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Effect of Heat Treatments of Milk on Biogenic and Free Amino Acids of Low Fat Ras Cheese

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ABSTRACT

Four Ras cheese treatments were made by heating cheese milk 1.5 % fat for 65, 70, 75 and 80 ^{o}C. Fresh samples of mentioned treatments were analyzed for moisture, fat, total nitrogen, salt and acidity. Analyzed for free amino acids and biogenic amines was carried out when fresh, and after 3 and 6 months. Total biogenic amines and free amino acids increased as ripening period progressed in all cheese treatments. Total biogenic amines of cheese made from 1.5% fat milk increased by raising the temperature of heat treatment up to 75 ^{o}C (T3). However, the total free amino acids and biogenic amines of cheese made from 1.5% fat milk decreased by raising the temperature of heat treatments up to 80 ^{o}C (T4).

Keywords: Biogenic amines – Free amino acids – Ras cheese

INTRODUCTION

Cheese is considered as a good source of proteins, vitamins and minerals. It is also considered as one of the most important fermented foods, commonly associated with biogenic amines contamination. These amines are usually resulted in by several microorganisms, mainly by the decarboxylation of amino acids, and also by deamination 0f aldehydes and ketons (Loizzo *et al.,* 2012, 2013).

Ras cheese is the most popular hard cheese in Egypt. Regarding the health as affected with the presence of fat, considerable and substantial interest have been focused on the development of a know dairy products similar to the existing ones, but are of considerable lower fat content. Such new products, I,e low fat cheese, are usually lack of flavor and characterized with weaker body and texture Williams . An increase of amino acids content usually accompanied with the proteolysis occurred during the ripening of cheese. Decaboxylation of Some of these free amino acids could associated with specific bacterial strains resulting in the formation of the amines. These amines are referred as biogenic, which possess harmful physiological effects in human and animals. These biogenic amines could be detected in cheese. The formation of biogenes (Histamine, tyramine, putrescine, cadaverine, spermidine, spermine, tryptamine and β-phenylethylamine) usually resulted in by decarboxylase^{+} bacteria, and frequently detected in cheese. Biogenic amines of poisoning effect are mainly histamine, tyramine, putrescine and cadaverine, (Edwards and Sanine, 1981, Joosten and Stadhouders 1987, Ten Brink et al.1990, Martuscelli *et al.,* 2005, Fernandez *et al.,* 2007 and Mercogliano *et al.,* 2010). Swiss, Emental, Cheddar, Gouda, Blue, Ras, Processed chesses and Hungarian hard cheese were all found to contain biogenic amines (and El- Sonbaty *et al.,* 1998). The presence and accumulation of these amines were the result of the specific bacterial cultures contaminating the cheese, the presence of responsible enzymes and the substrates for their formation, pH, moisture and salt content of the environment, and type of cheese, and the storage period of cheese (Edward and Sandine, 1981, Josten 1988, Degheidi *et al.,* 1992 Tawfik *et al.,* 1992, Darwish, 1993 , Abd- Alla *et al.,* 1996, and Petridis and Stenhart, 1996 a, b and El-Sonbaty *et al.,* 1998).

The objectives of this study were to investigate the effect of heat treatments of milk on the formation of free amino acids and biogenic amines in low fat Ras cheese.

MATERIALS AND METHODS

Fresh cow's milk was obtained from the farm, of the Faculty of Agriculture Al-Azhar Univ., Assiut. Calf rennet powder (Ha-La) and yoghurt culture consisting of *S. thermophillus* and *L. delbruckii ssp. bulgaricus* were obtained from CHR. Hansen's Lab. Denmark. The rennet was added at the rate of 3g/100 liter of milk cheese. Fine cooking salt was obtained from the local market of Assiut Egypt.

Low fat Ras cheese was made from raw cow's milk, being standardized to 3 and 1.5% fat to serve as control (1) and control (2), respectively. Other treatment of low fat Ras cheese was made from cow's milk 1.5% fat, and divided into four treatments as follows:

T_1: Heating the milk up to 65^{o}C
T2: Heating the milk up to 70^{o}C
T_3: Heating the milk up to 75^{o}C
T4: Heating the milk up to 75^{o}C

All cheeses were made according to the method described by Hofi *et al.,* (1970), with some modification, by adjusting the renneting temperature at 32^{o}C, calcium chloride was added at the rate of 0.015 and 0.020 % to milks heated up to 75 and 80^{o}C, respectively. The cheese were allowed to ripen under controlled condition of temperature 13±2^{o}C, and relative humidity of about 85%. cheese were sampled and analyzed when fresh, and after 90 and 180 days of ripening. Yield of fresh (before and after salting) and at the end of the ripening period were expressed as kg/100kg of milk.

