

Full Length Research Paper

The Influence of Starter culture on accumulation of Biogenic amines in Ripening period of Ras cheese

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Abstract

Four Ras cheese treatments were made by replacing traditional cheese starter with *Lactobacillus casei*. DSM2011. (T1)cheese made with traditional starter; (T2) cheese treatment made by using 0.75% traditional starter + 0.25% *Lbcasie* and (T3) cheese made by using 0.50% traditional starter +50%*Lbcasie* and (T4) cheese made by using 0.75% *Lbcasie* +0.25% traditional starter. Cheese treatments were analyzed for moisture content, total nitrogen, fat, ash, titratable acidity, soluble nitrogen (S.N), total volatile fatty acids (T.V.F.A), biogenic amine and organoleptic were evaluated when fresh and at 1, 2, 3 and 4 ripening months. Organoleptic properties increased by replacing the traditional cheese starter with *Lactobacillus casei* and this increase were proportional with the increasing replacing rate. Moisture content of all cheese treatments decreased during ripening period, while fat, total nitrogen, soluble nitrogen, T.V.F.A and scores of organoleptic properties increased as ripening period processed, also cheese treatments had different amount from different biogenic amines. Biogenic amines increased as ripening some period advanced, while some biogenic amines decreased.

Key words: Ras cheese, *Lactobacillus casei*, Biogenic amines

Introduction

Ras cheese is the most popular hard cheese in Egypt Starter cultures (lactococci and lactobacilli) used for cheese fermentation degrade milk proteins by means of proteases and peptidases to peptides and free amino acids. These breakdown products support the growth of starter cultures and are also important to achieve the characteristic texture and aroma of the cheese. On the other hand, they may increase the potential for amine formation during cheese fermentation when microorganisms are present which exhibit a decarboxylase activity (Leuschner and Hammes, 1998). Utilization of starter cultures enables producers to make food products with a standard quality in a shorter time. Selection of the starter culture, however, should not be only done considering the lactic acid production of the strains but also their activity in biogenic amine synthesis. (Halász et al., 1999). Biogenic amines are organic bases with an aliphatic, aromatic, or heterocyclic structure which have been found in many foods, such as fishery products, cheese, wine, beer, and other fermented foods (Stratton et al. 1991). Utilization in cheese making raw or pasteurized milk, higher ripening temperature, excessive proteolysis, high pH and low salt concentration may contribute to the ability of an organism to produce histamine (Gardini et al., 2001). Increases in the amine content of cheese may be attributed to various microorganisms. These microorganisms may make up the flora associated with the milk used to make the cheese, by contamination during cheese making and storage may be added to the cheese deliberately in starter cultures (Marino et al., 2000).

Cheese is considered a good source of proteins, vitamins and minerals biogenic amines (BAs) contamination. These compounds are basic nitrogenous compounds formed by series of microorganisms, mainly by decarboxylation of amino acids or “in vivo” also by deamination and trans-amination of aldehydes and ketones (Loizzo et al., 2012, 2013). Biogenic amines are compounds commonly present in living organisms in which they are responsible for many essential functions. They can be naturally present in many foods such as fruits and vegetables, meat, fish, chocolate and milk, but they can also be produced in high amounts by microorganisms through the activity of amino acid decarboxylases (Ten Brink et al., 1990). Excessive consumption of these amines can be of health concern because there is no equilibrate assumption in human organism, can generate different degrees of diseases determined by their action on nervous, gastric and intestinal systems and blood pressure (Suzzi and Gardini, 2003). Consumption of food containing high levels of BAs is considered undesirable since it can be associated with several toxicological problems such as respiratory distress, headache, hyper- or hypo-tension or allergies (Ladero et al., 2010). These problems are especially severe in consumers with low levels of the enzymes involved in the detoxification system (mono and di-amine oxidases), either by genetic disorders (Caston et al., 2002) or medical treatments (Halász et al., 1994). The importance of observing BAs content lies in potential toxicity to human, mainly when the concentration is up to 100 mg/kg (or up to 100 mg/L). Thus, the presence of BAs significantly influences the food quality and safety (Smit et al., 2005). The presence of relevant amounts of BAs in cheeses has been documented (Martuscelli et al. 2005; Kung et al. 2007; Pintado et al. 2008; Ladero et al. 2009; Mercogliano et al. 2010). In cheeses, BAs formation is caused by curdling and cheese decarboxylase-positive microorganisms. Histamine (HIS), tyramine (TYR), putrescine (PTR), cadaverine (CAD), spermidine (SPD), spermine (SPR), tryptamine (T), and β -phenylethylamine (PE) are frequently found in these products. Cheese is one of the fermented foods most commonly associated with BAs poisoning; mainly HIS, TYR, PTR and CAD. Indeed, the term “cheese reaction” is refer to it (Ten Brink et al., 1990). Tyramine and histamine are the most abundant and frequent BAs in cheese (Fernández et al., 2007). The content of biogenic amines and polyamines significantly differed according to the technology of ripening. The cheeses unwashed during ripening had much higher contents of all observed amines and polyamines in comparison with the washed-rind cheeses.

