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Free Amino Acid Profiles of Macedonian White Brined Cheese

BORCHE MAKARIJOSKI[1], GORDANA DIMITROVSKA[1], VESNA K. HRISTOVA[1], ELENA JOSHEVSKA[1], MAHMOUD A. ABDELAZIZ MAHMOUD[2], SYED KHALID MUSTAFA[2,*] and M. AYAZ AHMAD[3]

[1]Faculty of Biotechnical Science, University of St. Kliment Ohridski, Bitola, Macedonia
[2]Department of Chemistry, Faculty of Sciences, University of Tabuk, Tabuk, Kingdom of Saudi Arabia
[3]Department of Physics, Faculty of Sciences, University of Tabuk, Tabuk, Kingdom of Saudi Arabia

*Corresponding author: E-mail: khalid.mustafa938@gmail.com

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The present work is focussed to determine the Macedonian white brined cheese's free amino acid profile. Four variants of the Macedonian white brined cheese to analyze and determine free amino acid concentration; cheese samples define the Macedonian white brined cheese as a typical cheese. All free amino acids were specified, except the amino acid tyrosine. The detected amino acids in the tested cheese variants were present in different but approximate parameter values. The estimated essential free amino acids, the concentration of lysine was found highest in all examined samples with values from 26.40 ± 0.02 mg% to 28.20 ± 0.04 mg% and the concentration of threonine was the lowest from 3.19 ± 0.02 mg% to 3.32 ± 0.02 mg%). In the detected unessential free amino acids, the concentration of aspartic amino acid was highest in all the samples with values from 11.02 ± 0.05 mg% to 11.32 ± 0.03 mg% and the concentration of proline was at the lowest level from 4.16 ± 0.06 mg% to 4.22 ± 0.04 mg%.

Keywords: White-brined cheese, Free-amino acids, Concentration, Fermentation.

INTRODUCTION

Cheese and its product are familiar product used in our everyday lives and something new for the modern world, originating from ancient times. However, cheese production has a long history full of various myths, legends and historic evidence that reveal its origin and development [1]. The cheese manufacturers and experts initially try to enhance the quality to get a worthwhile and safe product for the people eating. These skills were conventionally transferred from generation to generation, from knee to knee, which continued white brined cheese production.

The white brined cheese belongs to the ferments' collection in a brine solution in anaerobic conditions, characterized as the acid-salty flavour, no rind, usually white colour. Sometimes, with a yellowish tint, anaerobic brine fermented in plastic cans and pieces are generally in the form of cubes with dimensions 10 cm × 10 cm × 10 cm [2]. Many small milk processors and large industrial plants that process milk have white brined at most due to its wide demand on the domestic markets.

Fox & McSweeney [3] reported that the mode of ripening of white brined cheese is because of various factors such as starter cultures, coagulants, manufacturing and ripening conditions. Proteolysis is the primary biochemical process during the ripening of white brined cheese and responsible for the developing typical cheese flavour and texture. Proteins hydrolyzed during proteolysis produce lower molecular weight compounds and further break down by peptidases into various nitrogenous substances, such as proteose, peptone, amino acids amines [4]. Complex microbiological and biochemical changes occur during white brined cheese fermentation, significantly inducing the final product's organoleptic characteristics. Proteolytic enzymes broke down casein into larger and smaller peptides followed by free amino acids. The amount of corresponding amino acids and the soluble peptides significantly affect the texture and organoleptic properties, especially the specific aroma of different types of cheese [5].

The presence of amino acids in white brined cheese is in low quantity. It correlates with the specific fermentation process and more significant breakdown of proteins in width and less

in-depth [6]. Amino acid catabolism is the essential process for forming the taste and aroma of white brined cheese [7]. Herein, the authors have determined the amino acids profile of Macedonian white-brined, which is the most used dairy product in Macedonia.

EXPERIMENTAL

Four white brined cheese variants (WBC1, WBC2, WBC3 and WBC4) were selected for the free amino acids determination. Produced fixed white brined cheese variants were obtained from the different dairy industries situated in Macedonia and the consumers mostly use these cheese variants.

