International Journal of Multidisciplinary Research and Development
www.allsubjectjournal.com
Online ISSN: 2349-4182, Print ISSN: 2349-5979
Received: 02-03-2022, Accepted: 17-03-2022, Published: 02-04-2022
Volume 9, Issue 4, 2022, Page No. 1-5

Lactose metabolism and proteolysis during ripening of Liqenasi goat's milk cheese

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Abstract
Local breeds can be considered cultural assets because of their role in local traditions and the fact that they have often played a central part in the social life of rural populations. They also can be linked to local culture due to their contribution to the preservation of ancient local traditional knoweldge and food products. To evaluate these diminishing local breeds that historically produced niche cheese products, the study of their qualitative traits are of high importance.
The aim of thi study was to evaluate the lactose metabolism and proteolysis through determination of the content of lactose and free amino acids during cheese ripening, coagulated with chymosin and starter culture and only with chymosin, with and without salt, from indigenous sources. The cheese samples were produced according to traditional methods. Cheese samples were ripening for 1, 3 (5), 7 days for non-salted batches and for 12, 22, and 32 for salted batches. The cheese produced were analyzed for physicochemical properties moisture (AOAC Method 926.08), pH (UB-10 pH-meter Denver Instrument), NaCl (IDF Standard 88A:1988) and acidity (by potentiometric titration), Lactoseby the phenol colorimetricmethod and proteolytic activity by determining the content of free amino acids in cheese (Cadmium-ninhydrin method) for ripening period.
The resultes shows that the use of microbial cultura (LAB) promotes lactose metabolism and proteolysis of cheese proteins (free amino acid content), from the first days of ripening cheese, obtaining flavor and aroma, as well as improved quality of cheese obtained. Lactose metabolism and free amino acid contend result to be lower for salted varieties and higher for non-salted one.

Keywords: ripening, lactose metabolism, proteolysis, Liqenasi goat, cheese

Introduction
Rennet coagulated cheeses are ripened for periods ranging from about two weeks to two or more years depending on the variety. During ripening biochemical and microbiological changes occur which result in characteristic developments of aroma, taste and texture of the variety. [1] Ripening of cheese is a process that involves a series of microbiological, biochemical and chemical reactions. Biochemical changes in cheese during ripening can be grouped into primary (lipolysis, proteolysis and metabolism of residual lactose, lactate and citrate) or secondary (metabolism of fatty acids and amino acids) [1, 2]. Residual lactose is rapidly metabolized in L- lactate during the early stages of maturation. Lactate is an important precursor to a range of reactions including racemization, oxidation or microbial metabolism [1, 3, 4]. Lipolysis is one of the biggest biochemical changes that occur during cheese ripening. The free fatty acids (FFAs) released during lipolysis contribute, along with the volatile compounds and proteolysis products, directly to the taste of the cheese. Lipolytic agents in cheese are lipolytic enzymes found naturally in milk (milk lipase), rennet (pregastric esterase) (PGE) and microflora [5, 6]. Proteolysis is associated with complex biochemical changes that occur during cheese maturation and is catalyzed by enzymes of coagulant residue, milk (plasmin), proteinase and peptidase by lactic acid bacteria. Proteolysis in cheese ripening plays a role in the development of texture and taste. It contributes to changes in the texture of the cheese mass, due to the breakdown of the protein network, the decrease of water activity (Aw) through the binding of water by carboxylic and amine groups and the increase of pH (especially in varieties ripened with mold on the surface), which facilitates the release of flavor compounds during mastication.[7]
At the global scale the process of erosion of animal genetic resources for food and agriculture has been considered by FAO.[8] Further on the European Union recognizes the importance of conserving animal genetic resources, and since 1992 started a policy of economic incentives for farmers keeping endangered breeds under EC Regulation 2078/92, followed by EC Regulation 1257/ 99. Despite the erosion during the last decades, Europe still hosts a large variety of local cattle breeds, although many are endangered. [9] The Lake Prespa region represents an example where high biodiversity values is well combined with human culture and traditions coexisting from immemorial time [10] Further on there are numerous local breeds that is found to be undervalued or not integrated into conservation policy. [11] The local goat and cattle are amongst most notable breeds well-known for the morphological features, adaptation and coexistence with local sub-populations in three bordering countries i.e. Albania, North Macedonia and Greece.