The effect of storage on the amine formation was not confirmed (Samková et al. 2013). Physiologically, histamine is one of the most effective BAs; it has vasoactive and psychoactive effects (Repka Ramirez and Baraniuk, 2002). Moreover, it is the main BAs involved in food poisoning and it is limited in some foodstuffs by law. At non-toxic doses, food borne histamine can cause intolerance symptoms such as diarrhoea, hypotension, headache, pruritus and flushes. Just 75 mg of histamine, a quantity commonly present in some meals, can induce symptoms in the majority of healthy persons with no history of histamine intolerance (Whrletal., 2004). The ability of microorganisms to decarboxylase amino acid is highly variable. Due to strain-specific, it is important to count decarboxylase-positive microorganisms to estimate; the risk of BAs food content and to prevent BAs accumulation in food products. Presence and accumulation of BAs depends on many factors such as presence of specific bacteria (Enterococci, Micrococci, Enterobacteriaceae and Lactobacilli) and enzymes, availability of free amino acids, presence of suitable cofactors, that is, pH level, water activity, temperature and salt content, type of cheese, ripening and storage period (Galgano et al., 2001). Some controversial results have been reported on the contribution of *Enterococci sp.* in BAs production in cheeses, and in particular in histamine (Sumner and Taylor, 1989). Some LAB generally used as starter cultures, may have specific amino acid decarboxylase activities and thus, the potential to synthesize BA that could be accumulated in the dairy products. Belonging to this group are lactococci, lactobacilli and streptococci.

Another way to reduce the accumulation of BA in dairy products, could be the use of adjunct cultures that include bacteria capable to degrade BA (Leuschner and Hammes, 1998 and Naila et al. 2010). The objective of this study were to study the effect of replacing traditional starter milk *Lactobacillus casei* on the formation of biogenic amines in Ras cheese and evaluate the equality of Ras cheese made with *Lactobacillus casei* and to monitor the changes in cheese quality during ripening period of Ras cheese. (Gardini et al., 2002; García-Ruiz et al., 2011; Zaman et al., 2011).

Materials and Methods

Bacteria strains and propagation:

Active *Streptococcus thermophilus* ENCC1043 *Lactobacillus delbruechii* subsp. *bulgaricus* EMCC1102 and *Lactobacillus casei* were obtained from the Egyptian Microbial Culture Collection (EMCC) at Cairo Microbiological Resources Center (Cairo Mircen), Faculty of Agriculture, Ain Shams University. These strains were activated individually by three successive transfers in sterile 10% reconstituted non-fat dry milk.

Cheese making

Bulk fresh cows' milk (obtained from the herd of Toch Tanbash farm, Minufia, Egypt) was pasteurized at 63° for 30 min. cooled to 35°C and divided to five batches. Control cheese treatment (T1) was made by adding 1.0% of traditional starter (50% *Streptococcus thermophilus* + 50% *Lactobacillus delbruechii* subsp. *bulgaricus*) and the other three treatments made by adding 0.75% traditional starter + 0.25% *Lactobacillus casei* DSM 2011 (T2); 0.50% traditional starter + 0.50% *Lactobacillus casei* DSM2011 (T3); 0.25% traditional starter + 75% *Lactobacillus casei* (T4) respectively. Ras cheese treatments were made according to Abdel-Tawab (1963) except that calcium chloride was added at the rate of 0.02% of milk.

