

Amino Acid Composition and Nutritional Evaluation of Proteins in Goat Cheeses Produced with Different Starter Cultures

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Abstract This study investigated the influence of mesophilic starter (M), thermophilic starter (T), *Lactobacillus Plantarum* ssp. *Plantarum* ATCC 14917 (*Lp*), a mix of the M and T (M1), and a mix of the M, T, and *Lp* (M2) on protein amino acid composition and nutritional evaluation in goat cheeses during maturation. The results of our SDS-PAGE analysis showed that the high-molecular-weight proteins in M2 cheese mostly degraded, producing a relevant amount of low-molecular-weight proteins. We detected 16 amino acids in all cheeses. The total amount of essential amino acids/total amino acids (TEAA/TAA) value ranged between 38.98%–44.63%. The nutritional evaluation showed that the TEAA of all 60-day-old cheeses exceeded the recommended WHO/FAO value and the score of amino acid ratio coefficient (SRC) and essential amino acid index (EAAI) result indicated that cheeses produced using M and M2 exhibited higher nutritional value than other cheeses. In conclusion, M2 cheese contained a high nutritional value with various molecular-weight proteins.

Keywords: goat cheese, starter culture, protein SDS-PAGE, amino acid composition, nutritional evaluation

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1. Introduction

Since the early 21st century, our society has witnessed increasing health awareness when choosing food products. In this context, dairy products are attracting interest for their nutritional properties and health benefits [1]. Milk can be processed into various types of dairy products such as cheese, a protein-rich product with multiple flavors, sizes, and forms. Among them, hard and semi-hard cheeses, including Chihuahua, Cheddar, Gouda, and Parmesan cheese, need aging [2]. Several biochemical changes occur, including the metabolism of residual lactose and lactate, citrate, lipolysis, and proteolysis [3]. Protein breakdown is mainly catalyzed by rennet, endogenous milk enzymes (blood cellulase, cathepsin D, and other somatic cell proteases), starter cultures, and nonstarter microbial enzymes [4], and it is possibly the most significant biochemical event. Specifically, casein in cheese is the most important protein to be degraded into polymer peptides under the combined action of rennet and starter protease present in the cheese. Studies show that rennet plays a major role in the degradation process [5]. Degradation continued for a long time. Polymer polypeptides gradually degraded into low-molecular

proteins, amino acids, amines, sulfur-containing compounds, and other flavor substances.

Meanwhile, amino acid transaminase, decarboxylase, and lyase catabolize amino acids produced by protein degradation. Protein catabolites such as peptides, free amino acids, ammonia, organic acids, sulfur compounds, and ester compounds play the most important role in cheese flavor and texture. Studies showed that protease in starter cultures could degrade cheese proteins, thus forming peptides, peptones, amino acids, etc., and making its tissue uniform and delicate [6]. Kongo [7] also reported that LAB starter cultures involved at the beginning of the fermentation process had a dominant role in determining several biochemical processes of the products during cheese ripening.

SDS-PAGE separates proteins in samples according to molecular weight and is widely used to identify and qualitatively determine different kinds of proteins. Tan et al. [8] used SDS-PAGE to study protein degradation during cheese maturation. They determined the degradation degree mainly by the number and color depth of different molecular weight bands in the gel. In addition, most proteins are composed of 20 amino acids, some of which are necessary for the human body in large quantities, whereas others are not. Amino acid composition and proportion are thus the most important

factors to evaluate protein quality. Meanwhile, as mentioned above, protein degradation produces abundant metabolites with a relevant impact on the quality of mature cheese, especially its amino acid composition, which significantly affects cheese's nutritional value and is considered an important nutritional value index. Amino acid analysis and evaluation methods mainly include amino acid score (AAS), essential amino acid index (EAAI), amino acid ratio coefficient (RC), or ratio coefficient score [9]. These methods are simple, feasible, and credible. Several studies focus on how starter cultures affect the physiochemical properties and volatile components of cheeses or the proteolysis in certain cheeses [10,11]. However, few studies that evaluate cheese quality from a nutritional perspective are available [12,13].

