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Kinetics of Free Amino Acid Profile in Various Levels of NACL Concentration of Cheese Protein Whey

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KEYWORDS

kinetics, free amino acid profile. Nonessential and essential amino acids

ABSTRACT:

Introduction: The appearances of free amino acids (FAAs) in the proteins of cheese are essential for a wide range of important biological activities in the human body. occurrence in several lines of evidence suggests that the FAAs, in cheese proteins play a role in specific nutritional functions. The process of metabolizing and using amino acids acquired from nutrition, together with the existence of released amino acids in dietary constituents and the presence of NaCl may be having an impact on kinetics of the FAAs, in the proteins of cheese.

Objectives. to investigate the kinetics of free amino acid in cheese protein that is beginning to mature in salted whey with various levels of sodium chloride (0%, 5%, and 10%).in the cheese whey, and their affected on the FAA kinetics during 60 days, ripping time(RT) at 5±2°C.

Methods: The composition of amino acids in the samples was measured using the HPLC separation technology and detection, techniques were used to identify and quantify amino acids in protein samples utilizing an automated protein amino acid analysis, the mixture was applied to the amino acid analyser manufactured by Shimadzu in Japan.

Results: The result, was shown that the analytical variance of FAAs in cheese protein transformed significantly ($P \leq 0.05$) at three different levels of whey NaCl concentration. The reference change values for Glu, Leu, Ala, and Lys in the FAA were 18.13, 12.78, 7.70, and 5.17 nmol /ml, respectively. A notable difference ($P \leq 0.05$) was observed in the quantity of free amino acids when comparing 5% and 10% salted whey to 0% salted whey. The highest quantities of the FAAs, Glu (23.41 nmol/ml), Leu (15.39 nmol/ml), and Lys (8.88 nmol/ml) were remarked in protein samples, the FAA with the lowest concentrations, in contrast, were Met, Thr and Ileu, respectively. Significant quantities of the amino acids Glu, Lys, Leu, Ala, and phe, were identified in 5% salted whey respectively

Conclusions The study of the kinetics of free amino acids is very important scientific research, because it enhances our knowledge of complex biological processes, especially those involving enzymatic reactions. The amino acids Glu, Lys, Leu, Ala, and Phe were detected in significant amounts in 5% salted whey, respectively. It is a major source of essential amino acids needed for human health. To avoid degradation of amino acids in cheese protein, we recommend not aging it for a long period of maturation in high-salt whey. However, the findings provide sufficient data to create a comprehensive amino acid database that can be used to support and enhance nutritional research.

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1. Introduction

Amino acids play a crucial role in a diverse array of vital biological processes in the human body. The process of breaking down and utilizing amino acids obtained from food, as well as the presence of free-form amino acids in dietary components, serve as the primary sources of the essential amino acids required for human metabolism. For a comprehensive list of the amino acid acronyms referenced [1]. In their released form, amino acids possess the inherent capability to serve as messengers, potentially targeting specific receptors. The nutritional composition of cheese is subject to influence not solely by the type of milk employed, but also by the intricacies of the manufacturing procedure and the modality of cheese ripening. The interplay of various factors imposes constraints on microbial growth in cheese, affecting biochemical processes, enzymatic reactions, and water potential. The utilization of salt, the constitution of the salt within the product, and the process by which it undergoes transformation during the ripening of the product are all noteworthy factors [2]. The salinity level exerts a significant influence on the growth and reproduction of microorganisms, the functionality of enzymes, and the biochemical transformations occurring during the maturation phase. These alterations delineate the gustatory perception, olfactory sensation, tactile sensation, and overall excellence thereof. The phenomenon of cheese ripening stands as a highly intricate process within the realm of food biochemistry. It encompasses the enzymatic degradation of proteins, lipids, and carbohydrates, leading to the liberation of gustatory compounds and exerting an influence on the cheese's texture modification [3]. Key factors to consider encompass the employed salt concentration, the salt profile within the product, and the salt's maturation progression [2]. The salinity level exerts an influence on the growth and reproduction of microorganisms, the functioning of enzymes, and the various biochemical transformations that take place during the maturation phase [29: 30]. These modifications delineate the gustatory, olfactory, and tactile characteristics of the specimen, alongside its holistic excellence. The preservation of cheese is facilitated by various factors including salt, temperature, pH, water activity, redox potential, and microbial flora. Salt plays a crucial role in the preservation of cheese by mitigating degradation and inhibiting the proliferation of pathogens. The user's text does not provide any information for me to rewrite in a biologist's perspective. Distinguishing between these various aspects can pose challenges due to their intricate interdependencies. Due to their significance as quantitative indicators of proteolysis activity throughout the ripening process, the dynamics of amino acids in cheese during the ripening process hold particular scientific interest. This phenomenon can be attributed to the involvement of amino acids in the formation of the flavour profile exhibited by cheese. In conjunction with temperature, pH, water activity, redox potential, and microbial flora, salt plays a pivotal role in the preservation of cheese. Its presence aids in diminishing the rate of cheese degradation and impeding the proliferation of pathogenic microorganisms. Furthermore, it should be noted that the intricate interplay among these various elements necessitates careful discernment, as distinguishing between them may pose certain challenges [31]. The investigation into the kinetics of amino acids in cheese during the ripening phase holds great importance due to their pivotal contribution to the development of cheese flavour and their substantial value as quantitative markers for proteolysis activity throughout the ripening procedure [4].Due to their inherent significance as quantitative indicators of proteolysis activity throughout the ripening period, the dynamics of amino acids in cheese during the maturation process hold particular scientific intrigue. The involvement of amino acids in the formation of cheese flavour is a result of their inherent biochemical properties.