The resultant cheese was ripened for four months. All cheese treatments were sampled and analyzed when fresh and at 1, 2, 3 and 4 months for chemical and biogenic amine sensory properties. The whole experiment was duplicated.

Chemical Analysis

Cheese samples were analyzed for moisture, salt, fat, ash according to A.O.A.C (2000). Titratable acidity, pH, soluble nitrogen (S.N), and total nitrogen (TN) were determined as described by Ling (1963). Total volatile fatty acid (T.V.F.A) were estimated by the method of Kosikowski (1966). Biogenic amines (Tryptamine, B-phenyl ethyl amine, Putrescine, Cadaverine, Histamine, Tyramine, Spermidine and Spermine) were extracted and determined according to Mietz and Karmas (1977), Ayesh.etal (2012), Sultan and Marrez 2014 with some modifications as follows by using High performance liquid chromatography (HPLC) used for dansylamines determination was an Agilent 1100 system equipped with quaternary pump model G1311A, UV detector model G1314A set at 254 nm wavelength, autosampler model G1329A. Agilent Zorbax Eclipse XDB C18 4.6 mm × 150 mm, 5m column was used for biogenic amines separation. Data were integrated and recorded using Chemstation Software program.

Sensory Evaluation

Cheese samples were evaluated for appearance, flavor and body & texture according to the scoring sheet of El-Shafei et al., (1995) by the staff members of Dairy Science Dept., Food Technology Institute Agriculture Research Center Giza, Egypt.

Statistical analysis

2X3 factorial design was used to analyze all the data and the student Newman Keuls' test was used to make the multiple comparisons (Steel and Torric, 1980). Using Costat program, significant differences were determined at $p \leq 0.05$.

Results and Discussion

Each value represent the mean value (mg. kg⁻¹) ±SD. C: fresh samples, T1: samples with traditional starter, T2: samples with 75% traditional starter + 25% *Lb.casei*, T3: samples with 50% traditional starter + 50% *Lb.casei*, T4: samples with 25% traditional starter + 75% *Lb.casei*. The effect of adding *Lb. casei* on biogenic amine formation in Ras cheese is shown in Table (1). From such data it could be noticed that the total biogenic amines was significantly increased ($P \leq 0.05$) with the elongation of the ripening period. The total biogenic amines content of fresh Ras cheese samples was recorded 2.32±0.31mg.kg⁻¹. The average concentration of tryptamin, ethylamine, putrasin, Cadavrein, histamine, Tyramine, Spermidine and Spermine were 0.04, 0.75,

0.26, 0.86, 0.10, 0.23, 0.03 and 0.04 mg. kg⁻¹, respectively. After four months of storage, the average concentration were increased by the 400, 153, -30, -88, 58, 1810, 200 and 150% for all of these amines, respectively. On the other side, the adding of starter culture leads to significant decreasing on the formation rate of biogenic amines in Ras cheese samples. Such decreasing rates were increased with the increasing of starter culture concentration. The most significant decreasing was recorded for both putrasin and cadavrein.

Table 1. Effect of adding *Lb. casei* on biogenic amine formation in Ras cheese (mg. kg⁻¹)