Goat milk is a valuable food source of animal protein, phosphorus, fat, protein, lactose, and mineral (especially calcium) and possesses various health benefits [14]. Its lipids provide better digestibility with small fat globule size and high short- and medium-chain fatty acids content. In addition, the proteins in goat milk, not like bovine milk, are not prone to allergy as life-threatening. The β -CN/ α _{s1}-CN ratio (70%/30%) of goat milk proteins is the most similar to human milk, which results in more digestibility compared to the bovine milk in relation to higher sensitivity of β -casein to the protease enzymes. Oligosaccharide-rich goat milk is also important in its protective function of intestinal flora against pathogens and in brain and nervous system development. As the largest goat milk-producing province in China, Shaanxi Province had 2.4 million dairy goats in 2021, with an output of 700,000 tons of goat milk and a total output of 129,000 tons of goat milk-based products. However, at present, goat milk-based products are mainly formula goat milk powder with the market share more than 90% in China, and goat milk cheese is rarely present. Therefore, this study aimed to investigate how starter cultures affect cheese quality and provide a suitable starter culture for goat cheeses of various molecular-weight proteins and high nutritional value.

2. Materials and Methods

2.1. Starter Preparation

The mesophilic starter (**M**) contained *Lactococcus lactis* ssp. *cremoris* and *Lactococcus lactis* ssp. *lactis* (R-704, Chr. Hansen, Horsholm, Denmark), whereas the thermophilic starter (**T**) *Lactobacillus delbrueckii* subsp. *bulgaricus* and *Streptococcus thermophiles* (YO-MIX 495, Danisco DuPont, Dangé-Saint-Romain, France). Both starter powder were inoculated into sterilized, reconstituted skim milk, incubated at 42°C to develop, and was then maintained at 0 to 5°C before cheesemaking. *Lactobacillus plantarum* ssp. *plantarum* ATCC 14917 (**Lp**) was activated and mixed with physiological saline solution for use. Mixed starter 1 (**M1**) was a mixture of M and T at a ratio of 1:1 (wt/wt). Mixed starter 2 (**M2**) was a mixture of M, T, and Lp at a ratio of 1:1:1 (wt/wt/wt).

2.2. Cheese Manufacture

We produced goat cheeses according to a previous study [15]. Briefly, 10 kg of fresh goat milk for each trial was pasteurized at 63°C for 30 min and inoculated with 2% (wt/wt) starter culture at 32°C. Next, we supplemented the milk with 0.4 g calcium chloride dissolved in 5 mL distilled water. Based on its activity, we evaluated chymosin (1:100,000 strength, calf rennet enzyme; CAS No. 9001-98-3; Zhengzhou Universe Food Ingredient Limited Company, Henan, China). After the coagulation catalyzed by chymosin for 40 min, the curd samples were carefully cut into 1 cm³ cubes and then heated to 42°C at a rate of 1°C min⁻¹ with stirring. We drained the whey by filtration and mixed the obtained curd samples with salt at a final concentration of 2.5% (wt/wt). Then we placed the mixture in a cheese mold with the pressure of 30 g cm⁻² for 6 h at room temperature. The cheese samples matured at 11.5°C with 70% relative humidity for 7 days. We divided the cheese samples into 12 fan-shaped structures, vacuum-sealed them, and allowed them to continue maturing under the original conditions.

We used the cheese samples that matured for 1, 7, 15, 30, 45, and 60 days for further analysis, and cheese samples produced by M, T, Lp, M1, and M2 were named **MC**, **TC**, **LC**, **M1C**, and **M2C** respectively.

2.3. Protein SDS-PAGE

Proteins in all cheese samples were analyzed by SDS-PAGE using a vertical casting system (*Solarbio*, Beijing, China). Acrylamide concentrations in the gels were 3.75% (w/v) for the stacking and 13.5% (w/v) for the separating gels. The mixture of 0.5 g of cheese with 3 mL of 40°C distilled water was homogenized for 6 min at 6,000 rpm then incubated in a water bath at 45 °C for 40 min. Next, the test tubes were removed and centrifuged at 5,000 rpm for 15 min. The supernatant was separated and chilled for further use.

