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Effect of Heat Treatment and Salt Concentration on Free Amino Acids Composition of Sudanese Braided (*Muddaffara*) Cheese during Storage

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ABSTRACT

The aim of this study was to assess the effect of heat treatment and salt concentrations (0, 5, and 10%) on the free amino acids (FAA) composition of Sudanese braided cheese (BC) ripened for up to 3 months at 5±2°C. Heat and salt concentration significantly affected the FAA of braided cheese. The free amino acids concentrations of BC ripened in 0%, 5%, and 10% salted whey (SW) were significantly fluctuated. Under ripening conditions tested (salt level + time), braided cheese made from pasteurized milk (BCPM) had consistently lower values of FAA than braided cheese made from raw milk (BCRM). In fresh cheese, the major FAA in BCRM were Glu (36.12 nmol/ml), Leu (26.77nmol/ml) and Lys (14.51 nmol/ml) while the major ones in BCPM were Lys (2.94 nmol/ml) and Ala (2.45 nmol/ml). BCPM stored in 10% SW had shorter quality life compared to that stored in 5% salted whey.

Introduction

Cheese is the most diverse group of dairy products that is widely produced and consumed worldwide. It provides a high concentration of nutrients relative to its energy content. The nutritional composition of cheese depends on the type of milk used and the manufacturing and ripening procedures (Ucuncu, 2004). The gross composition of cheese milk, especially the concentrations of protein, casein and fat, has a major influence on several aspects of cheese manufacture and ripening, including rennet coagulability, gel strength, curd syneresis, cheese composition, yield and final quality (Fox and McSweeny, 2004). Cheese ripening is one of the most complex phenomena in food biochemistry. It is about the breakdown of proteins, lipids and carbohydrates which could release flavor compounds and modify cheese texture (Zaharia and Gabriela, 2011). The kinetics of amino acids in cheese during ripening is of particular interest because of their role in cheese flavor development and their significance as quantitative indicators of proteolytic activity during the ripening process (Buruiana and Zeydan, 1982).The concentrations of free amino acids correlate significantly with flavor development in cheese and are considered a reliable indicator of the rate of flavor production (McSweeney and Sousa, 2000). The amino acids increase with ripening time, and flavor development coincides with the appearance of certain specific amino acids, primarily glutamic acid, methionine and leucine (McSweeney, 2004). Various authors have demonstrated that leucine is a good indicator of both cheddar cheese maturation and extent of proteolysis (McSweeney, 2004). While free amino acids increased with the age of the cheddars cheeses, the relative proportion of each amino acid remained essentially constant. Glutamic acid, leucine, phenylalanine, valine and lysine were detected in large quantities in all cheese (McSweeney, 2004). Cheese making in Sudan is the major preservation method for surplus milk in rural areas especially during the rainy season when plenty of milk is available (El Owni and Hamid, 2008). In Sudan, most popular cheese types are white soft cheese locally known as Jibna beyda and braided cheese locally known as Muddaffara. The popularity of braided cheese is not only in Sudan but also in many Middle Eastern countries where the production and consumption of this type of cheese is relatively high. It is a semi hard cheese with hard texture, yellowish color, and slightly acid and salt taste (El-Sheikh, 1997). About

7500 tons of braided cheese is manufactured in Sudan each year and sold in the local markets (FAO, 2003). To date, there is no study on the Sudanese braided cheese as affected by use of raw or pasteurized cow milk and different level of salt on the amino acids profile during ripening. It is necessary to obtain best specifications and best quality attributes of Sudanese braided cheese. Therefore, the aim of this study was to investigate the synergistic effect of heat and salt concentration on free amino acids content of Sudanese braided cheese during ripening periods.

Materials and Methods

Materials

Fresh cow's milk was obtained from Khartoum University Farm, Khartoum, Sudan. Rennet, (Chr. Hansen's, Denmark), $CaCl_2$ was a product of Sigma Chemical Company, (St Louis, MO, USA). Salt (NaCl) and Black cumin (*Nigella sativa*) were obtained from the local market, Khartoum, Sudan. All chemicals and reagents used were of technically recommended analytical grade.

Cheese manufacture

Braided cheese was manufactured according to Altahir et al. (2014). Briefly, the obtained fresh cow milk (10 kg) was divided in two equal portions. One was used as raw milk without pasteurization and the other was pasteurized. Both milks were warmed to 40°C, and then starter culture with a combination of 1 : 1 *Lactobacillus bulgaricus* and *Streptococcus thermophilus* (0.02%), rennet (0.5g/5kg milk) and $CaCl_2$ were added to both milks. After complete coagulation (about 45 min), the curd was cut or broken to small parts and incubated until the required acidity (0.49-0.67 lactic acid %) for kneading was reached. The curd was put on a wooden table, and left for (5 min) to drain off the remaining whey. The curd was then cooked in 500 mL of whey at 70-80°C for five minutes. A natural flavoring ingredient such as black cumin was added (0.5%) to the hot paste, then cut into small pieces and flattened-like a circle shape. The curds formed where then braided, divided into three equal portions, each packed in plastic container assigned randomly to one of the three salted whey (0%, 5%, and 10% salt) in a ratio of 1:1 (cheese: whey) and stored for up to 90 days at 5±2°C.