Biogenic amine (BA)	C (Mean±SD)		Four months ripening samples											
			T1		T2		T3		T4					
			Mean±SD	% of change	Mean±SD	% of change	Mean±SD	% of change	Mean±SD	% of change				
Tryptamine	0.04±	0.01	2.82±	0.47	6950	2.77±	0.88	6825	1.40±	0.22	3400	0.20±	0.04	400
Ethyl amine	0.75±	0.07	4.48±	0.74	497	6.40±	1.94	753	4.08±	0.80	444	1.90±	0.24	153
Putrasin	0.26±	0.03	12.63±	3.45	4795	2.56±	0.63	892	1.14±	0.27	342	0.18±	0.07	-30
Cadavrein	0.86±	0.11	7.82±	1.89	807	1.37±	0.45	59	0.40±	0.16	-54	0.10±	0.03	-88
Histamine	0.10±	0.01	3.91±	0.65	3771	3.82±	0.99	3682	0.81±	0.22	702	0.16±	0.02	58
Tyramine	0.23±	0.02	9.35±	1.54	3913	6.23±	1.44	2574	2.43±	0.36	943	4.45±	0.55	1810
Spermidine	0.03±	0.01	4.00±	0.76	13233	0.98±	0.33	3167	0.16±	0.04	433	0.09±	0.01	200
Spermine	0.04±	0.02	4.38±	0.72	9855	1.11±	0.43	2423	0.72±	0.13	1536	0.11±	0.03	150
Total	2.32±	0.31	49.39±	10.15	2031	25.24±	7.27	989	11.14±	1.99	381	7.19±	0.91	210

C: fresh samples

T1: samples with traditional starter

T2: samples with 75% traditional starter + 25% *Lb.casei*

T3: samples with 50% traditional starter + 50% *Lb.casei*

T4: samples with 25% traditional starter + 75% *Lb.casei*.

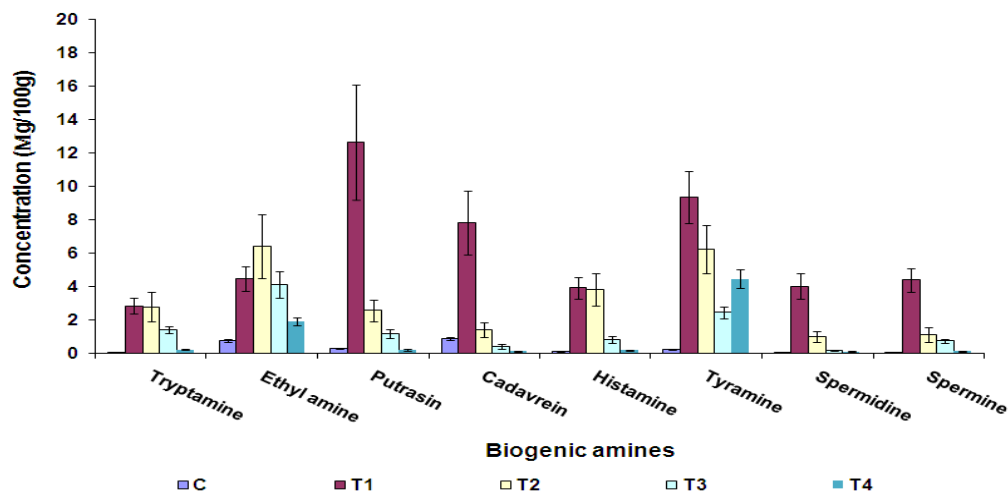


Fig 1. Effect of adding *Lb. casei* on biogenic amine formation in Ras cheese.

In similar study, Nour El-Din (2014) found that the total biogenic amines detected in cheese samples was increased by the ratio of 3278.92% as the result of storage for 6 months at 15°C and 85% relative humidity. The biogenic amine, tyramine, was constituted 70.98% of the total amines content detected in both fresh and storage cheese samples. Also, Similar result was reported for other cheeses (Joosten 1988; Darwish 1993; Abdalla *et al.*, 1996 and Petridis and Steinhart 1996a).

Many authors have reviewed factors affecting amine formation in cheese. These factors include: storage temperature (Stratton *et al.*, 1991; Halasz *et al.*, 1994; Ragab, 2003 and Nour El-Din, 2014), air redox potential of the medium (Halasz *et al.*, 1994), pH (Diaz *et al.*, 1992; Pogorzelski, 1992; and Majjala *et al.*, 1993), salting (Babu *et al.*, 1986; Joosten, 1988; Yatsunami and Echigo, 1993 and Teodorovic *et al.*, 1994) and microorganisms present (Joosten and Northolt, 1987; Koehler and Eitenmiller, 1978; and Joosten, 1987). From such studies it could be noticed that increasing of storage temperature and degree of salting, reducing the redox potential of the medium, and acidic environment, leads to increase the formation of biogenic amines in Damiatta cheese. This information's gives an explanation for the larger concentrations of biogenic amines reported in ripening Ras cheese than the fresh ones. Additionally to these factors, the present study confirmed that the adding of starter culture leads to significant decreasing on the formation rate of biogenic amines in Ras cheese samples. Such decreasing rates were increased with the increasing of starter culture concentration.