Materials and Methods

Cheese samples were produced acording to traditional production coagulated (Batch A) with chymosin with 1/10000 (150mg / 5 liters milk) salted, (Batch B) withchymosin + microbial culture (30g yogurt) salted, (Batch C) with chymosin with 1/10000 (150mg / 5 liters) unsalted, (Batch D) chymosin + microbial culture (30g yogurt) unsalted.

Physical andchemical parameters of the cheese

In the cheese samples obtained with salting (on days 12, 22 and 30) and without salting (with and without microbial culture-yoghurt) (on days 1,3,5 and 7), the analyzes of the indicators of moisture, pH, lactic acid, sodium chloride, lactose and free amino acids.

- **Moisture-Dried material**: with the method IDF Standard 4A:1982 [12]
- **Acidity, with the potentiometric method [13]**

The acidity of cheese is determined by end point titration using 0.1 eq/l NaOH. The end point value is generally fixed at pH 8.4 and the result is expressed in % of lactic acid, which has a MW of 90.08 g/mol.

pH was measured using pH-meter UB-10 and combined electrodes by Denver instruments, which is calibrated with series of standards solution ph-4.00, 7.00 and 10 from VWR. A known amount of cheese 20 g was place in a 250 ml beaker, adds 100 ml of distilled water at 40°C and homogenized with a high speed homogenizer. The mixture was filter or centrifuge and dilute to 250 ml using a volumetric flask. An aliquot of 50 ml for example was titrate. Results are expressed as % of lactic acid.

- **pH, with the potentiometric method [12]**

Grated cheese is packed in a cylinder (h=3 cm, o.d. =1.5 cm) and put in close contact with a combined electrode thoroughly calibrated with two buffer solutions at pH 4.01 and 7.00. The pH value to the nearest 1/100 unit is recorded 30 s later. The measurement is repeated consecutively using three different test portions. Between each measurement, the electrode is wiped to remove most of the cheese paste, soaked a few seconds in ethanol/ether (50/50), rinsed with water, then re-equilibrated in buffer 4.01 and wiped.

- **Cadmium-ninhydrin method for determination of free amino groups in cheese** [12]

Although this reagent reacts to some extent with all amino groups, it reacts very intensely with the amino group of free amino acids and hence appears to be a simple method for estimating the concentration of free amino acids in cheese. [14]

Cadmium Ninhydrin reagent. Ninhydrin (0.8 g) is dissolved in a mixture of ethanol (80 mL) and acetic acid (10 mL). To the resulting solution is added 1 g CdCl2 dissolved in 1 mL H2O.

Procedure. A water soluble extract of cheese is prepared as outlined above. An aliquot of the WSN extract (10-100 uL depending on the concentration of free amino acids expected) is diluted to 1 mL with H2O and 2 mL of Cd-ninhydrin reagent added. The mixture is heated at 84°C for 5 min, cooled and the absorbance at 507 nm determined against a reagent blank, containing no WSN. Results are expressed as A507 or as mg Leu / g cheese by reference to a standard curve.