2. Objectives

The correlation between the concentration of free amino acids and nutrient values development in cheese is highly significant, making it a reliable indicator of the rate at which substance is produced [5]. The observed occurrence of increased levels is observed to be positively correlated with the duration of ripening, while the concurrent progression of nutrient values attributes aligns with the emergence of select essential amino acids, namely methionine, and leucine [6]. Multiple researchers have provided evidence indicating that leucine serves as a reliable marker for assessing the progression of Cheddar cheese maturation and the degree of proteolysis [6]. Cheese serves as the principal preservative agent for surplus milk in rural regions of Sudan, particularly in times of heightened milk abundance such as the rainy

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season [7]. Cheese, such as muddaffra, is widely recognized as a highly favoured dairy product in Sudan, as well as in various other Middle Eastern nations. The aforementioned product can be classified as a semi-hard cheese, characterized by its firm texture, yellowish hue, and a subtle combination of acidic and salty flavours. This particular type of cheese has gained recognition primarily within the urban regions of Sudan [8]. Regrettably, the available literature pertaining to the amino acid composition of fresh cow milk cheese is rather limited. Moreover, the potential ramifications of diminished sodium concentrations on the intricate interplay of amino acids within the maturation process of cheese remain unexplored, as no empirical investigations have been undertaken in this regard. It is of utmost importance to acquire the optimal cheese specifications and characteristics, while also endeavouring to prolong the cheese's expiration date. The study of free amino acids kinetics is a very significant scientific search, since it enhances our knowledge of intricate biological processes, particularly those involving enzymatic reactions, and the intricate sequence of reactions linked to the preservation techniques of biological characteristics. The objective of this investigation was to evaluate the impact of varying NaCl concentrations on the free amino acids kinetics in cheese protein throughout the preservation process involving enzymatic reactions.

3. Methods

The bovine lacteal secretion, colloquially known as fresh cow's milk, was procured from the esteemed Khartoum University Farm. Rennet, sourced from Chr. Hansen in Denmark, calcium chloride ($CaCl_2$) obtained from Sigma Chemical Company, sodium chloride (NaCl) derived from Nigella saliva, and black cumin were procured from local sources. The chemicals and reagents employed in the study were exclusively sourced from the producer and adhered strictly to the analytical grade criteria.

Samples preparation

To sum up, the samples were made from fresh cow's milk that was warmed up at 40 °C and mixed with 0.02% starting culture, 1 tablet of rennet per 100 L, of milk, and $CaCl_2$. Next the curd had been coagulated for about 45 minutes, then the curds were put together. After the samples were cut up, they were put into plastic bottles with 0.5L of salted whey in three salt levels: 0% ,5%, and 10%. The samples were then kept at 5±2C for up to 60 days [9]. During Ripening time (RT), samples were taken and kept at -20°C until they could be scientifically tested. Triplicates of each sample were used for analysis.