When compared the obtained data with the limits of biogenic amines permissible in foods for human consumption it could be noticed that all Ras cheese samples recorded values less than the permissible limits mentioned by Sandler *et al.*, (1974), Taylor,

(1985) and Luten *et al.*, (1992). So, it couldn't be threaten the human health consumed these cheese. In this concern, Sandler *et al.*, (1974) reported that 3 mg of phenylethylamine cause migraine headaches in susceptible individuals, while 6 mg total tyramine intake was reported to be a dangerous dose for patients receiving monoamine oxidase inhibitors. While, Taylor, (1985) mentioned that the level of 1000 mg/kg (amine/food) is considered dangerous for health. This level is calculated on the basis of food borne histamine intoxications related to amine concentration in food. The discrepancy in the toxic histamine level in food might be due to the absence or presence of other synergistic biogenic amines like putrescine and cadaverine. Also, Luten *et al.*, (1992) reported that the European Community has recently proposed that the average content of histamine should not exceed (0 - 20 mg/100 gm of fish). On the other side, Halasz *et al.*, (1994) and Santos, (1996) reported that the exact toxicity threshold of biogenic amines in individuals is extremely difficult. The toxic dose is strongly dependent on the efficiency of the detoxification mechanisms of different individuals. Upper limits of 100 mg histamine/kg in foods, 2 mg histamine/ L in alcoholic beverages, and 100 - 800 mg/kg tyramine and 30 mg/kg phenylethylamine in foods have been suggested (Brink *et al.*, 1990).

In general, the data of this study proved the importance of using selected starter culture as natural potent components in both therapy and food technology. Also, the present study with the others recommended the Egypt government should be applied a critical control point during the cheese manufacture and selected the suitable starter culture unable to formation of biogenic amines and to obtain cheeses with low or moderate levels of biogenic amines as well as with high quality safety.

Chemical composition of Ras cheese

Changes in moisture content during ripening of Ras cheese are presented in Table (2) at the end of this paper. Moisture content of Ras cheese decreased significantly ($P \leq 0.05$) by replacing the traditional cheese starter with *Lb. casei* and this decrease was proportional with increasing the rate of replacement. Cheese treatments that made by adding the highest ratio of *Lb. casei* (75%) contained the lowest moisture content (Tables 2, 5). These results might be due to the increase of cheese acidity, which consequently helps to expel the whey from the cheese curd.

Moisture content of all probiotic Ras cheese decreased as ripening period proceeded. Moisture content decreased rapidly during the first months, and then decreased gradually up to the end of ripening period table (2,5). These results are in agreement with those reported by Badawi (1998), Hussein *et al.* (2006), Mehanna *et al.* (2002) and Kebary *et al.* (1996).

Total nitrogen, fat and ash contents of probiotic Ras cheese increased slightly ($P \leq 0.05$). By increasing the rate of replacing traditional cheese starter with *Lb. casei* and as ripening period progressed. These results could be attributed to the loss of moisture content (Table 2,5). Similar trends were reported by Badawi (1998); Mehanna *et al.* (2002), Kebary *et al.* (1996), Chen *et al.* (2009) and Fayed *et al.* (2006).

Titrate acidity of Ras cheese treatments is illustrated in Table (3) at the end of this paper. Titrate acidity of Ras cheese increased significantly ($P \leq 0.05$) by replacing the traditional cheese starter with *Lb. casei* (Tables 3,5). There were positive correlation between cheese acidity and the rate of replacement, which means that the increase of cheese acidity was proportional to the rate of replacing traditional cheese starter with *Lb. casei*. These results are in accordance with those reported by Mehanna *et al.* (2002), who reported that this increase in cheese acidity could be attributed to the ability of *Lb. casei* to produce lactic acids from lactose. On the other hand titrate acidity of all cheese treatments increased significantly ($P \leq 0.05$) as ripening period advanced. (Tables 3,5). Similar trends were reported by Badawi (1998), Mehanna *et al.* (2002) and Fayed *et al.* (2006).