Next, we boiled 14 μ L of 4 \times protein loading buffer and 42 μ L of the above-described supernatant for 5 min. After adding the electrode buffer to the electrophoresis system, we injected 8 μ L of the boiled mixture. The gels were subsequently stained with 1 g Coomassie blue (R-250) dissolved in a mixture of 45% (v/v) water, 45% (v/v) methanol, and 10% (v/v) acetic acid for 2 h, then destained with a solution consisting of 10% (v/v) methanol, 10% (v/v) acetic acid, and 80% (v/v) distilled water. We scanned the gels with the Chemi Doc-It System (UVP) for grayscale analysis and calculated the values with their own blanks as 0.

2.4. Amino Acid Composition and Nutritional Evaluation

We put 1 g of grated cheese into the digestive tube, supplemented it with 10 mL of 6 mol L⁻¹ hydrochloric acid, sonicated the mixture for 2 min, purged it with nitrogen for 2 min, and finally sealed. We took out the digestive tube after it baked in an oven at 110°C for 22 h and diluted it to a 100 mL volumetric flask with ultrapure water. We filtered the solution with a 0.45- μ m inorganic

filter after mixing, and 1 mL of filtrate was diluted to 10 mL again. Then, a 0.45- μm inorganic filter was used for another filtration cycle. The amino acid type and content in the filtrate were determined by an L-8900 automatic amino acid analyzer (Hitachi Ltd., Tokyo, Japan) for three times.

Based on previous studies [12] and the guidelines of the Food and Agriculture Organization/World Health Organization [16], we evaluated the nutritional content of amino acids in the cheese samples by means as follows:

AAS:

$$\text{AAS} = \frac{\text{AA}}{\text{AA(FAO/WHO)}} \times 100\% \quad (1)$$

where AA represents the amino acid content in the test sample ($\text{mg g}^{-1} \text{N}$) and AA (FAO/WHO) the amino acid content in the FAO/WHO scoring model ($\text{mg g}^{-1} \text{N}$).

Ratio Coefficient of Amino Acid (RC):

$$\text{RC} = \frac{\text{AAS}}{\text{Average(AAS)}} \quad (2)$$

Score of RC (SRC):

$$\text{SRC} = 100 \times (1 - \text{CV}) \quad (3)$$

$$\text{CV} = \frac{\sigma}{\mu} \quad (4)$$

where CV represents the coefficient of variation, σ the standard deviation, and μ the average.

Essential amino acid index (EAAI):

$$\text{EAAI} = \sqrt[n]{\frac{100a}{A} \times \frac{100b}{B} \times \dots \times \frac{100h}{H}} \quad (5)$$

where EAAI was calculated using whole egg protein as a reference protein (RP), "a, b, ..., h" represent the essential amino acid (EAA) content in the measured sample protein, ($\text{mg g}^{-1} \text{N}$); A, B, ..., H the EAA of whole egg protein, ($\text{mg g}^{-1} \text{N}$); and n the species number of EAA in this study ($n = 7$).

2.5. Statistical Analysis

We used SPSS software (Version 17.0, SPSS Inc., Chicago, IL) for statistical analysis and plotted the figures using Adobe Illustrator (Adobe Systems, USA).