Amino acids Determination

The composition of amino acids in the samples was assessed using the methodology outlined in a separate publication [10]. HPLC separation technology and detection techniques were used to identify and quantify amino acids in protein samples utilizing an automated protein amino acid analysis, the mixture was applied to the amino acid analyser made by Shimadzu in Japan.

Statistical analysis

Statisticians used a fully randomized design (CRD) with three replications. A 5% significance threshold was used for statistical analysis for science (SPSS, 2020).

4. Results

The kinetics of free amino acid composition of cheese protein at varying concentrations of salty whey is depicted in Figure 1 and summarized in Table 1. Table 2 and Fig. 1: Essential and None Essential amino acids of cheese's protein (nmol/mL) in three levels of salted (0%, 5%, and, 10%) whey concentration.

Table 1.
Kinetics of free amino acid composition in cheese protein (nmol/ml) at three levels of salted whey concentration

Types of FAA	FAA	NaCl (%)		
		0%	5%	10%
Essential amino acids	Leu	12. 78^{a}	11. 90^{b}	10. 58^{c}
	Ileu	2.40^{a}	1.86^{b}	2.41^{a}
	Thr	3.78^{a}	2.77^{b}	2.38^{c}
	Lys	5.17^{c}	4.40^{a}	4.25^{b}
	Val	3.59^{c}	3.86^{b}	3.99^{a}
	Met	1.54^{b}	1.72^{a}	1.41^{c}
	His*	1.87^{b}	2.85^{a}	.70^{c1}
	Arg*	.15^{c1}	.59^{a1}	.36^{b1}
Non-Essential amino acids	Glu	18.13^{a}	13.76^{b}	12.66^{c}
	Ala	7.70^{a}	4.32^{b}	3.28^{c}
	Ser	5.61^{a}	3.16^{b}	2.15^{c}
	Asp	4.32^{a}	2.45^{b}	1.94^{c}
	Gln	2.94^{a}	1.09^{c}	1.23^{b}
	Gly	2.43^{b}	2.50^{a}	1.99^{c}
	Asn	2.52^{a}	1.95^{b}	1.68^{c}
	Cit	1.03^{b}	1.01^{b}	1.07^{a}
	Cys	1.61^{c}	1.83^{b}	2.28^{a}
	Tyr	2.60^{a}	1.40^{b}	1.03^{c}

Mean followed by different superscripts letters in each row is significantly different $P \leq 0.05$.

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Fig 1. Essential amino acids of cheese's protein in salted whey concentration (0%)(nmol/mL)

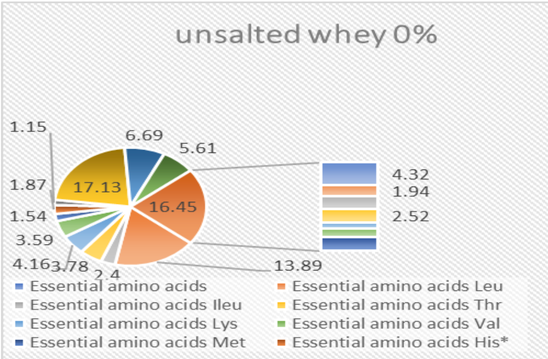
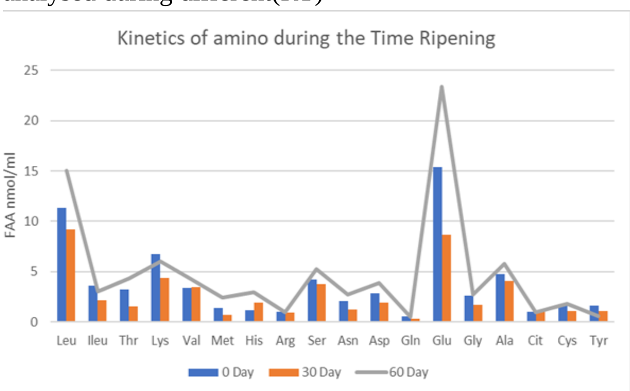


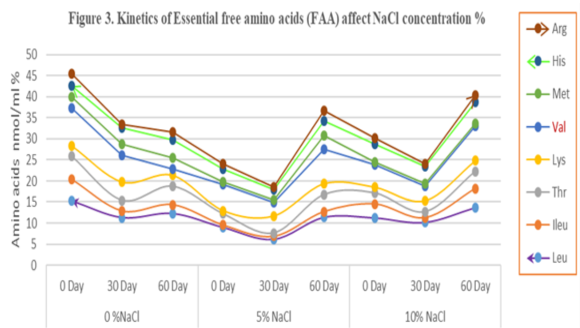
Table.2 Free amino acids kinetics during different (RT) (nmol/mL).