Salt content of all Ras cheese treatments increased as ripening period advanced (Table 3). These which results are in agreement with those reported by Badawi (1998); Hussein (2000); Taha *et al.* (2007); AbdAlla *et al.* (2008); Mehanna *et al.* (2009) significant ($P < 0.05$) as ripening period progressed (Tables 3,5). These results are in accordance with those reported by Badawi (1998); Hussein (2000).

Changes in ripening indices {water soluble nitrogen (WSN), total volatile fatty acids (TVFA), during ripening of Ras cheese are shown in Table (3). WSN, TVFA and increased significantly ($P \leq 0.05$) by increasing the rate of replacing traditional starter with *Lb. casie* (Tables, 3 and 5). There were positive correlation between the rate of replacement and the values of WSN, T.V.F.A. These results are in agreement with those reported by Chen *et al.* (2009) and Mehanna *et al.* (2002). These results could be attributed to the presence of proteolytic and lipolytic system of *Lbcasie* (Bergamini *et al.*, 2009). Also WSN and TVFA of all cheese treatments increased significantly ($P \leq 0.05$). Throughout the ripening period (Tables, 3,5).

These results are in agreement with those reported by Badawi (1998), Mehanna *et al.* (2002); Fayed *et al.* (2006) and Chen *et al.* (2009). Cheese treatment (T4) that made with adding 1.0% *Lb. Casei* showed the highest values for WSN, TVFA and (Tables, 3,5).

Organoleptic evaluation of Ras cheese

Scores of organoleptic properties (flavour, body& texture, appearance and total scores are presented in Table (4) at the end of this paper. Scores of flavour, body& texture, appearance and total scores of all Ras cheese treatments followed similar trends. Scores of organoleptic properties (flavour and total scores) of all cheese treatments increased as ripening period progressed (Tables,4,5). Similar results were reported by Badawi (1998); Fayed *et al.* (2006) and Mehanna *et al.*, (2002). On the other hand scores of organoleptic properties increased with replacement of traditional starter with *Lb. casei* (Tables ,4,5). There were positive

correlation between the rate of replacement and scores of organoleptic properties. Cheese made with 25% traditional starter + 75% *Lb.casei* gained the highest score.

Table 5: Statistical analysis of properties of Ras cheese as effected by *Lb. casei* bacteria

Cheese Properties	Effect of treatment					Effect of ripening period (Month)					
	Mean Squares	Multiple Comparisons				Mean Squares	Multiple Comparisons				
		T1	T2	T3	T4		0	1	2	3	4
Chemical properties											
Moisture (%)	0.278	A	B	C	D	67.227*	A	B	AC	C	D
Fat (%)	0.040	A	A	A	A	4.403	B	A	A	A	A
TN (%)	0.032*	B	A	A	A	0.039	A	A	A	A	A
Acidity	0.445*	B	A	A	A	7.213*	E	D	C	D	A
SN (%)	0.583*	B	B	AB	A	0.131	A	A	A	A	A
TVFA (ml 0.1N NaOH/100gm)	351.19*	B	B	B	A	3565.69*	D	C	B	A	A
Salt (%)	0.146*	B	B	AB	A	1.719*	C	C	C	B	A
Ash (%)	1.190*	C	B	A	BA	0.043*	B	AB	B	AB	A
Organoleptic properties											
Flavour (50)	15.533*	D	C	B	A	75.142*	D	C	C	B	A
Body and texture (40)	13.000*	C	B	B	A	124.350*	D	C	B	B	A
Color & appearance (10)	0.549	A	A	A	A	3.525	A	A	A	A	A
Total Score (100)	63.749*	D	C	B	A	415.725*	E	D	C	B	A

T1: samples with traditional starter,

T2: samples with 75% traditional starter + 25% *Lb.casei*,

T3: samples with 50% traditional starter + 50% *Lb.casei*,

T4: samples with 25% traditional starter + 75% *Lb. casei*.Data with different letters on the same raw have significantly different at $p \leq 0.05$.