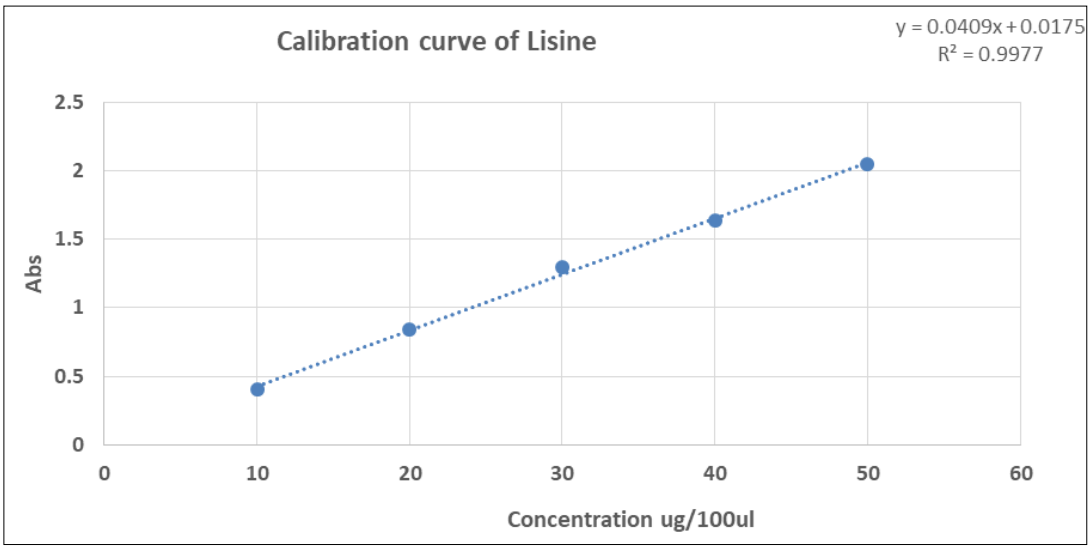


Fig 1: Calibration Curve of Lisine

- **Determination of lactose in cheese by the phenol colorimetric method**

An aqueous extract of cheese is clarified with sodium hydroxide and zinc sulphate and treated with aqueous phenol and sulphuric acid. The absorbance of the resulting solution is measured and the lactose concentration is calculated from a standard curve prepared similarly using solutions of lactose monohydrate. Weigh accurately

4.8-5.3 g of cheese into a blender jar. Add 20 ml 0.5M sodium hydroxide from a measuring cylinder and about 100 ml distilled wster. Blend until the sample is dispersed. Transfer to a 250 ml volumetric flask, add 20 ml of 10% zinc sulphate solution from a measuring cylinder and shake. Make up to the mark with distilled water. Filter through a fast filter paper and discard the first few millilitres of filtrate. Prepared standard solutions containing 0, 1.0,2.0,4.0, 6.0 and 8.0 mg/l00 ml lactose by dilution of the standard stock solution containing 1 gil in 100 ml volumetric flasks. Pipette 1.00 ml of each of these standard solutions into a series of labelled test tubes and, into a separate test-tube, pipette 1.00 ml ofthe cheese filtrate. To each of these tubes add 1 ml of phenol suolution and 5 ml of concentrated sulphuric acid from a dispenser ensuring that the acid is added directly to the solution and not down the sides of the test tubes. Place all the tubes in a boiling water bath for 5 min. Measure the absorbance of each at 490nm [15].