3. Results and Discussion

3.1. Protein SDS-PAGE

Starter cultures reportedly exhibited limited physicochemical properties but an obvious effect on textural characteristics and volatile compounds in our previous study [15]. The total protein content of the cheese samples at 60 days of maturity, determined by the micro-Kjeldahl method according to AOAC [17], was 21.84%, 25.77%, 25.16%, 22.85%, and 25.84%, for MC, TC, LC, M1C, and M2C, respectively. These results were consistent with the protein content of rennet cheese ranging between 20%-30% in the study of Eroglu et al. [18]. Based on the

variance analysis, the protein contents of MC produced with the M was significantly lower than the other cheeses ($p < 0.05$). The lowest contents may be due to the fast acidification rates caused by the starter M. It is reported that rapid acidification could accelerate the dissolution of calcium in the matrix and inhibit the protein-protein interactions [19], resulting in increasing protein loss during cheese making and maturation. However, the synergistic effects of T and *Lp* were beneficial to reducing protein loss and could produce goat cheese with the increased protein contents such as M1C, and M2C.

Starter culture types reportedly exhibit an obvious influence on cheese protein degradation [20]. In this study, SDS-PAGE and grayscale scanning analyses evaluated protein degradation during cheese ripening. Figure 1 and Table 1 show that the macromolecular protein content with relative molecular weights above 45 kDa (mainly LF and BSA) in all cheese samples decreased with the increasing maturity time, especially in M2C macromolecular proteins almost disappeared after 15 days of maturity. The results indicated that M2 had a strong ability to break down high-molecular-weight proteins in cheese. However, the LF content in 60-day-old TC increased by 14.52% when compared with 1-day-old TC, which may be due to the degradation and accumulation of high molecular proteins. In addition, IgG with specific activity was almost undetected in this work.

β -CN is one of the four main types of casein, the main milk protein. Alichanidis et al. reported that the relative content of β -CN to total casein in goat milk (34–64%) was higher than that in cow milk (28–38%) [21]. Its content in MC, TC, and M2C showed an increasing then a decreasing trend with a slight final recovery during the subsequent maturation, which may be related to both the degradation and accumulation of polymeric proteins during cheese ripening and the continuous degradation of β -CN to form low molecular weight peptides. Among them, the proteases and peptidases that lead to the degradation of cheese proteins, especially casein, are mainly derived from starter and non-starter lactic acid bacteria [22,23], and the peptides derived from β -CN could be further analyzed by peptidomics. However, the β -CN change in M1C was in contrast with that in MC and M2C, consistently with the results of Ren [24]. Compared with 1-day-old samples, β -CN content in 60-day-old LC, M1C, and M2C decreased. Among them, the percent decrease of β -CN in LC and M2C (74.49% and 67.79%, respectively) was higher than that in M1C (52.07%), which might be attributed to the presence of *Lp* in LC and M2C. *Lp* exhibited a high ability to degrade β -CN during cheese maturation rapidly. In addition, the less degradation by starter cultures accounted for the higher κ -CN content, another main milk protein, in MC and TC than that of the other three cheese samples during their ripening.

The electrophoretic bands of β -LG and α -LA in LC were darker than those of β -CN and κ -CN (and displayed higher gray values in Table 1). This result potentially indicated that a significant amount of high-molecular-weight proteins in LC degraded by *Lp* into low-molecular-weight proteins below 20 kDa, such as β -LG and α -LA. The obvious changes in the electrophoretic bands of M1C and M2C during cheese maturation (60 days) also

indicated that more high-molecular-weight proteins were available for degradation in these two goat cheeses than in MC and TC individually. Compared with the other four goat cheese samples, cheeses fermented by M2 produced more low-molecular-weight proteins (the most were low-molecular-weight electrophoresis bands), suggesting that *Lp* combined with M and T played the most important role in protein degradation during cheese ripening.

Nevertheless, no obvious α_{s1} -CN or α_{s2} -CN appeared in the five goat cheeses throughout the maturation period. One of the potential reasons is that the α_{s1} -CN (5.60%) and α_{s2} -CN (19.2%) contents were low in the goat milk, which is also why goat milk is less allergenic compared with cow milk [25]. Another reason might be that most proteases in cheese could easily hydrolyze α_s -CN, whereas β -CN in cheese was less susceptible to hydrolysis. The results of our previous study showed that goat milk α_s -CN was more unstable and could be digested faster under the same *in vitro* simulated digestion conditions when compared to β -CN and κ -CN: after 30 min of simulated *in vitro* gastric digestion, followed by 2 min of intestinal digestion, the digestibility of α_{s1} -CN and α_{s2} -CN was higher than that of β -CN and κ -CN (99.82, 99.86,

99.76, and 99.05%, respectively) [26]. In general, the protein degradation in cheeses might involve complex mechanisms and processes. Further research efforts should focus on these aspects.