Cheeses were analyzed in triplicates for the moisture content at 120^{o}C (IDF, 1993). Salt by titration with $AgNo_3$, fat by Gerber method , total nitrogen (TN) and titratable acidity, TA using the methods of AOAC (2002).The pH of milk and cheese samples was detected using pH- meter with combined electrode, HANNA HI 8014 pH meter (HANNA, Instrument Portugal). Free amino acids and biogenic amines were assayed according to the methods of (Krause *et al.,* 1995) and

modified by (Pinho *et al.,* 2001). All results were statistically analyzed using analysis of variance (ANOVA) and the differences between means were tested using Duncan's test as well as average computer program (SPSS, 1999) AT <0.05, level of significance.

RESULTS AND DISCUSSION

Results in Table (1) indicate that fresh Ras cheese made by heating milk at 80°C (T_4) contained higher moisture content, compared with other treatments, followed by those of T_3, T_2, C_1(control $_1$), T_4, then came C_2 (control $_2$). The increase in moisture content of cheese made by heating milk might be due to impairing of whey syneresis from curd. These results are in agreement with those of Badawi (1985), Walstra *et al.,* (1985), Kebary *et al.,* (1999), Hussein (2000), Okasha (2001), Abd- Alla (2008) and El- Zahar (2014).

Results in Table (1) demonstrate the Fat content of cheese made from different milk fat levels. It is evident that fat content of cheese decreased by decreasing milk fat levels. These results are in harmony with those of Hussein (2000), Kebary *et al.,* (2002), Ghita *et al.,* (2004), Ragab (2014) and Amer (2015). Table (1) also indicated that the samples of treatment$_4$ had The highest total nitrogen content in the fresh cheese was detected, compared with the other treatments, followed by those of treatments 3, 2, 1 then control 2 and 1 (Table 1). Similar results were also stated by Badawi (1998), Abou El-Nour *et al.,* (2004), Abd- Alla (2008), Ragab (2014) and Amer (2015). Salt content of fresh Ras cheese with different fat level is given in Table (1). It was noticed that samples of the control (2) had the highest salt content at the beginning of ripening. Samples of C_2 had the highest acidity in the fresh cheese, compared with the other treatments, followed T_1, T_2, C_1, T_3 and T_4. These results are in accordance with those reported by Osman *et al.,* (2001), Sakr and Mehana (2011) and Amer (2015).

Results in the same Table, On the other hand, show the variation in the cheese yield of various treatments. As could be seen from these data, control (1) was of

Table 1. Effect of heat treatments of milk on yield and some chemical properties of fresh Ras cheese.

Property	C_1	C_2	T_1	T_2	T_3	T_4	LSD
Moisture	45.89D	44.39F	45.58E	46.87C	47.69B	49.90A	0.1779
Fat	20.21A	17.83B	17.24C	16.85D	15.99E	14.35F	0.2754
Total nitrogen	3.10F	3.71E	3.92D	4.04C	4.17B	4.42A	0.1082
Salt	1.75AB	1.83A	1.79A	1.75AB	1.70AB	1.64B	0.1488
Acidity	1.25C	1.31A	1.28B	1.25C	1.21D	1.19D	0.0299
pH	5.61AB	5.54B	5.58AB	5.61AB	5.64AB	5.66A	0.1089
			Cheese yield				
Before salting	12.34A	9.03F	9.24E	9.86D	10.12C	10.55B	0.1772
After salting	11.72A	8.51F	8.92E	9.32D	9.75C	10.10B	0.1277
After 3 mont.	9.35A	7.66E	7.83D	7.96D	8.33C	8.6B	0.1508
After 6 mont.	9.11A	7.42D	7.61D	7.75CD	8.12CD	8.31B	0.4040

Of the highest yield when fresh and at the end of ripening period, compared with all other treatments, followed by T(4), T(3),T(2),T(1) and control92). Cheese made from heat treated milk had higher yield than that made from untreated milk. These results came in accordance with Abd-Alla (2008), Sakr and Mehana (2011) and Amer (2015). Figures (1, 2 and 3) show that the total free amino acids (FAA) content of fresh Ras cheese samples were 20.52, 17.17, 16.07, 16.02, 15.66 and 14.54 mg/100g for T(3), T(2), T(1), C(1), C(2) and T(4), respectively, and were 28, 23.82, 20.84, 20.78, 19.32 and 17.4 mg/100g after 3 months (Table 3). While were 38.74, 33.27, 26.94, 26.75, 23.46 and 20.85 mg/100g after 6 months (Table 4) for the same treatments, respectively. Total free amino acids of cheese made from 1.5% fat milk increased by raising the temperature of heat treatment up to 75°C (T_3), which could be attributed to the effect of bacterial growth, especially, when increasing the water activity (Weber and Ramet, 1983). Conversely raising the temperature of heat treatments to 80°C (T_4) resulted in a considerable decrease in the total free amino acids content, which might be due to the pronounced destruction of indigenous milk proteases, proteolytic bacteria and to the formation of whey protein casein complex. Similar trends for free amino acids were reported by Khader *et al.,* (1995), Kebary *et al.,* (1999) and Amer (2015).