Determination of amino acids

Amino acids content of the cheese samples were analyzed following the method described elsewhere (Schurr et al., 1950). Briefly, to 0.1 g of the sample, about 5 ml water was added and vortex mixed. The samples were incubated in boiling water bath for 30 min. After cooling down to room temperature, samples were centrifuged at 14,000 × *g* for 20 min. The supernatant was transferred to a new tube. To the precipitate 3 ml water was added, vortex mixed and centrifuged again at 14,000 × *g* for 20 min. The supernatant had been taken and combined with the first extract (Total volume 8 ml). Then, the lipids had been extracted from the combined extract by using diethyl ether for two times. After centrifugation at 12,000 × *g* for about 15 min at 4°C, the volume of supernatant was raised to 20 ml with distilled water and then kept at -20°C until used. About 0.3 ml of the sample solution was taken and filtrated with membrane filter (0.45 um, Millex Millipore), and used to determine amino acid concentrations. The filtered samples were mixed with phosphate buffer (1:1) and run on an Amino Tac JLC-500/V amino acid analyzer (JEOL, Tokyo, Japan). Standard mixtures of amino acids were used to identify and quantify the samples.

Statistical analysis

A factorial experiment in a completely randomized design (CRD) with three replications was used. Collected data were subjected to analysis of variance (ANOVA) and whenever appropriate ($P<0.05$) Duncan multiple range test (Steel et al., 1997) was employed to separate the treatments means using the SAS program (SAS, 2007).

Results and Discussion

Effect of heat treatment on amino acids composition of braided cheese

Free amino acids composition of braided cheese made from raw milk (BCRM) and braided cheese made from pasteurized milk (BCPM) was shown on Table 1. The amino acid concentrations (nmol/ml) of BCRM were significantly ($P <0.05$) higher than that of BCPM. Almost the specific essential amino acids, measured, namely, (Thr, His, Lys, Arg, Val, Met, leu, and Ileu) and non-essential amino (Glu, Asp, Asn, Gly, Ala, Cys, Ser, Tyr, and Pro) of BCRM showed remarkably ($P<0.05$) superior values in comparison with that of BCPM. In BCRM, the highest value was 26.11 nmol/ml for Glu followed by 19.69 nmol/ml for Leu. On the other hand, the lowest value was found in Met of BCPM that was not detected. This could be due to the effect of heat treatment (pasteurization) eliminates some of the bacteria that are the major cause of amino acids production in cheese (Beresford and Williams, 2004). The higher concentration of amino acids of raw cheese could be attributed to high microbial count on raw milk, it is well known that microorganism of cheese-produce proteases that are responsible for the degradation of cheese protein and hence release of free amino acid (Beresford and Williams, 2004). These results are in agreement with Puchades et al., (1989) who reported that raw milk cheese showed higher concentration of amino acids during ripening than those made from pasteurized milk. Raw milk cheese showed remarkably arise of a specific amino acids like, methionine, glutamic , leucine, phenylalanine, valine and lysine, compared to pasteurized milk cheese, to be characteristic of the treatment, because of some typical metabolic pathways triggered by the activity of specific enzymes (Beuvier and Buchin, 2004).

Effect of salt concentration on amino acids composition of braided cheese

Amino acids composition of BCRM as affected by the different levels of salt concentration was shown in Table 2. The free amino acids concentration (nmol/ml) of braided cheese ripened in (0%, 5% and 10%) salted whey was significantly ($P<0.05$) varied. Although, increase in salt concentration level had caused concomitant ($P<0.05$) decreases in Thr, Asn, Glu, Ala, Asp, Ser, Tyr, Ilue, and Lue values, it is also associated with significant ($P<0.05$) increases in the values of Val, Cys, Pro and Cit. The

concentration of the rest of the amino acids (His, Lys, Arg, Meth, P-ser, Gln, Gly, and Hypro) of braided cheese ripened in different levels of salt showed a mixed change i.e increase with the salt level at (5%) then decreased at high salt level(10%). Saleem et al. (1978) found that the white soft cheeses stored in low salted whey had higher tyrosine content than that stored in high salt concentration. Kristiansen et al. (1999) reported a decrease in tyrosine content as level of salt increased in a semi-hard cheese. The significant decrease in tyrosine content of braided cheeses as a result of ripening in different level of salt was probably due to break down of protein by proteolytic agent at low salt concentration (Hayaloglu et al., 2002). Hamid et al. (2008) reported that the Tyrosine and Tryptophan content of the cheese with 4% salt were significantly higher in comparison with those of 6% salt. This result agreed with the findings of the current study and those reported by Nuser (2001). Possibly high salt level (10%) had a negative effect on proteolyic enzymes activity.

Effect of storage periods on amino acids composition of braided cheese

Table 3 shows the changes in amino acids (nmol/ml) content of BCRM during storage period. The results fluctuated as the storage time progressed. With exception of few of amino acids tested, the general trend was a decrease ($P<0.05$) in concentration at 30 and 90 days, and an increase ($P<0.05$) at 60 days. The amino acids Glu, Ser, Asp, Gln, Gly Met, Ala, Lue, and Tyr values at the beginning of storage period were significantly ($P<0.05$) decreased compared to their initial on day 30, then increased after day 60 and then decreased at the end of storage period (day 90). The increase at 60 day of storage may be due to excessive proteolysis of protein. The highest values 20.51 and 13.04 nmol/ml were found for Glu and Lue, respectively on day 60 of storage periods. The lowest values of 0.0 and 0.17 nmol/ml were reported for Lys and Pro, respectively at the end of storage period. The high concentrations of His 7.22 nmol/ml after 90 days of storage, may lead to the production of histamine if the appropriate bacteria particularly those that possess decarboxylases. Most biogenic amines in cheese can be formed by decarboxylation, as is the case with the production of histamine from His and tyramine from Tyr. However, the formation of secondary and tertiary amines cannot be explained readily (Fox and McSweeney, 1996). Omar and El-Zayat (1986) reported that the concentrations of glutamic acid, serine, aspartic acid, threonine, proline, alanine and lysine were higher in 4-month-old Kashkaval cheese than in fresh one but the concentrations of valine, methionine, isoleucine, leucine and tyrosine were lower in all cheeses that have been investigated (Omar and El-Zayat, 1986). Levels of free amino acids in a number of cheese varieties, relative to the level of water-soluble N, cheddar contains low concentrations of amino acids; the principal amino acids are Glu, Leu, Arg, Lys, Phe, and Ser, as reported by Fox and Wallace (1997).