Differences in white brined cheese variants (WBC1, WBC2, WBC3 and WBC4) were found. WBC1, WBC2 and WBC4 were produced from non-standardized milk and the variant WBC3 was produced from standardized milk, which contains protein 32-35 g/kg and milk fat 37-40 g/kg. The temperature of pasteurization was different for each cheese variant, WBC1: 72-74 ºC for 15 min, WBC2:72-74 ºC for 10 min, WBC3: pasteurization I:74-77 ºC for 60 s, pasteurization II: 74-77 ºC for 5 min and WBC4: 72 ºC for 5 s. The added starter cultures were also different in white brined cheese variants. In WBC1 the following starter cultures were added: *Lactococcus lactis* ssp. *Lactis*, *Str. thermophilus*, *Lact. delbrueckii* ssp. *bulgaricus*. WBC2 contained *Lactobacillus helveticus*, *Str. thermophilus*, *Lactobacillus. delbrueckii* ssp. Bulgaricus; WBC3 sample contains *Str. thermophilus*, *Lactobacillus bulgaricus*; while WBC4 contains *Lactobacillus bulgaricus*, *Str. thermophilus*, *Lactobacillus acidophilus*.

The addition of $CaCl_2$ was also different in the variants' production process. In WBC1, $CaCl_2$ contained 0.035-0.050%, WBC2 contained 0.030-0.045% of $CaCl_2$; WBC3 contained 0.010-0.020% of $CaCl_2$ and in WBC4, $CaCl_2$ was added in 0.010-0.015%. The coagulation time for each variant was the same (45-60 min). Brine is a salt solution ranging from about 8% to 12%. The fermentation process lasts 45 days.

The quantity of free amino acids was calculated by using an automatic amino analyzer Hd –1200 E, Czech Republic and by the method of Spackman *et al.* [8] by using an automated amino analyser Pharmacia LKB, model Alpha Plus Series 2 (Amersham Pharmacia Biotech Europe GMBH, Orsay, France). Processing the analysis results was performed using Nelson Analytical type 2600 (Perkin Elmer S. A. - Saint Quentin en Javelins -France).

The free amino acids were pre-extracted from 5 g of cheese in 50 mL of double distilled water. By adding 3 g of CCl_3COOH, casein precipitation was generated. The sample was filtered, while the resulting filtrate was used to determine the free amino acids using automatic amino analyser Hd - 1200 E.

RESULTS AND DISCUSSION

The composition and content of free amino acids were determined on the 60th day, after the fermentation process, in all four different varieties of the white brine cheese. The results obtained from the laboratory analyses characterize the white brined cheese as a typical cheese in which all free amino acids were determined, except for the amino acid tyrosine. The detected amino acids in analyzed white brined cheese variants were with different but approximate prevalence.

In WBC1 after the fermentation period, a total amount of free amino acids of 142.30 ± 0.44 mg% was determined, of which 109.33 ± 0.25 mg% were essential amino acids, while 32.97 ± 0.19 mg% were non-essential. This variant of BSS has the highest total amount of free amino acids and the highest amount of essential amino acids in its composition compared to the other three variants. In WBC2 after 60 days fermentation period, a total amount of free amino acids of 137.71 ± 0.48 mg% was determined, of which 104.29 ± 0.28 mg% were essential amino acids and 33.02 ± 0.20 mg% were non-essential amino acids. This variant has the highest non-essential amino acids content and the lowest range of essential amino acids in its composition compared to the other three variants. In WBC3, after 60 days fermentation period, a total amount of free amino acids of 140.17 ± 0.35 mg% was determined, of which 107.58 ± 0.21 mg% were essential amino acids and 32.59 ± 0.24 mg% were non-essential. In WBC4 after 60 days fermentation period, a total amount of free amino acids of 137.70 ± 0.30 mg% was determined, of which 105.11 ± 0.16 mg% were essential amino acids and 32.59 ± 0.14 mg% were non-essential amino acids. This variant has the lowest total free amino acid content and the lowest non-essential amino acid content than the other three variants tested (Table-1). The significant differences between variants for amino acid content at level $p < 0.05$ are also listed in Table-1.

