}	1.341
Histamine	1.36±0.075^{A}	1.39±0.081^{A}	0.01±0.001^{B}	0.01±0.003^{B}	0.1799
Serotonine	ND	0.28±0.017^{A}	ND	0.01±0.001^{B}	0.0283
Tyramine	0.22±0.012^{B}	0.28±0.012^{A}	ND	0.3±0.028^{A}	0.0541
Spermidine	0.28±0.012^{B}	0.97±0.035^{A}	ND	0.16±0.012^{C}	0.0624
Spermine	0.24±0.012^{A}	0.02±0.001^{BC}	0.01±0.001^{C}	0.03±0.001^{B}	0.019

* ND: not detected.

According to many earlier studies, Mehanna et al. (1989) and Ibrahim & Mehanna (2016) reviewed the factors which may affect BAs formation in cheese which included availability of free amino acids for decarboxylation, decarboxylative properties of starter and existence of a proper environment in the cheese as pH, temperature, salt, and water activity. On the other hand, the prementioned authors reviewed some important useful functions of BAs if presented in the human body in certain quantities, which can cause adverse effects if the amounts of many BAs are high enough.

Briefly, it is worthy to mention that the addition of probiotic bacteria (*L. plantarum*), in the present study, to the traditional starter containing *L. lactis* led to the decrease of BAs formation in Gouda cheese. The ability of lactic acid bacteria (such as *L. Lactis*, *L. Plantarum*, *L. parabuchneri*, *S. thermophilus*, *Ln. mesenteroides*, *Lc. lactis*, and *Ln. lactis*) to decrease the formation of BAs was reported in the literature (Fadda et al., 2001; Callejón et al., 2014; Guarcello et al., 2016). However the lower BAs concentration in T3 than in control samples could be attributed to a synergistic effect between *L. lactis* and *L. plantarum*.

Conclusion

Results of the present study exhibited the importance of the partial usage of buffalo's milk on the nutritive value and the economical benefit for the countries where the buffalo's milk is more produced than that of cow. Also, the usage of probiotic bacteria, as in T3, led to a significant decrease in biogenic amines content. This result ensured the safety of the produced Gouda cheese containing *L. Plantarum*. So, these findings enable us to conclude that the treatment 3 (25% buffalo's milk + 75% cow's milk + *L. plantarum* ATCC14917) showed the best quality and safety characteristics of Gouda cheese. Additionally, it is recommended to market the Gouda cheese at 60 days old for better safety properties concerning the formation of biogenic amines which increases by time.

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تأثير استخدام اللبن الجاموسي مع اللبن البقري والسلالات البكتيرية المنتقاه علي خواص وسلامة جبن الجودا

قامت الدراسة الحالية بتقييم التغيرات في التركيب الكيميائي والخواص الريولوجية للجبن الجودا نتيجة الاستبدال الجزئي للحليب البقرى بـ ٢٥٪ حليب جاموسى بالإضافة لإستخدام أنواع مختلفة من بكتريا البروبيوتيك. كذلك ، تم تقدير الأمينات الحيوية فى الجبن الناتج من المعاملات المختلفة لتقييم مدى سلامته. أشارت النتائج إلي ارتفاع تدريجي في محتوي الأحماض الأمينية الحرة فى عينات الجبن لمختلف المعاملات خلال فترة النضج. علاوة على ذلك، فقد أدى الاستبدال الجزئي لحليب البقر بنسبة ٢٥٪ من حليب الجاموس في الجبنة الجودا (المعاملة رقم ١) إلى زيادة صفات الصلابة والتماسك فى الجبن بشكل ملحوظ عن الصفات القياسية. بينما لوحظ بعض التحسن في المعلاملات رقم (٢) ، (٣) أثناء النضج. وعموما ، في نهاية فترة النضج ، تم تسجيل اختلافات كبيرة بين الكنترول والمعاملة رقم (١) من ناحية وبين الجبن من المعاملتين رقم (٢) ، (٣) مما يشير إلى مزيد من التحسن الناتج عن تأثير استخدام بكتيريا البروبيوتيك. وأخيرًا، فقد أظهرت النتائج أن المستويات المكتشفة من الأمينات الحيوية في عينات الجبن كانت ضمن الحدود الآمنة التي أوصت بها منظمة الغذاء والدواء (FDA) ، ومع ذلك فقد إحتوت المعاملة رقم (٣) (المحتوية على *L. plantarum*) على أقـل محتوى من الأمينـــات الحيويـة من البكتيريـــا لأن إضافة بكتيريا البروبيوتيـــك (*L. Plantarum*) أدت إلى انخفاض تكوين الأمينات الحيوية في الجبنة الجودا.