Table 1 Effect of heat treatment on the free amino acids composition (nmol/ml) of braided cheese

Amino acids	BCRM	BCPM	Change
Thr	5.88 a	0.08 b	-ve
His	3.26 a	0.35 b	-
Lys	7.21 a	1.33 b	-
Arg	0.46 a	0.28 b	-
Val	6.66 a	0.97 b	-
Met	3.11 a	0.00 b	-
p-ser	6.88 a	2.38 b	-
Asn	4.04 a	0.05 b	-
Glu	26.11 a	1.66 b	-
Ser	6.65 a	0.63 b	-
Asp	5.26 a	0.55 b	-
Gln	0.86 a	0.14 b	-
Gly	3.05 a	0.66 b	-
Ala	7.26 a	2.26 b	-
Cit	1.08 a	1.00 a	-
Cysta	1.58 a	0.22 b	-
Ileu	4.32 a	0.13 b	-
Leu	19.69 a	3.40 b	-
Tyr	2.59 a	0.43 b	-
bAla	7.73 a	2.21 b	-
Hypro	1.37 a	1.03 b	-
Pro	0.36 a	0.08 b	-
Total	125.43	19.84	-

"ab" Means followed by different superscripts letters in each row are significantly different $P<0.05$; BCRM=braided cheese processed from raw milk; BCPM=braided cheese processed from pasteurized milk.

Table 2 The changes in free amino acids composition (nmol/ml) of BCRM at different levels of salt concentration.

Amino acids	Salt concentration (%)		
	0	5	10
Thr	3.78 a	2.77 b	2.38 c
His	1.87 b	2.85 a	0.70 c
Lys	4.16 c	4.40 a	4.25 b
Arg	0.15 c	0.59 a	0.36 b
Val	3.59 c	3.86 b	3.99 a
Met	1.54 b	1.72 a	1.41 c
p-ser	4.14 c	5.01 a	4.73 b
Asn	2.52 a	1.95 b	1.68 c
Glu	17.13 a	12.76 b	11.77 c
Ser	5.61 a	3.16 b	2.15 c
Asp	4.32a	2.45 b	1.94 c
Gln	1.94 a	0.09 c	0.23 b
Gly	2.43 b	2.50 a	1.99 c
Ala	6.69 a	4.32 b	3.28 c
Cit	1.03 a	1.01 a	1.07 a
Cysta	0.61 c	0.83 b	1.28 a
ileu	2.40 a	1.86 b	2.41 a
Leu	12.20 a	11.91 b	10.53 c
Tyr	2.60 a	1.10 b	0.83 c
bAla	5.46 a	5.20 b	4.25 c
Hypro	0.89 b	2.71 a	0.00 c
pro	0.17 c	0.28 a	0.21 b
Total	85.23	73.33	63.22

"abc" Means followed by different superscripts letters in each row are significantly different $P<0.05$.

Table 3 Effect of the storage periods on free amino acids composition (nmol/ml) of BCRM.

Amino acids	Storage periods (days)			
	0	30	60	90
Thr	3.24 b	1.56 d	4.34 a	2.78 c
His	0.00 b	0.00 b	0.00 b	7.22 a
Lys	6.74 a	4.34 c	6.00 b	0.00 d
Arg	0.00 c	0.00 c	0.93 a	0.54 b
Val	3.38 d	3.44 c	4.27 a	4.17 b
Met	1.40 c	0.66 d	2.40 a	1.76 b
p-ser	4.19 b	3.78 c	5.27a	5.27 a
Asn	2.07 c	1.22 d	2.73 a	2.17 b
Glu	14.50 b	8.65 d	20.51 a	11.89 c
Ser	4.06 b	2.52 d	4.74 a	3.24 c
Asp	2.80 c	1.91 d	3.85 a	3.05 b
Gln	0.57 b	0.27 d	0.45 c	0.74 a
Gly	2.59 b	1.66 d	2.72 a	2.26 c
Ala	4.76 b	4.03 d	5.7`8 a	4.47 c
Cit	1.00 c	1.05 a	0.99 c	1.11 b
Cysta	1.64 a	0.10 d	0.77 c	1.10 b
Ileu	2.63 a	1.17 c	2.62 a	2.47 b
Leu	11.30 c	9.18 d	13.04 a	12.67 b
Tyr	1.57 c	1.04 d	1.77 a	1.65 b
bAla	4.66 c	4.14 d	5.21 b	5.89 a
Hypro	0.00 d	2.74 a	1.73 b	0.33 c
Pro	0.00 d	0.43 a	0.28 b	0.17 c
Total	73.10	53.90	80.40	73.94

"abcd" Means followed by different superscripts letters in each row are significantly different P<0.05.

Effect of heat treatment and salt concentration on amino acids composition of braided cheese