The several essential amino acids were detected in the white brined cheese, *e.g*. lysine, histidine, arginine, threonine, valine, methionine, isoleucine, leucine and phenylalanine (Fig. 1a), while the detected non-essential amino acids were aspartic acid, proline, glycine, alanine and cysteine (Fig. 1b). The statistical significance observed no significant deviations for level $p < 0.05$ between amino acids in different variants as shown in Table-1.

Among all the detected essential amino acids, the amount of amino acid lysine was most present in all the variants white brined cheese with values from 26.40 ± 0.02 mg% to 28.20 ± 0.04 mg%, then amino acid phenylalanine with values from 15.11 ± 0.01 to 15.36 ± 0.07 mg%, valine with values from 14.05 ± 0.02 mg% to 14.55 ± 0.01 mg%, histidine with values from 13.10 ± 0.01 mg% to 14.50 ± 0.03 mg% and leucine from 13.28 ± 0.06 mg% to 13.66 ± 0.04 mg%. The essential amino acid threonine was determined at the lowest level in all the examined white brined cheese variants with the values range from 3.19 ± 0.02 mg% to 3.32. 0.02 mg%.

Among the detected non-essential amino acids, aspartic acid was present in all examined white brined cheese variants with values range from 11.02 ± 0.05 mg% to 11.32 ± 0.03 mg%, whereas proline was present at the lowest level in all the examined white brined cheese variants with values from 4.16 ± 0.06 mg% to 4.22 ± 0.04 mg%.

Present results for the amino acid content of white brined cheese are similar to the results presented by Balabanova *et al.* [6]. Bulgarian white cheese produced from cow milk has detected the several amino acids *viz*. leucine, serine, phenylalanine, lysine valine and arginine. The total amount of free

TABLE-1
FREE AMINO ACIDS CONTENT OF EXAMINED CHEESE VARIANTS

Free amino acids mg (%)	White brined cheese variants (60 day)			
	WBC1	WBC2	WBC3	WBC4
Lysine	28.20 ± 0.04[a]	26.40 ± 0.02[b]	28.10 ± 0.01[ac]	27.10 ± 0.02[d]
Histidine	14.50 ± 0.03[a]	13.80 ± 0.02[b]	14.20 ± 0.01[ac]	13.10 ± 0.01[d]
Arginine	5.06 ± 0.01[a]	4.09 ± 0.01[b]	5.02 ± 0.01[a]	5.06 ± 0.01[a]
Threonine	3.32 ± 0.02[a]	3.28 ± 0.01[a]	3.19 ± 0.02[a]	3.30 ± 0.01[a]
Valine	14.55 ± 0.01[a]	14.05 ± 0.02[b]	14.33 ± 0.02[b]	14.28 ± 0.01[b]
Methionine	8.10 ± 0.01[a]	7.92 ± 0.02[a,b]	7.68 ± 0.01[c]	7.78 ± 0.02[c,d]
Isoleucine	6.58 ± 0.02[a]	6.32 ± 0.03[a]	6.38 ± 0.01[a]	5.94 ± 0.03[b]
Leucine	13.66 ± 0.04[a]	13.28 ± 0.06[b]	13.37 ± 0.08[b]	13.44 ± 0.04[a,b]
Phenylalanine	15.36 ± 0.07[a]	15.15 ± 0.09[a]	15.31 ± 0.04[a]	15.11 ± 0.01[a]
Total essential free amino acids	109.33 ± 0.25	104.29 ± 0.28	107.58 ± 0.21	105.11 ± 0.16
Asparginic acid	11.32 ± 0.03[a]	11.24 ± 0.06[a]	11.09 ± 0.04[b]	11.02 ± 0.05[b]
Proline	4.18 ± 0.06[a]	4.21 ± 0.02[a]	4.16 ± 0.06[a]	4.22 ± 0.04[a]
Glycine	6.22 ± 0.08[a]	6.12 ± 0.07[a]	6.31 ± 0.05[a]	6.11 ± 0.01[a]
Alanine	4.43 ± 0.01[a]	4.54 ± 0.01[a]	4.25 ± 0.08[b]	4.33 ± 0.02[b]
Cysteine	6.82 ± 0.01[a]	6.91 ± 0.04[a]	6.78 ± 0.01[a]	6.91 ± 0.02[a]
Tyrosine	NF	NF	NF	NF
Total non-essential free amino acids	32.97 ± 0.19	33.02 ± 0.20	32.59 ± 0.24	32.59 ± 0.14
The total content of free amino acids	142.3 ± 0.44	137.71 ± 0.48	140.17 ± 0.35	137.70 ± 0.30