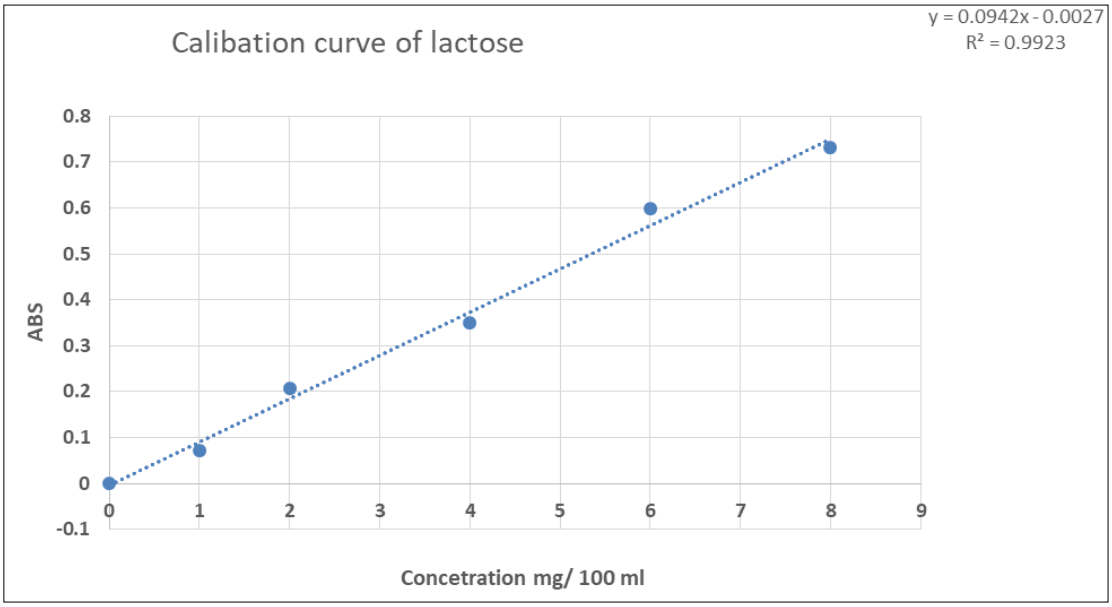


Fig 2: Calibration curve of lactose

Sodium chloride: *Official methods* Chloride: IDF Standard 88A:1988; AOAC Method 983.14 (Potentiometric titration) [12] Grated cheese (2 g) is placed into a 150 mL beaker and 100 mL dilute nitric acid solution added (1.5 mL cone. HNO3 made up to 1 L). This solution is then heated at 60°C for 1 h and titrated with 0.1 N $AgNO_3$. The end point is determined potentiometrically and is reached when a difference of +255mV is obtained between the silver electrode and the reference electrode. [16]

Results and Discussion

1. **Changes in the content of lactose and free amino acids in cheese samples coagulated with chymosin and chymosin + microbial culture, with salting, during ripening for 12, 22 and 32 days.**

In the table 1 are shown the data of humidity, pH, acidity, sodium chloride, lactose and free amino acid content of cheese samples, coagulated with chymosin and chymosin + microbial culture and treated with salt, during ripening for 12, 22 and 32 days.

The data in table 1 show that the lactose content decreases from day 12, 22 and 32, respectively 100, 93 and 83 mg / 100 g for chymosin coagulated cheese and 130, 30 mg / 100 and lower than the limit of detection of the method forchymosin+Starter coagulated cheese. Whereas, the content of free amino acids does not undergo significant changes for chymosin coagulated cheese. It results in approximately 3.35, 4.03 and 3.43 mg / 100g of cheese on days 12, 22 and 32 of ripening, respectively.

Table 1: Physico-chemical indicators during the maturation of cheese coagulated with chymosin and chimosin and starter culture (salted variant)

Sample/Analysis	Ripeining days	Humidity, %	Ph, unit	Acidity, % Lactic Acid	NaCl, %	Lactose, mg/100 gr	AAM mg/100 gr
Chymosin coagulated cheese (S)	12	44.3	6.5	0.3	20.7	100	3.35
	22	40.9	6.4	0.2	19.1	93	4.03
	32	40	6.3	0.14	19.1	83	4.43
Chymosin&Starter coagulated cheese (S)	12	48.4	5.39	0.55	23	130	13
	22	40	5.36	0.7	22	30	17
	32	44	5.3	0.68	22	0	22

(S-salted)

Whereas, the content of free amino acids undergoes changes for chymosin+Starter coagulated cheese. It increases with respectively 13, 17 and 22 mg / 100g of cheese on days 12, 22 and 32 of ripening. Our dada shown that the activity of lactic acid bacteria, which come from milk, is low for lactose metabolism throughout the ripening period.

The same phenomenon is observed on content of free amino acid, where the proteolysis of cheese proteins is low throughout the ripening period. Whereas the addition of microbial culture (yoghurt) significantly increases lactose metabolism. The amount of lactose is reduced to a minimum at the end of the ripening period.On the other hand, the proteolysis of proteins for the cheese, coagulated with chymosin and starter, during storage is higher than that of cheese coagulated only by chymosin.

Changes in the content of lactose and free amino acids of cheese samples coagulated with chymosin and chymosin + microbial culture, without salting, during ripening for 1, 3 (5) and 7 days.