The changes in amino acids (nmol/ml) content of braided cheese as affected by heat treatment and salt concentration were presented in Table 4. The combination of heat treatment and salt concentration had caused substantial changes in the free amino acids contents of BCPM. Most of the observed changes during the combination of both treatments could be attributed to the effect of heat treatment rather than salt concentration. Particularly, comparing the Lys content of both types of cheese (BCPM and BCRM) ripened in 0% salted whey revealed a 54.5% reduction of its content BCPM. However, a 78.8% reduction in Lys content was observed when BCPM was ripened in 5% salted whey. It is thus appeared that only 24.3% of the reduction in Lys content was resulted from the ripening in 5% salted whey compared to 54.5% of the same type of cheese ripened in 0% salted whey. It also worth to note that the Lys content of BCRM increased with the increase in salt concentration, while the opposite was true in the case of BCPM. Only in the case of Hypro, heat treatment had led to an increase in any amino acids content where BCPM ripened in 0% salted whey had greater (P<0.05) Hypro than BCRM. The Thr, Asn, Glu, Ser, Asp, Gly, Ala, and Tyr content of BCRM were significantly (P<0.05) decreased with increase in salt concentration. The Lys and P-Ser content of BCRM increased (P<0.05) with increase in salt concentration. On other hand, the Thr, Lys, Ser, Asn, Ala and Hypro content of BCPM were significantly (P<0.05) decreased with increase in salt concentration. In general the amino acids contents of BCRM stored in salted whey were significantly (P<0.05) higher in comparison with BCPM stored in salted whey. According to Frau et al. (1997) asparagine and glutamine would be denaturized by pasteurization. Aspartic acid was higher in raw cheese than in pasteurized cheeses (Frau et al., 1997; Ordonez et al., 1999; Albenzio et al., 2001). Hamid et al. (2008) reported that the tryptophan and tyrosine content of the cheese with 4% salt were significantly higher in comparison with those of 6% salt. Serine and tyrosine were also found preferentially in pasteurized milk cheeses of some varieties, as well as in pasteurized or MF Swiss-type cheeses (Skie and Ardo, 2000). Hayaloglu et al. (2002) reported that the tyrosine content was decreased significantly (P<0.05) of braided cheeses as a result of ripening in different levels of salt, probably due to break down of protein by proteolytic agent at low salt concentration.

Effect of salt concentration and storage periods on amino acids composition of braided cheese

Amino acids composition of braided cheese as affected by the different levels of salt, during storage periods for 90 days is shown in Table 5. The free amino acids concentration (nmol/ml) of braided cheese ripened in (0%, 5%, and 10%) salted whey was significantly (P<0.05) varied during storage periods. The values of His, Val, Gly, Cysta, Ilue, and Lue of the braided cheese stored in 5% salted whey was being higher (P<0.05) at the end of storage than those stored in 0% and 10% salted whey. On the other hand, the lower concentrations of Thr, Lys, Met, Asn, Asp, Ala, and Tyr were found with the braided cheese stored in 10% salted whey at the end of storage period. Similarly, a significant difference (P<0.05) in tryptophan and tyrosine content of Mudaffara cheese during storage as level of salt concentration increased was reported previously (Abd El-Razig et al., 2002). Also Hamid et al. (2008) reported that the tyrosine and tryptophan content of the cheese with 4% salt were significantly (P<0.05) higher in comparison with those of 6% salt from zero day to 240 days of storage. Omar and El-Zayat (1986) reported that during ripening of Kashkaval cheese, the distribution pattern of free amino acids and free fatty acids changes due to the complexity of the maturation process.

Effect of heat treatment and storage periods on amino acids composition of braided cheese

Table 6 shows the effect heat treatment and storage period on free amino acids composition of Sudanese braided cheese. Generally, the results revealed that most of the essential (EAA) and non- essential (NEAA) amino acids of BCRM reached their peak values after 60 days of storage compared to those of BCPM. Except for His, Lys, Gln, ILue, Hypro, and Pro were lower in comparison of the rest of the EAA. The higher values of amino acids in BCRM could be due to the fact that proteolyic bacteria were able to grow and function in BCRM and hence released the free amino acids. The higher values of amino acids detected were Lys, Gln, Cit, Cysta and Ilue in BCRM at the beginning of storage. Moreover the amino acid His was only detected at late storage time (90 days) in both types of cheese. As expected, the lowest values of Thr, Arg, Val, Met, Asn, Glu, Asp, Gln, Gly, Ala, Ilue,

Lue and bAla, were found in fresh BCPM. These results may be due to the protein degradation that leading to accumulation of amino acids during the storage. These results were contradictory with those of Frantisek and Kracmar (2004) who reported that the amino acid would continue to decrease during storage of the processed cheese depending on the storage duration and temperature. During ripening, the distribution pattern of free amino acids and free fatty acids changes due to the complexity of the maturation process, resulting in the formation of the characteristic flavor of Kashkaval cheese (Omar and El-Zayat, 1986).

Table 4 Effect of heat treatment and salt concentration on free amino acids composition (nmol/ml) of braided cheese.