FAA	Ripening Time(RT) (days)		
	First day	30days(1Month)	60days(2Month)
Leu	11.30 ᶜ	9.18ᵈ	15.05 ᵃ
Ileu	3.63 ᵃ	2.17 ᶜ	2.99 ᵃ
The	3.24 ᵇ	1.56 ᵈ	4.34 ᵃ
Lys	6.74 ᵃ	4.34 ᶜ	6.00 ᵇ
Val	3.38 ᵈ	3.44 ᶜ	4.27 ᵃ
Met	1.40 ᶜ	0.66 ᵈ	2.40 ᵃ
His	1.12 ᵇ	1.95 ᵇ	2.91 ᵇ
Arg	1.00 ᶜ	0.94 ᶜ	0.93 ᵃ
Ser	4.19 ᵇ	3.78 ᶜ	5.27ᵃ
Asn	2.07 ᶜ	1.22 ᵈ	2.73 ᵃ
Asp	2.80ᶜ	1.91 ᵈ	3.85 ᵃ
Gln	0.57 ᵇ	0.27 ᵈ	0.47 ᶜ
Glu	15.40 ᵇ	8.65 ᵈ	23.41 ᵃ
Gly	2.59 ᵇ	1.66 ᵈ	2.72 ᵃ
Ala	4.76 ᵇ	4.03 ᵈ	5.78 ᵃ
Cit	1.00 ᶜ	1.05 ᵃ	0.99 ᶜ
Cys	1.64 ᵃ	1.10ᵈ	1.77 ᶜ
Tyr	1.57 ᵃ	1.04 ᵇ	0.57 ᶜ

Mean followed by different superscripts letters in each row is significantly different P ≤ 0.05.

Figure 2. Kinetics of 18 free amino acids (FAA) were analysed during different(RT)





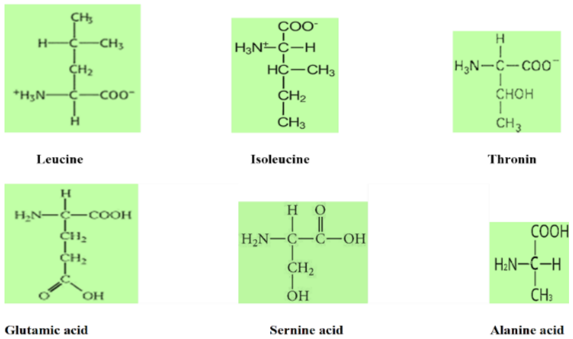


Fig .5. Structures of some amino acids

Table 4. Kinetics of essential free amino acids (FAA) within different salted whey levels (%) (nmol/mL).

	0% NaCl	5% NaCl	10% NaCl
Leu	6.05	6.05	10.15
Ileu	0.82	0.82	1.15
Thr	0.77	0.77	1.39
His	4.03	4.03	2.61
Lys	3.21	3.21	3.47
Arg	0.66	0.66	0.62
Val	2.49	2.49	4.08
Met	0.49	0.49	0.58

Mean followed by different superscripts letters in each row is significantly different P ≤ 0.05

Fig.6. Kinetics of free amino acids (FAA) within different salted whey levels (%) (nmol/mL).