3.2. Amino Acid Composition Analysis

Table 2 shows the results of the amino acid composition analysis of the five investigated goat cheeses. There were 16 kinds of amino acids in each cheese, including eight EAAs: leucine, lysine, phenylalanine, valine, threonine, isoleucine, methionine, and tyrosine (conditional EAA) and eight kinds of non-EAAs: glutamic acid, proline, aspartic acid, serine, alanine, histidine, arginine, and glycine. Tryptophan and cysteine were not detected in our study, possibly because tryptophan in cheese was degraded during acid hydrolysis, whereas cystine was degraded and oxidized, according to You [27]. The amino acid type and content in cheese depend to a large extent on the maturation time, the milk type (cow, sheep, and goat milk, or a mixture), the refrigeration conditions of milk (frozen and nonfrozen), the use of lactic acid bacteria coculture, and the cheese production process [28].

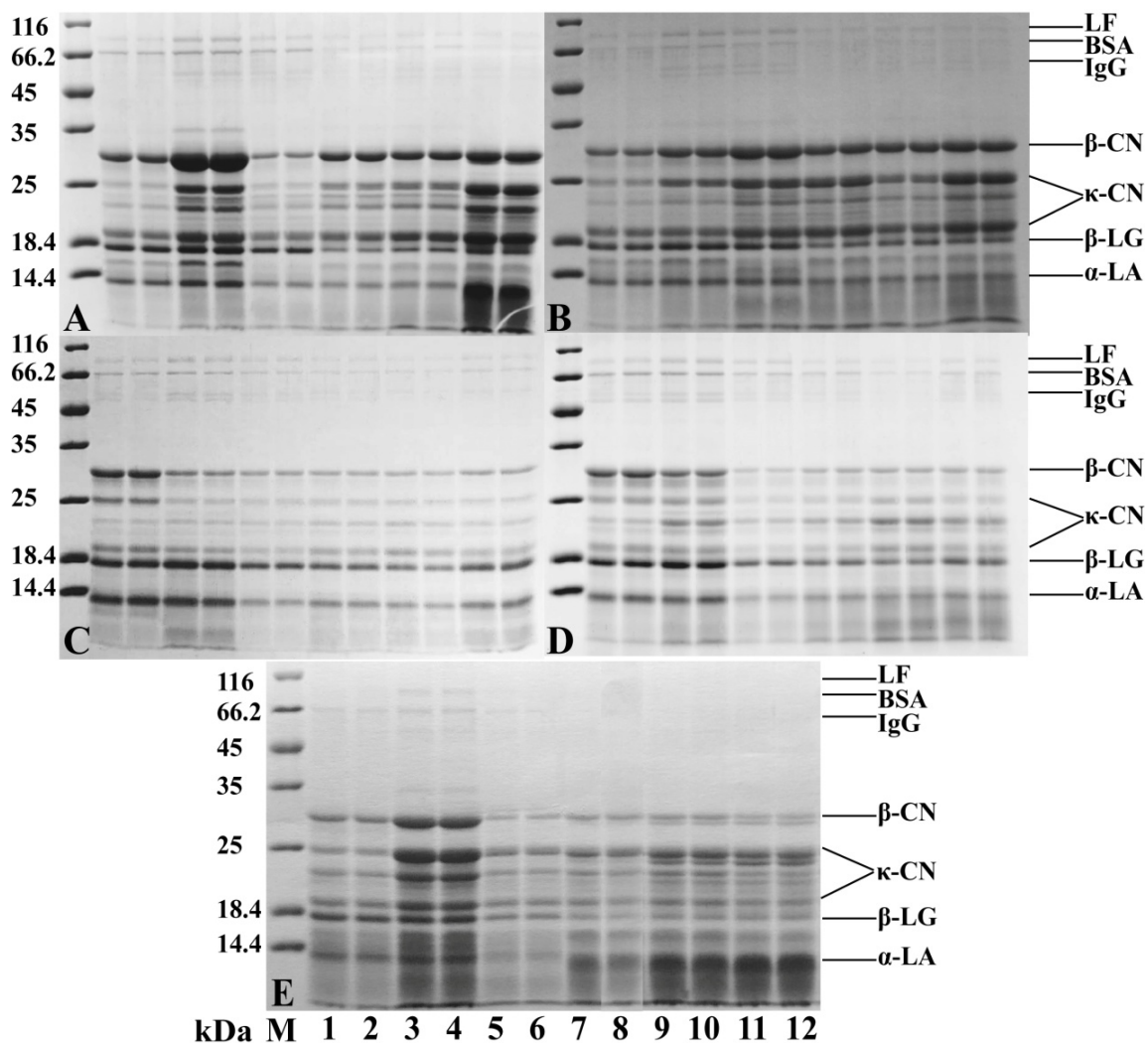


Figure 1. SDS-PAGE of goat cheeses with five starter cultures at different maturation periods. MC (A, cheese made with M), TC (B, cheese made with T), LC (C, cheese made with *Lp*), M1C (D, cheese made with M1), and M2C (E, cheese made with M2). From left to right, the leftmost M was the standard protein band; the protein electrophoresis bands were 1 (1, 2), 7 (3, 4), 15 (5, 6), 30 (7, 8), 45 (9, 10), and 60 days (11, 12), respectively. The period was two sets of parallels

Table 1. Grayscale scanning analysis of goat cheeses with five starter cultures at different maturation periods