Amino acids	BCRM			BCPM		
	Salt concentration (%)					
	0	5	10	0	5	10
Thr	7.33 ^{a}	5.55 ^{b}	4.77 ^{c}	0.23 ^{d}	0.00 ^{f}	0.00 ^{f}
His	3.53 ^{b}	4.86^{a}	1.40^{c}	0.20^{f}	0.85^{d}	0.00^{g}
Lys	5.72^{c}	7.60 ^{b}	8.32^{a}	2.60^{d}	1.21^{f}	0.17^{g}
Arg	0.16 ^{b}	0.81 ^{a}	0.40 ^{b}	0.15 ^{b}	0.36 ^{b}	0.32 ^{b}
Val	6 .27 ^{b}	6.21^{c}	7.51^{a}	0.91^{f}	1.52 ^{d}	0.47 ^{g}
Met	3.09 ^{b}	3.43^{a}	2.81^{c}	0.00^{d}	0.00^{d}	0.00^{d}
p-ser	6.03^{c}	6.40 ^{b}	8.20 ^{a}	2.25 ^{d}	3.63 ^{f}	1 .26 ^{g}
Asn	5.03 ^{a}	3.90 ^{b}	3.20 ^{c}	0.00 ^{f}	0.00 ^{f}	0.16 ^{d}
Glu	31.89 ^{a}	24.28 ^{b}	22.16 ^{c}	2.40 ^{d}	1.24 ^{g}	1.38 ^{f}
Ser	10.45 ^{a}	5.69 ^{b}	3.80 ^{c}	0.78 ^{d}	0.62 ^{f}	0.49 ^{g}
Asp	8.00 ^{a}	4.27 ^{b}	3.50 ^{c}	0.63 ^{d}	0.63 ^{d}	0.39 ^{f}
Gln	2.39 ^{a}	0.00 ^{f}	0.21 ^{c}	0.00 ^{f}	0.19 ^{d}	0.25 ^{b}
Gly	4.46 ^{a}	3.91 ^{b}	3.49 ^{c}	0.40 ^{g}	1.10 ^{d}	0.49 ^{f}
Ala	10.29 ^{a}	6.26 ^{b}	5.25 ^{c}	3.09 ^{d}	2.37 ^{f}	1.30 ^{g}
Cit	1.09 ^{a}	1.03 ^{b}	1.11 ^{a}	0.98 ^{b}	1.00 ^{b}	1.03 ^{b}
Cysta	1.21 ^{b}	1.13^{c}	2.41^{a}	0.00^{g}	0.52^{d}	0.15^{f}
ileu	4.62 ^{b}	3.51^{c}	4.82^{a}	0.18^{d}	0.20^{d}	0.00^{f}
Leu	21.52^{a}	18.43 ^{c}	19.12 ^{b}	2.87^{f}	5.39^{d}	1.93^{g}
Tyr	4.77^{a}	1.55 ^{b}	1.44^{c}	0.42^{f}	0.64 ^{d}	0.22 ^{g}
bAla	9.12^{a}	7.05 ^{b}	7.03 ^{b}	1.81^{d}	3.36^{c}	1.48 ^{f}
Hypro	0.59 ^{d}	3.52^{a}	0.00^{f}	1.20^{c}	1.90 ^{b}	0.00^{f}
pro	0.33 ^{b}	0.32 ^{c}	0.42^{a}	0.00 ^{f}	0.25 ^{d}	0.00 ^{f}

"a-g" Means followed by different superscripts letters in each row are significantly different (P < 0.05). BCRM=braided cheese processed from raw milk. BCPM= braided cheese processed from pasteurized milk

Table 5 Effect of salt concentration and storage periods on free amino acids (nmol/ml) of BCRM

Amino acids	Salt concentration (%)											
	0				5				10			
	Storage period (days)											
	0	30	60	90	0	30	60	90	0	30	60	90
Thr	4.45^{b}	2.52^{j}	4.37^{c}	3.77^{f}	2.63^{h}	0.77^{i}	4.03^{d}	3.66^{g}	2.62^{h}	1.39^{k}	4.61^{a}	0.91^{e}
His	0.00 ^{d}	0.00 ^{d}	0.00 ^{d}	7.46^{b}	0.00 ^{d}	0.00 ^{d}	0.00 ^{d}	11.41^{a}	0.00 ^{d}	0.00 ^{d}	0.00 ^{d}	2.79^{c}
Lys	8.72^{a}	6.34^{c}	1.56 ^{k}	0.00 ^{j}	6.18 ^{d}	3.21^{h}	8.23 ^{b}	0.00^{j}	5.31^{f}	3.47 ^{g}	8.22^{b}	0.00 ^{j}
Arg	0.00 ^{d}	0.00 ^{d}	0.00 ^{d}	0.61 ^{b}	0.00 ^{d}	0.00 ^{d}	2.16 ^{a}	0.18 ^{c}	0.00 ^{d}	0.00^{d}	0.62 ^{b}	0.82 ^{b}
Val	2.79^{k}	3.76^{g}	4.19^{d}	3.62^{e}	3.07 ^{i}	2.49^{j}	3.49^{h}	6.40^{a}	4.28^{c}	4.08^{f}	5.13 ^{b}	2.47^{i}
Met	1.63^{f}	0.92^{h}	1.85^{c}	1.79^{d}	1.32 ^{g}	0.49 ^{k}	2.51 ^{b}	2.54 ^{b}	1.25 ^{e}	0.58^{j}	2.85 ^{a}	0.95^{h}
p-ser	3.83^{e}	3.57^{h}	4.38^{f}	4.79^{d}	4.80^{d}	3.99^{j}	5.18^{c}	6.08^{b}	3.94^{j}	3.79^{e}	6.26^{a}	4.93^{g}
Asn	2.86^{c}	1.58^{h}	2.84^{bc}	2.79^{b}	1.74^{f}	0.88^{e}	2.39^{d}	2.78^{b}	1.62^{g}	1.21^{k}	2.98^{a}	0.93^{e}
Glu	18.06^{d}	14.74^{e}	19.32^{c}	16.40^{f}	11.77^{i}	2.85^{L}	20.13^{b}	16.29^{g}	13.66^{h}	8.35^{j}	22.09^{a}	2.98^{k}
Ser	6.58^{a}	4.11^{d}	6.38^{b}	5.38^{c}	2.88^{h}	2.14^{j}	4.01^{f}	3.60^{e}	2.70^{i}	1.30^{k}	3.84^{g}	0.74^{L}
Asp	4.66^{b}	3.44^{f}	4.87^{a}	4.29^{c}	1.79^{e}	0.76^{j}	3.26^{g}	4.00^{d}	1.96^{h}	1.51^{k}	3.43^{f}	0.86^{i}
Gln	1.71^{a}	0.80^{d}	0.93^{c}	1.34 ^{b}	0.00 ^{j}	0.00 ^{j}	0.00 ^{j}	0.37 ^{h}	0.00 ^{j}	0.00 ^{j}	0.41^{g}	0.50 ^{f}
Gly	2.61^{d}	1.78^{h}	2.76 ^{c}	2.58^{d}	2.61^{d}	1.89^{g}	2.36^{f}	3.16 ^{a}	2.55^{d}	1.31^{j}	3.05^{b}	1.04^{k}
Ala	6.81^{a}	6.35^{b}	6.61^{ab}	6.99^{a}	3.93^{d}	2.84^{g}	5.50 ^{c}	5.00^{c}	3.54^{f}	2.90^{g}	5.24^{c}	1.43^{h}
Cit	0.92^{d}	1.02^{c}	0.95^{d}	1.25^{a}	1.04^{bc}	1.04^{bc}	0.97^{cd}	1.01^{c}	1.05 ^{b}	1.10^{b}	1.06 ^{b}	1.06^{b}
Cysta	2.42^{b}	0.00 ^{g}	0.00 ^{g}	0.00 ^{g}	0.00 ^{g}	0.00 ^{g}	0.00 ^{g}	3.31^{a}	2.49^{c}	0.30 ^{f}	2.32^{d}	0.00 ^{g}
Ileu	3.94^{c}	1.55 ^{g}	2.04^{f}	2.07^{f}	0.61 ^{L}	0.82 ^{j}	1.33 ^{h}	4.67 ^{a}	3.32 ^{d}	1.15 ^{i}	4.48^{b}	0.68^{k}
Leu	13.73^{c}	11.33^{f}	12.33^{d}	11.41^{f}	8.95^{h}	6.05^{i}	11.39^{f}	21.26^{a}	11.22^{f}	10.15^{g}	15.39^{b}	5.34 ^{j}
Tyr	3.10 ^{a}	2.27 ^{c}	2.63 ^{b}	2.40 ^{d}	0.74 ^{e}	0.49 ^{h}	0.92 ^{g}	2.23^{d}	0.87 ^{g}	0.35 ^{j}	1.77 ^{f}	0.34 ^{j}
bAla	5.60^{b}	5.28^{d}	5.60 ^{b}	5.38 ^{c}	4.02 ^{e}	2.69 ^{h}	4.44 ^{f}	9.66^{a}	4.34 ^{g}	4.45^{f}	5.59 ^{b}	2.62^{j}
Hypro	0.00 ^{g}	1.18 ^{d}	1.41 ^{c}	0.99 ^{f}	0.00 ^{g}	7.04 ^{a}	3.79 ^{b}	0.00 ^{g}	0.00 ^{g}	0.00 ^{g}	0.00 ^{g}	0.00 ^{g}
Pro	0.00 ^{f}	0.66 ^{b}	0.00 ^{f}	0.00 ^{f}	0.00 ^{f}	0.64 ^{c}	0.00 ^{f}	0.50^{d}	0.00 ^{f}	0.00 ^{f}	0.85 ^{d}	0.00 ^{f}