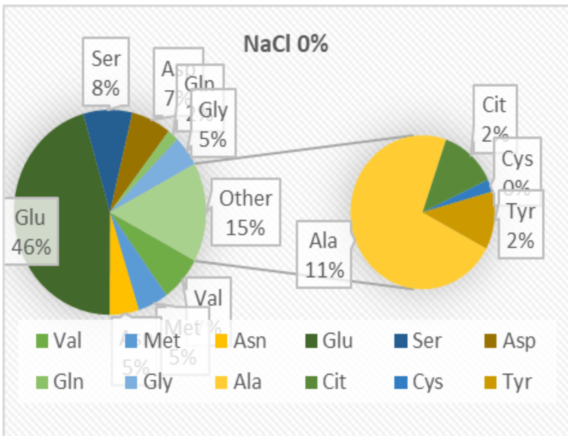
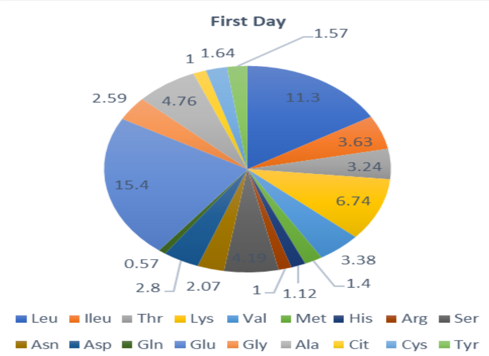
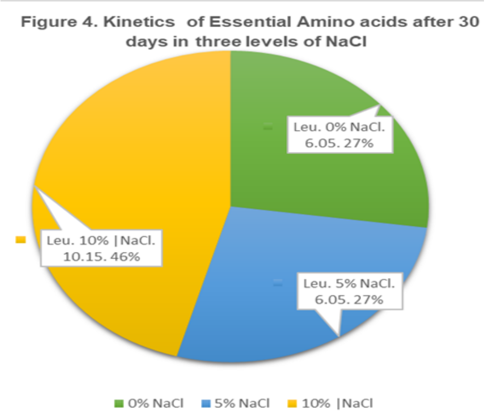


Fig.7.Kinetics of free amino acids (FAA) within different salted whey levels (5 %) (nmol/mL).





Discussion

The kinetics of free amino acids composition Present in cheese protein

The figures 1, & 2, provide an overview of both essential and non-essential amino acids found in cheese protein. The quantification of these amino acids is conducted in Nano moles per millilitre. A statistically significant variation ($P \leq 0.05$) was noted in the concentration of free amino acids (nmol/ml) in cheese protein subjected to maturation in salted whey at concentrations of 0%, 5%, and 10%. Upon careful consideration, it is evident that the prevailing trend observed pertains to a decrease in the quantity of certain amino acids Fig7. An increase in the concentration of salt has led to significant decreases ($P \leq 0.05$) in the concentrations of Threonine (Thr), Asparagine (Asn), Glutamate (Glu), Alanine (Ala), Aspartate (Asp), Serine (Ser), Tyrosine (Tyr), Blue (Blue), and Lue (Lue). Contrarily, it has been observed that the concentration of amino acids, including Valine, and Cysteine, exhibited a significant increase ($P \leq 0.05$) in response to the elevation of salt concentration in braided cheese. The concentration of the remaining amino acids, namely Leu. Ileu. Thr, His, Lys, Arg, Meth, P-ser, Gln, and Gly, exhibited a diverse shift when subjected to maturation in different salt concentrations T. The observed phenomenon exhibited a positive correlation between the concentration and the salt level at 5%, whereas a negative correlation was observed when the salt level was elevated to 10%. The tyrosine content of soft white cheeses that were subjected to low salted whey exhibited a higher concentration compared to cheeses that underwent storage in a high salt concentration [11]. Conversely, it has been observed that the tyrosine levels in semi-hard cheeses exhibit a decline in response to an increase in salt concentration [12]. The tyrosine content in cheese protein undergoes a notable reduction during the ripening process at different stages. The observed occurrence of salt is likely attributed to the enzymatic action of the proteolytic agent, which is known to catalyse the hydrolysis of proteins even at low salt concentrations [13]. In comparing the cheese variants, it is observed that the cheese containing 4% salt exhibited a notable increase in the concentrations of Tyrosine and Tryptophan, whereas the cheese containing 6% salt displayed a marked decrease in the levels of these particular amino acids [14]. The findings of this investigation were in line with the observations made in the present study, suggesting that a 10% salt concentration may have hindered the activity of proteolytic enzymes [15].

Kinetics of free amino acids composition affected by NaCl concentration and during RT.