Sample	d	α-LA	β-LG	β-CN	α ₂ -CN	IgG	BSA	LF
MC	1	105.99±4.60 ^c	144.71±10.23 ^a	125.44±7.82 ^{cd}	ND	ND	39.11±2.95 ^b	10.18±5.05 ^b
	7	140.13±4.67 ^b	166.27±2.18 ^a	193.27±0.16 ^a	39.10±3.32	ND	62.16±0.40 ^a	32.42±0.06 ^a
	15	80.09±6.43 ^d	109.94±8.20 ^b	64.50±5.07 ^e	ND	ND	32.40±5.67 ^b	12.41±2.45 ^b
	30	73.48±2.62 ^d	92.71±1.64 ^b	124.53±6.49 ^d	ND	ND	7.70±1.83 ^c	3.54±1.75 ^b
	45	86.70±9.13 ^{cd}	112.38±6.70 ^b	145.45±5.47 ^b	ND	ND	5.91±1.13 ^c	2.57±2.64 ^b
	60	191.39±0.51 ^a	161.78±10.51 ^a	163.98±1.87 ^b	ND	ND	ND	ND
TC	1	73.50±0.45 ^{ab}	90.11±2.45 ^{ab}	52.85±3.96 ^d	ND	ND	16.47±1.10 ^c	15.95±0.22 ^b
	7	82.47±8.80 ^{ab}	105.49±6.89 ^a	100.64±1.55 ^{bc}	ND	ND	29.41±1.09 ^a	33.15±3.65 ^a
	15	87.05±4.42 ^a	104.61±5.05 ^a	112.71±1.26 ^a	ND	ND	21.48±0.27 ^b	26.99±1.06 ^a
	30	73.09±3.74 ^{ab}	78.25±6.10 ^b	100.97±0.24 ^{bc}	ND	ND	13.21±1.44 ^c	14.09±1.39 ^b
	45	67.08±5.68 ^b	81.48±0.89 ^b	99.20±0.38 ^c	ND	ND	13.87±1.71 ^c	16.26±1.44 ^b
	60	79.96±1.10 ^{ab}	88.70±1.87 ^{ab}	107.86±1.98 ^{ab}	ND	ND	15.37±0.85 ^c	18.27±1.28 ^b
LC	1	155.31±1.38 ^a	154.81±6.46 ^a	151.48±3.91 ^a	ND	27.72±5.83 ^a	44.38±1.35 ^a	27.52±2.88 ^a
	7	143.19±8.50 ^a	153.62±6.47 ^a	84.27±5.18 ^b	ND	24.83±6.64 ^a	36.46±6.40 ^a	27.13±6.60 ^a
	15	61.20±7.12 ^b	111.33±4.27 ^b	46.15±7.91 ^c	ND	3.89±3.78 ^b	10.92±3.81 ^b	7.30±1.17 ^b
	30	65.57±6.02 ^b	92.93±4.42 ^b	30.88±0.23 ^{cd}	ND	0.14±0.08 ^b	1.23±0.93 ^b	0.32±0.16 ^b
	45	50.29±0.64 ^b	62.82±2.16 ^c	22.18±5.90 ^d	ND	ND	0.33±0.00 ^b	0.21±0.03 ^b
	60	68.96±2.52 ^b	103.84±9.70 ^b	38.65±5.70 ^{cd}	ND	ND	1.26±0.16 ^b	0.11±0.08 ^b