"a-l" Means followed by different superscripts letters in each row are significantly different (P<0.05)

Table 6 Effect of heat treatment and storage periods on free amino acids composition (nmol/ml) of braided cheese

Amino acids	BCRM				BCPM			
	Storage period (days)							
	0	30	60	90	0	30	60	90
Thr	6.47 b	2.92 d	8.57 a	5.56 c	0.00 e	0.19 f	0.11 g	0.00 e
His	0.00 c	0.00 c	0.00 c	13.04a	0.00 c	0.00 c	0.00 c	1.40 b
Lys	11.26 a	6.96 c	10.63 b	0.00 h	2.21 d	1.73 f	1.37 g	0.00 h
Arg	0.00 c	0.00 c	1.37 a	0.45 b	0.00 c	0.00 c	0.48 b	0.62 b
Val	6.47 b	5.64 c	8.07 a	6.46 b	0.28 h	1.25 f	0.47 g	1.87 d
Met	2.80 c	1.32 d	4.80 a	3.52 b	0.00 f	0.00 f	0.00 f	0.00 f
p-ser	6.97 c	5.44 d	7.98 a	7.11 b	1.41 e	2.12 h	2.57 g	3.42f
Asn	4.15 c	2.23 d	5.47 a	4.34 b	0.00 g	0.22 f	0.00 g	0.00 g
Glu	28.23 b	14.12 d	40.01 a	22.09 c	0.76 e	3.17 f	1.02 h	1.69 g
Ser	7.15 b	4.42 d	8.70 a	6.31 c	0.97 f	0.61 h	0.78 g	0.17 e
Asp	5.36 c	2.94 d	7.14 a	5.59 b	0.25 e	0.87 f	0.57 g	0.51 h
Gln	1.14 a	0.54 d	0.89 b	0.89 b	0.00 f	0.00 f	0.00 f	0.58 c
Gly	4.22 b	2.73 d	5.16 a	3.69 c	0.96 f	0.59 h	0.28 e	0.83 g
Ala	7.62 b	5.34 d	9.44 a	6.66 c	1.90 h	2.72 f	2.12gh	2.28 g
Cit	1. 07 a	1.10 a	1.02 a	1.11 a	0.93 b	1.01 a	0.96 b	1.10 a
Cysta	2.28 a	0.00 g	1.55 b	1.51 c	0.00 g	0.20 f	0.00 g	0.70 d
Ileu	5.25a	2.09 d	5.16 b	4.75 c	0.00 j	0.26 f	0.07 h	0.19 g
Leu	21.18 b	13.42 d	25.18 a	18.36 c	0.78 h	4.93 g	0.90 h	6.97 f
Tyr	2.85 b	1.49 d	3.31 a	2.70 c	0.29 g	0.59 f	0.24 h	0.61 f
bAla	8.40 b	5.59 d	9.43 a	7.51 c	0.91 e	2.69 g	0.99 h	4.26 f
Hypro	0.00 d	5.48 a	0.00 d	0.00 d	0.00 d	0.00 d	3.47 b	0.66 c
Pro	0.00 d	0.87 a	0.57 b	0.00 d	0.00 d	0.00 d	0.00 d	0.33 c