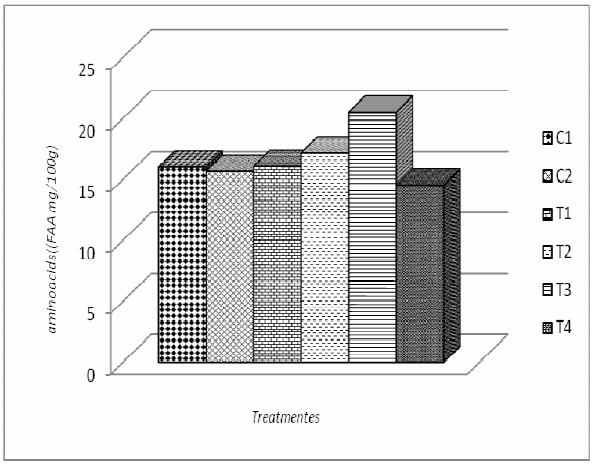

Figure 1. Effect of heat treatments of milk on free amino acids (FAA) of fresh low fat Ras cheese.

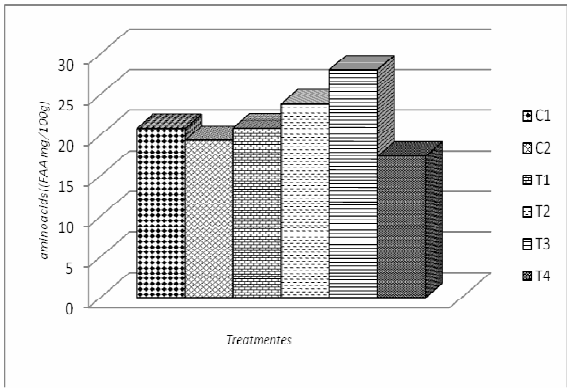

Figure 2. Effect of heat treatments of milk on free amino acids (FAA) of low fat Ras cheese ripened for 3 months.

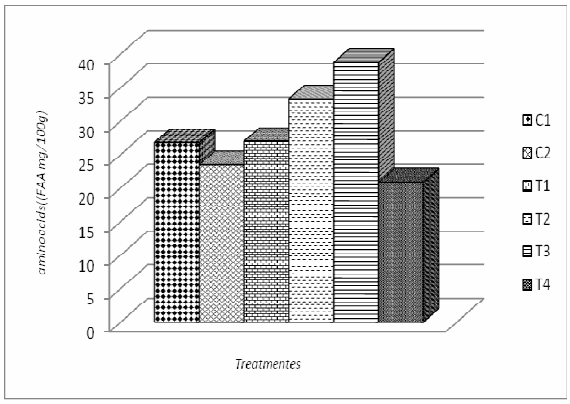

Figure 3. Effect of heat treatments of milk on free amino acids (FAA) of low fat Ras cheese ripened for 6 months.

Figures (4, 5 and 6) demonstrate the change in the biogenic amines of full and low fat Ras cheese during ripening. The total biogenic amines content of fresh Ras cheese samples were of 15.27, 12.7, 13.0, 14.65, 16.3 and 9.52 mg/100g for C_1, C_2, T_1, T_2, T_3 and T_4, respectively. The average concentration of Spermine, Spermidine, Pyenylethylalanine, Histamine, Putrescine, Cadaverine, Tyramine and Tryptamine, while they were of 21.36, 16.48, 18.0, 20.46, 23.34 and 11.39 mg/100g after 90 days, and 29.87, 21.4, 24.26, 8.6, 33.08 and 12.29 after 180 days for the same treatments, respectively.

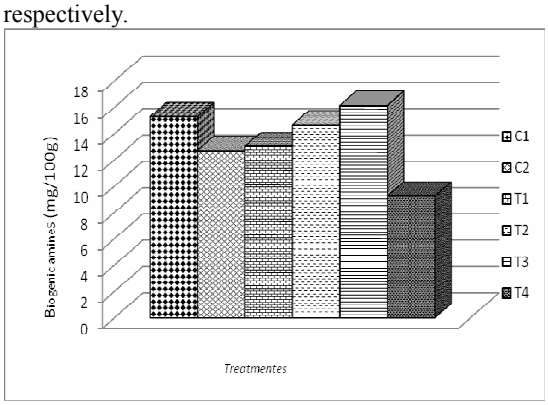

Figure 4. Influence of heat treatments of milk on biogenic amines of fresh low fat Ras cheese.