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Table 2. Effect of adding *Lb. casei* on the gross chemical composition of Ras cheese (g. 100g⁻¹)

Treatment	Ripening period (months)																			
	Moisture					Fat					Ash				Total nitrogen (TN)					
	fresh	1	2	3	4	fresh	1	2	3	4	fresh	1	2	3	4	fresh	1	2	3	4
T1	40.83	38.66	38.19	38.36	33.86	39.12	40.18	40.25	40.32	40.60	5.25	5.25	5.38	5.52	5.62	3.20	3.25	3.28	3.30	3.33
T2	40.73	39.38	38.77	37.5	33.70	39.14	40.19	40.35	46.2	40.70	5.30	5.34	5.45	5.58	5.76	3.25	3.28	3.30	3.32	3.35
T3	40.72	38.57	37.45	37.20	33.45	39.15	40.20	40.20	40.8	40.75	5.42	5.65	5.68	5.70	5.78	3.32	3.34	3.36	3.38	3.40
T4	40.72	39.86	38.43	37.85	34.24	39.15	40.22	40.65	40.2	40.77	5.35	5.40	5.60	5.69	5.98	3.16	3.20	3.25	3.27	3.42

T1: samples with traditional starter

T2: samples with 75% traditional starter + 25% *Lb.casei*

T3: samples with 50% traditional starter + 50% *Lb.casei*

T4: samples with 25% traditional starter + 75% *Lb. casei*.

Table 3. Effect of adding *Lb. casei* on salt, acidity, soluble nitrogen (SN) and total volatile fatty acids (TVFA)

Treatment	Ripening period (months)																			
	Salt				Acidity				SN (g.100g ⁻¹)				TVFA (ml 0.1N NaOH/100g)							
	fresh	1	2	3	4	fresh	1	2	3	4	fresh	1	2	3	4	fresh	1	2	3	4
T1	3.6	3.92	3.96	3.98	4.35	0.42	1.16	1.3	1.20	2.33	0.185	0.201	0.225	0.290	0.380	24.60	35	50	56	58
T2	3.6	3.52	3.62	3.80	4.34	0.39	1.9	1.98	2.25	2.3	0.186	0.245	0.305	0.347	0.440	24	35	55	62	66
T3	3.5	3.43	3.65	3.90	4.20	0.40	1.60	1.9	2	2.7	0.183	0.260	0.331	0.440	0.520	24	42	54	63	72
T4	3.55	3.34	3.50	3.85	4.96	0.41	1.5	1.8	2.15	2.6	0.180	0.280	0.372	0.450	0.535	26	55	59	73	82

T1: samples with traditional starter

T2: samples with 75% traditional starter + 25% *Lb.casei*

T3: samples with 50% traditional starter + 50% *Lb.casei*

T4: samples with 25% traditional starter + 75% *Lb. casei*.

Table 4. Effect of adding *Lb. casei* on score of organoleptic properties of Ras cheese

Treatments	Ripening period (months)																			
	Flavor (out of 50)					Body and Texture (out of 40)					Appearance (out of 10)					Total Score (100)				
	fresh	1	2	3	4	fresh	1	2	3	4	fresh	1	2	3	4	fresh	1	2	3	4
T1	38	40	41	42	44	28	30	32	32	34	7	7	7	7	8	71	77	80	81	86
T2	39	41	42	43	45	27	31	32	33	35	7	7	8	8	8	73	79	82	84	88
T3	39	42	42	44	46	28	32	33	35	305	7	7	7	8	8	74	81	82	87	89
T4	39	43	43	45	47	28	32	34	34	37	7	7	7	8	9	74	82	84	87	92

T1: samples with traditional starter

T2: samples with 75% traditional starter + 25% *Lb. casei*

T3: samples with 50% traditional starter + 50% *Lb. casei*

T4: samples with 25% traditional starter + 75% *Lb. casei*.