"a-j" Means followed by different superscripts letters in each row are significantly different (P <0.05).
BCRM=braided cheese processed from raw milk.
BCPM= braided cheese processed from pasteurized milk

Effect of heat treatment, salt concentration, and storage periods on amino acids composition of braided cheese

The results in Table 7 show the effect of heat treatment and salt concentration on the free amino acids (nmol/ml) of braided cheese during the storage periods for 90 days. When the three factors of heat treatment, salt concentration and storage period were considered the results revealed that most of essential amino acids (EAA) of BCRM reached their peak values after 60 days of ripening in 10% salted whey. While, His and Arg reached their peak values after 90 days and 60 days of ripening in 5% salted whey, respectively. The general trend for most of the non-essential amino acids (NEAA) of BCRM was decrease with the increase of ripening periods at any one salt concentration tested. Except for His and Arg, comparison of the EAA contents of BCRM and BCPM at 0% salt concentration and day 0 of ripening revealed that BCRM had always lower EAA content than BCRM. Such observation continued as the salt concentration and ripening time increased. For most of the amino acids tested, the observed changes in their contents could be attributed to the effect of the three factors (heat treatment, salt concentration, and storage period) examined; out of these three factors, the effect of pasteurization is by far the greatest. The effect of heat treatment could be attributed to low activity of proteolysis and high salt concentration, which suppress activity of bacterial proteases. Several factors may contribute to amino acids formation in cheese (Gardini et al., 2001). Utilization in cheese making, raw or pasteurized milk and low salt concentration may contribute to the ability of on organism to produce amine while free amino acids increased with the age of the cheddars cheeses, the relative proportion of each amino acid remained essentially constant. In the current study, glutamic acid, leucine, alanine, valine, and lysine were detected in large quantities in both types of cheese. These results are similar with Hickey et al. (1983) who reported that the increase of these amino acids with ripening time, and flavor development coincides with the appearance of certain specific amino acids, primarily glutamic acid, methionine and Leucine, compared with pasteurized milk cheeses. Therefore, pasteurization of milk led to a decrease in final biogenic amine content of cheese as the result of the reduction of its microbial population. Several authors have attributed the intensity of cheese flavor largely to components of the water-soluble fraction (Aston and Creamer, 1986). The proteolysis process was shown to be affected by the ripening temperature as higher concentrations of free amino acids were found in cheese ripened at 15°C than at 6°C (Puchades et al., 1989). The concentrations of glutamic, serine, aspartic, threonine, proline, alanine and lysine acid were higher in 4-month-old Kashkaval than in young cheese but the concentrations of valine, methionine, isoleucine, leucine, and tyrosine acid were lower (Omar and El-Zayat, 1986).

Conclusion

The free amino acids of braided cheese processed from raw milk were significantly superior in comparison with braided cheese processed from pasteurized milk. It is recommended that braided cheese be ripened in whey with low salt concentration, while avoiding ripening cheese in high salted whey for a prolonged period. Further study shall specifically focus on the microbiological aspects of braided cheese made from raw milk and ripened in low salt whey.

Table 7 Effect of heat treatment, salt concentration and storage periods on free amino acids (nmol/ml) of braided cheese

HT[1]	SC[2]	SP[3]	Amino acids										
			Thr	His	Lys	Arg	Val	Met	p-ser	Asn	Glu	Ser	Asp
BCRM	0	0	8.91b	0.00g	14.51c	0.00d	5.57j	3.26e	6.72c	5.73b	36.12d	12.32a	9.32a
		30	4.46j	0.00g	8.36 e	0.00d	5.29i	1.84g	4.37L	3.15g	22.14j	7.27d	5.03g
		60	8.42c	0.00g	0.00s	0.00d	7.44d	3.69d	6.11h	5.67b	37.36c	11.94b	9.07b
		90	7.54f	14.11b	0.00s	0.63c	6.78h	3.57c	6.93f	5.58c	31.96f	10.26c	8.59c
	5	0	5.26h	0.00g	9.36 f	0.00d	5.67g	2.64f	6.32g	3.48f	22.45i	4.44 f	3.16h
		30	1.54m	0.00g	5.58i	0.00d	4.99m	0.98L	5.85j	1.77j	5.70m	3.41i	1.52k
		60	8.07d	0.00g	15.46b	2.88a	6.52f	5.02b	6.37g	4.78d	39.15b	7.23 d	5.95f
		90	7.31 g	19.42a	0.00s	0.37c	7.66 c	5.09b	7.05e	5.57c	29.81g	7.19 d	6.46d
	10	0	5.24h	0.00g	9.92 d	0.00d	8.17 b	2.50i	7.88b	3.25h	26.13h	4.18 h	3.60i
		30	2.77i	0.00g	6.94 h	0.00d	6.65 e	1.15k	6.11h	1.76j	14.52k	2.60 j	2.28j
		60	9.22a	0.00g	16.44a	1.25b	10.27a	5.69a	11.46a	5.96a	43.51a	6.94 g	6.39e
		90	1.83 k	5.59 c	0.00s	0.37c	4.94m	1.90g	7.35 d	1.86i	4.49 n	1.49 e	1.73m
BCPM	0	0	0.00 r	0.00g	2.94 m	0.00d	0.00 t	0.00m	0.94 w	0.00L	0.00 y	0.84 m	0.00 r
		30	0.58 n	0.00g	4.32 j	0.00d	2.23 L	0.00m	2.77 q	0.00L	7.34 L	0.95m	1.86L
		60	0.33 p	0.00g	3.13 k	0.00d	0.94 p	0.00m	2.65 p	0.00L	1.29 p	0.83m	0.66 p
		90	0.00 r	0.81 d	0.00s	0.59c	0.46 r	0.00m	2.65 p	0.00L	0.85 t	0.50 p	0.00 r
	5	0	0.00 r	0.00g	3.00 L	0.00d	0.46 r	0.00m	3.29 n	0.00L	1.09 p	0.83m	0.42 v
		30	0.00 r	0.00g	0.85 n	0.00d	0.00 t	0.00m	2.12 s	0.00L	0.00 y	0.88m	0.00 r
		60	0.00 r	0.00g	0.99 p	1.45b	0.47 r	0.00m	4.00 m	0.00L	1.11 p	0.79 m	0.57 s
		90	0.00 r	3.40 f	0.00s	0.00d	5.15 k	0.00m	5.10 k	0.00L	2.77 y	0.00 r	1.54 k
	10	0	0.00 r	0.00g	0.00s	0.00d	0.39 s	0.00m	0.00 y	0.00L	1.19 p	1.23 k	0.32w
		30	0.00 r	0.00g	0.00s	0.00d	1.51 n	0.00m	1.47 t	0.00L	2.18 s	0.00 r	0.75 n
		60	0.00 r	0.00g	0.00s	0.00d	0.00 t	0.00m	1.05 v	0.00L	0.67 t	0.73 m	0.47 t
		90	0.00 r	0.00g	0.00s	1.27b	0.00 r	0.00m	2.52 r	0.00L	1.47 r	0.00 r	0.00 r