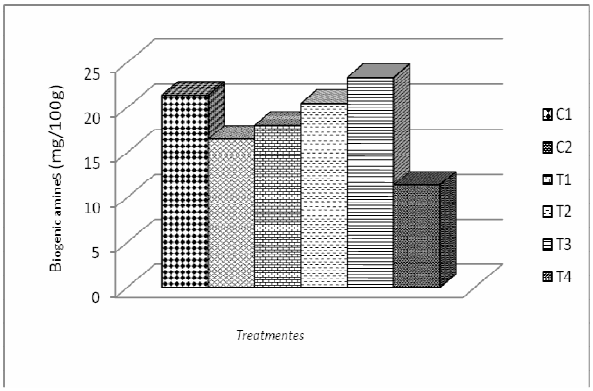

Figure 5. Influence of heat treatments of milk on biogenic amines of low fat Ras cheese ripened for 3 months.

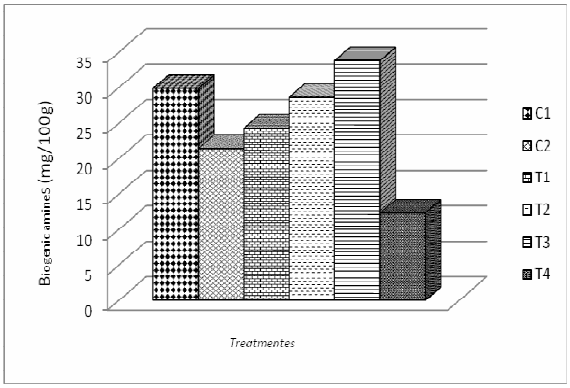

Figure 6. Influence of heat treatments of milk on biogenic amines of fresh low fat Ras cheese ripened for 6 months.

Total biogenic amines increased as ripening period progressed (Degheidi *et al.,* (1992). Total biogenic amines of cheese made from 1.5% fat milk (T_3) increased by heating the milk up to 75°C while they decreased in chesses made by rising heat treatment to 80°C (T_4). Similar results were reported for other cheeses Abd- Alla *et al.,* (1996), Ragab (2014) and Amer (2015).

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تأثير المعاملات الحراريه للبن على الأمينات والحيويه والأحماض الأمينيه الحره فى الجبن الراس المنخفض الدهن

وحيد ابراهيم الدسوقى محمد على

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اجريت هذه الدراسه لمعرفة تأثير المعاملات الحرارايه للبن البقرى على محتوى الجبن الراس منخفض الدهن من الأمينات الحيويه والأحماض الأمينيه الحره وقد تم تقسيم اللبن الى ٦ اقسام :لبن بقرى خام ٣% دهن كنترول (١) لبن بقرى خام ١,٥% دهن كنترول (٢) لبن بقرى معامل حراريا حتى ٦٥°م ١,٥% دهن (T_1) لبن بقرى معامل حراريا حتى ٧٠°م ١,٥% دهن (T_2) لبن بقرى معامل حراريا حتى ٧٥°م ١,٥% دهن (T_3) لبن بقرى معامل حراريا حتى ٨٠°م ١,٥% دهن (T_4) وقد تم تحليل الجبن الناتج من حيث محتواه من الرطوبه – النيتروجين الكلى – الدهن – الملح – الحموضه – الأمينات الحيويه – الأحماض الامينيه الحره وكانت اهم النتائج المتحصل عليها كما يلى :* ارتفاع نسبة الرطوبه والنيتروجين الكلى والأحماض الأمينيه الحره والأمينات الحيويه فى جبن المعاملات عن الكنترول .* زيادة نسبة الأمينات الحيويه والأحماض الأمينه الحره بزيادة مدة التخزين. * ارتفاع نسبة الأمينات الحيويه والأحماض الأمينيه الحره بارتفاع درجة الحراره حتى ٧٥°م بينما انخفضت هذه النسبه بارتفاع درجة الحراره الى ٨٠°م .* كانت افضل المعاملات من حيث محتواها من الأمينات الحيويه والأحماض الأمينيه الحره هى (T_3) بينما كانت المعامله (T_4) هى اقل المعاملات من حيث محتواها من الأمينات الحيويه والأحماض الأمينيه الحره.