The impact of varying levels of NaCl on the amino acid composition of protein cheese during a 60-day ripening period is depicted in Figures 1, 2, 3, and table.2. The concentration of free amino acids (expressed in nmol/ml) in cheese that underwent ripening in salted whey solutions of varying concentrations (0%, 5%, and 10%) exhibited significant variations ($P \leq 0.05$) over a period of 60 days, as depicted in Figure 7. The amino acid levels of Histidine (His), Valine (Val), Glycine (Gly), Cysteine (Cys), Isoleucine (Ile), and Leucine (Leu) in the protein cheese, which was stored in a 5% salted whey solution, exhibited significantly higher values ($P \leq 0.05$) at the conclusion of the storage period compared to those stored in 0% and 10% salted whey solutions (Fig3). In contrast, it was observed that the protein cheese stored in 10% salted whey exhibited decreased concentrations of Thr,

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Lys, Met, Asn, Asp, Ala, and Tyr at the conclusion of the storage duration. Previously, it has been reported that there was a notable distinction ($P \leq 0.05$) in the levels of tryptophan and tyrosine originate in Mudaffara cheese as the concentration of NaCl increased during the storage period [9]. Additionally, it is worth noting that the levels of the amino acids Tyrosine (Tyr) and Tryptophan (Trp) in the cheese containing 4% salt were found to be significantly higher ($P \leq 0.05$) when compared to the cheese with 5% salt during the entire ripening period ranging from zero-days to ≤ 60 days [14]. During the maturation period of Kashkaval cheese, alterations in the distribution pattern of free amino acids and free fatty acids occur as a consequence of the intricate progression of ripening [17].

The results explained that the majority of the vital amino acids (VAA) present in the protein composition of cheese attained their maximum levels subsequent to a period of 60 days of maturation in a solution of 10% saline whey. Table 1. The amino acids Histidine (His) and Arginine (Arg) exhibited their maximum concentrations following a 60-day maturation period in a 5% solution of whey that had been salted. The observed trend in the majority of non-essential amino acids (NEAA) exhibited a decline as the duration of ripening increased, regardless of the NaCl concentration under investigation. The aforementioned observation persisted as the concentration of sodium chloride (NaCl) and the duration of ripening were augmented. The alterations in the contents of the amino acids under investigation can be primarily ascribed to the influence exerted by the three examined factors, namely the concentration of NaCl, ripening time, and heat treatment. Among these factors, the impact of heat treatment can be attributed to the diminished activity of proteolysis and the elevated concentration of NaCl, which effectively inhibit the activity of bacterial proteases. The formation of amino acids in cheese may be influenced by various contributing factors [23]. Large quantities of glutamic acid, leucine, phenylalanine, valine, and lysine were observed to be present in all cheese samples. The findings presented in [24] demonstrate a resemblance in the patterns observed, wherein the augmentation of these amino acids aligns with the progression of ripening and the concurrent enhancement of flavour. Notably, specific amino acids such as glutamic acid, methionine, and leucine emerge prominently, exhibiting a discernible disparity when compared to cheeses made from pasteurized milk. Hence, the process of pasteurization applied to milk resulted in a notable reduction in the overall biogenic amine levels found in cheese. This outcome can be attributed to the concurrent decrease in the microbial population present within the cheese. The intensity of cheese flavour has been widely attributed by various authors to the constituents present in the water-soluble fraction [25]. The observed impact of ripening temperature on the proteolysis process is evident in the comparative analysis of cheese samples. Specifically, it was observed that cheese ripened at a temperature of 15°C exhibited higher concentrations of free amino acids in comparison to cheese ripened at 6°C [26]. The levels of glutamic, serine, aspartic, threonine, proline, alanine, and lysine acids were found to be elevated in 4-month-old Kashkaval cheese compared to young cheese. Conversely, the concentrations of valine, methionine, isoleucine, leucine, and tyrosine were observed to be diminished [17]. In the entirety of the examined samples, it was observed that the concentration of individual Free Amino Acids (FAA) generally increased during the ripening process, with the exception of Arginine (Arg), which exhibited a decrease in all batches after a period of 3 months. The reduction in Arginine (Arg) levels can be attributed to its utilization by bacteria. It is worth noting that several species of lactic acid bacteria (LAB) possess the ability to convert Arginine into Cytosine (Cit) [27: 28].

Conclusion

The study of the kinetics of free amino acids is very important scientific research, because it enhances our knowledge of complex biological processes, especially those involving enzymatic reactions. The amino acids Glu, Lys, Leu, Ala, and Phe were detected in significant amounts in 5% salted whey, respectively. It is a major source of essential amino acids needed for human health. To avoid degradation of amino acids in cheese protein, we recommend not aging it for a long period of maturation in high-salt whey. However, the findings provide sufficient data to create a comprehensive amino acid database that can be used to support and enhance nutritional research.

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