*Differences of values with different superscripts in the same row are statistically significant at level $p < 0.05$; NF = not found.

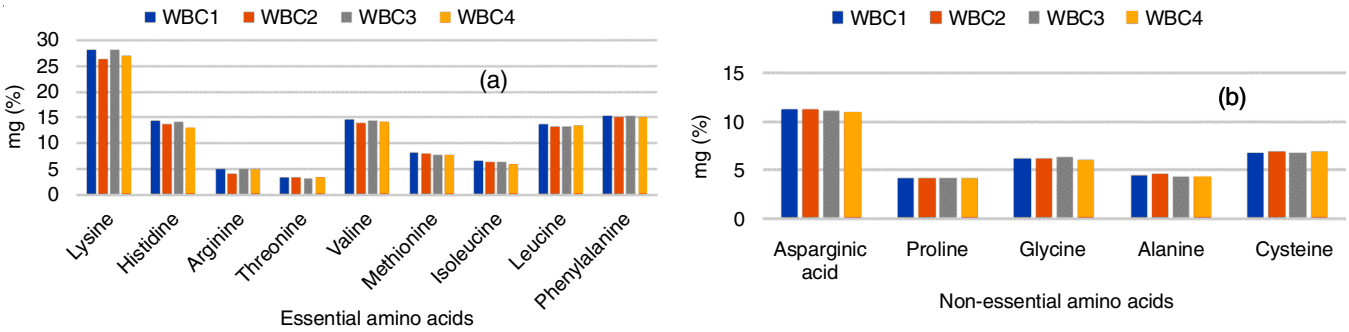

Fig. 1. A graphical representation of essential amino acids (a) and non-essential amino acids (b) after ripening after 60 days

amino acids after 45 days fermentation period was 139.1 mg%. Eren-Vapur & Ozcan [9,10] also determined the same essential free amino acids in Turkish white cheese (leucine, glutamic acid, phenylalanine, valine and lysine). Similar results were also reported by Alichanidis *et al.* [11] and Katsiari *et al.* [12] in Greek white cheese-Feta (leucine, lysine, glutamic acid and valine). These amino acids were also identified in the composition of four different white brined cheese variants of Macedonia. Moreover, Hayaloglu *et al.* [13] and Polychroniadou *et al.* [14] reported the level of free amino acids in the white brine cheese ranges from 1 to 7 g kg^{-1} cheese, which correspond to the results obtained in the present research.

Conclusion

The quantity of free amino acids in observed white brined cheese variants obtained from Macedonia after the 60 days of ripening period. The free amino acids were in the range of from 137.70 ± 0.30 mg% to 142.30 ± 0.44 mg%. The highest percentage of free amino acids in WBC1 was detected in this study. The several essential amino acids in examined cheese variants after the ripening period were ranged from 104.29 ± 0.28 mg% to 109.33 ± 0.25 mg%. The maximum percentage of vital free amino acids was present in WBC1. The quantity of non-essential amino acids in examined cheese variants after-ripening period ranged from 32.59 ± 0.14 mg% to 33.02 ± 0.20 mg%. The maximum percentage of non-essential free amino acids was present in the variant WBC2.

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CONFLICT OF INTEREST

The authors declare that there is no conflict of interests regarding the publication of this article.

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