[1]HT=Heat treatment; [2]SC=Salt concentration (%); [3]SP=Storage period (days); "a-w" Means followed by different superscripts letters in each column are significantly different (P <0.05); BCRM=braided cheese processed from raw milk; BCPM= braided cheese processed from pasteurized milk.

Table 7 Continue

HT[1]	SC[2]	SP[3]	Amino acids										
			Gln	Gly	Ala	Cit	Cysta	ileu	leu	Tyr	bAla	Hypro	Pro
BCRM	0	0	3.42a	5.22b	11.17a	0.90c	4.85b	7.87c	26.77b	6.19a	10.47b	0.00g	0.00f
		30	1.61d	2.68L	8.03c	1.11b	0.00	2.58h	14.47g	3.20 d	6.80k	2.36 d	1.32b
		60	1.85c	5.06c	10.80a	0.93c	0.00e	3.88g	23.27c	4.90 b	9.73 d	0.00g	0.00f
		90	2.68b	4.87d	11.13a	1.42a	0.00e	4.14f	21.57d	4.80 c	9.49 f	0.00g	0.00f
	5	0	0.00e	3.51h	5.82 d	1.20b	0.00e	1.22L	16.80h	1.16 j	6.93 j	0.00g	0.00f
		30	0.00e	3.30j	3.71h	1.11b	0.00e	1.40 e	11.72i	0.57 k	4.70 p	14.07a	1.28c
		60	0.00e	4.72f	8.38 c	1.00c	0.00e	2.65i	22.02d	1.83 h	8.42g	0.00g	0.00f
		90	0.00e	4.13g	7.12 f	0.80d	4.53 d	8.78 b	23.21c	2.64 f	8.13 h	0.00g	0.00f
	10	0	0.00e	3.95e	5.86 d	1.12b	4.99 a	6.66 d	21.85d	1.20 j	7.80i	0.00g	0.00f
		30	0.00e	2.22i	4.26 g	1.08b	0.00e	2.30 j	14.08L	0.71i	5.26L	0.00g	0.00f
		60	0.83f	5.72a	9.15 b	1.15b	4.64 c	8.97 a	30.24a	3.18 d	4.90 m	0.00g	1.70a
		90	0.00e	2.08k	1.74 j	1.11b	0.00e	1.35 k	10.32j	0.67 j	4.92 m	0.00g	0.00f
BCPM	0	0	0.00e	0.00y	2.45 e	0.94c	0.00e	0.00 s	0.68 m	0.00L	0.72 v	0.00g	0.00f
		30	0.00e	0.88p	4.66 g	0.93c	0.00e	0.52 p	8.19 k	1.34 g	3.77 n	0.00g	0.00f
		60	0.00e	0.46 r	2.42 e	0.98c	0.00e	0.21 r	1.39 m	0.35 m	1.47 s	2.83 c	0.00f
		90	0.00e	0.28s	2.84 e	1.08b	0.00e	0.00s	1.24 m	0.00L	1.27 w	1.98 f	0.00f
	5	0	0.00e	1.71n	2.03 j	0.88c	0.00e	0.00s	1.10 m	0.33 m	1.11 t	0.00g	0.00f
		30	0.00e	0.49 r	1.96 j	0.98c	0.00e	0.25 r	0.38 m	0.42 m	0.67 v	0.00g	0.00f
		60	0.00e	0.00y	2.63e	0.93c	0.00e	0.00 s	0.77 m	0.00L	0.45 y	7.58 b	0.00f
		90	0.74g	2.20i	2.88 e	1.22b	2.09 f	0.57m	19.30f	1.82 h	11.19a	0.00g	0.99d
	10	0	0.00e	1.16m	1.22 j	0.98c	0.00e	0.00s	0.58 m	0.53 k	0.89 q	0.00g	0.00f
		30	0.00e	0.41r	1.54 j	1.13 b	0.00e	0.00s	6.23 L	0.00L	3.64 r	0.00g	0.00f
		60	0.00e	0.39 r	1.33 j	0.97 c	0.00e	0.00s	0.55 m	0.36 m	1.04 t	0.00g	0.00f
		90	0.99h	0.00 y	1.12j	1.02c	0.00e	0.00s	0.37 m	0.00L	0.33 z	0.00g	0.00f

[1]HT=Heat treatment; [2]SC=Salt concentration (%); [3]SP=Storage period (days); "a-y" Means followed by different superscripts letters in each column are significantly different (P <0.05); BCRM=braided cheese processed from raw milk; BCPM= braided cheese processed from pasteurized milk.

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