Table 2: Physical andchemical indicators during the maturation of cheese coagulated with chymosin and chymosin and starter culture (non-salted variant)

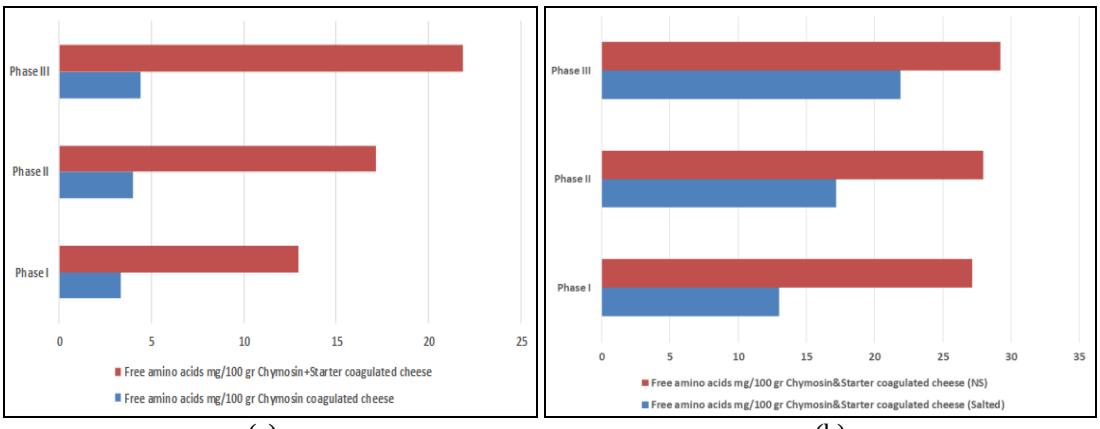
Sample/Analysis	Ripeining days	Humidity, %	Ph, unit	Acidity, % Lactic Acid	Lactose, mg/100 gr	AAM mg/100 gr
Chymosin coagulated cheese (NS)	1					
	5	58.6	6.36	0.56	190	6.3
	7	62.4	6.11	0.27	65	12.1
Chymosin&Starter coagulated cheese (NS)	1	57.6	6.15	0.82	110	27.2
	3	44.4	5.08	0.95	55	27.9
	7	43.5	5	0.99	>DL	29.2

(NS – non salted, DL-detection limit)

The data in Table 2 shows that the lactose content decreases significantly from day 1, 5 and 7, respectively, 190 and 65 mg / 100 g. Whereas, the content of free amino acids increases with respectively, 6.3 and 12 mg / 100g of cheese on days 5 and 7 of ripening for Chymosin coagulated cheese (NS). Dates for Chymosin & Starter coagulated cheese (NS) shown that lactose content decreases significantly from day 1, 3 and 7, respectively 110, 55 mg / 100 g and lower than the detection limit. Whereas, the content of free amino acids results with respectively 27.2, 27.9 and 29.2 mg / 100g of cheese on days 1, 3 and 7 of baking. Our data shows that the addition of starter culture (LAB) (yoghurt) in cheese significantly increases the activity of lactic acid bacteria, in the first 7 days of ripening, and therefore the metabolism of lactose. On the other hand, the activity of lactic acid bacteria during ripening of cheese (NS) coagulating only with chymosin increased significantly compared to salted cheese ripening.Proteolysis of proteins of non-salted without or with starter (LAB) cheese during storage for seven days increases significantly compared to the proteolysis of salt-treated cheese. The data shown that microbial culture (LAB) treatment promotes lactose metabolism and protein proteolysis during the period of salt-free cheese ripening and improves the taste and aroma of the cheese obtained. The data of our study are consistent with other studies [3, 4, 6, 17, 18].

From the data's of the study it is concluded that the use of microbial culture promotes lactose metabolism and proteolysis of cheese proteins (free amino acid content), from the first days of cheese ripening, obtaining flavors and aromas, as and improved quality of the cheese obtained.

From the results of the study we advise the further study of the processes of proteolysis and lipolysis and the standardization of the quality of the processes and products of cheese from cow, sheep and goat milk.



(a) (b)

Fig 3: The impact of (a) microbic culture, (b) salt on the concentration of free amino acids during ripening period.

Conclusions

The activity of lactic acid bacteria and proteolysis of proteins durin ripening of cheese with chymosin and without salting increases significantly compared to the ripening of cheese coagulated only with chymosin and salting. The use of microbial culture promotes lactose metabolism and proteolysis of cheese proteins (free amino acid content), from the first days of ripening cheese, obtaining flavor and aroma, as well as improved quality of cheese obtained.

The reserach appeals for the conservation demands of local bereeds and measures to be undertaken for promoting conservation development or integration of development practices into nature conservation approaches.

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