M1C	1	121.68±7.81 ^{ab}	165.96±1.47 ^a	139.18±9.07 ^a	ND	26.19±4.28 ^b	57.37±5.40 ^a	29.85±2.37 ^b
	7	133.67±10.38 ^a	175.69±7.76 ^a	111.59±1.05 ^b	ND	38.00±0.94 ^a	66.68±1.17 ^a	43.03±0.04 ^a
	15	71.24±2.62 ^d	124.93±4.61 ^{bc}	31.49±9.69 ^d	ND	20.35±2.74 ^b	40.20±5.66 ^b	28.86±4.25 ^b
	30	92.63±4.41 ^{cd}	111.24±2.10 ^c	62.16±0.29 ^c	ND	18.02±1.22 ^b	38.26±0.99 ^b	22.49±2.86 ^{bc}
	45	103.88±4.71 ^{bc}	115.51±0.05 ^{bc}	71.09±3.20 ^c	ND	ND	20.64±0.85 ^c	14.91±2.62 ^c
	60	104.56±5.95 ^{bc}	130.11±2.63 ^b	66.71±0.76 ^c	ND	ND	32.11±2.45 ^{bc}	21.85±1.60 ^{bc}
M2C	1	80.08±0.91 ^b	79.85±1.47 ^b	55.24±5.73 ^b	ND	ND	ND	ND
	7	111.42±0.78 ^a	105.34±0.54 ^a	112.53±1.14 ^a	ND	ND	ND	ND
	15	49.34±1.03 ^c	48.77±1.66 ^c	15.04±1.40 ^d	ND	ND	ND	ND
	30	75.56±8.03 ^b	32.75±8.25 ^d	24.13±1.00 ^{cd}	ND	ND	ND	ND
	45	110.38±7.74 ^a	46.59±0.29 ^{cd}	25.99±0.50 ^c	ND	ND	ND	ND
	60	122.50±0.33 ^a	42.30±0.92 ^{cd}	17.79±1.01 ^{cd}	ND	ND	ND	ND

^aValues represent the means (Average ± SD; n = 2) in MC (cheese made with M), TC (cheese made with T), LC (cheese made with *Lp*), M1C (cheese made with M1), and M2C (cheese made with M2). ND, not detected.

^{a,b,c,d} The same cheese in the column with different superscripts is significantly different ($p < 0.05$).

Table 2. Content of 16 amino acids of goat cheeses with five starter cultures during maturation (Expressed as mean; Unit: mg g⁻¹ sample)

Sample	d	Essential amino acids								Non-essential amino acids								TEAA	TAA	TEAA/TAA (%)
		Leu	Lys	Phe	Val	Thr	Ile	Met	Tyr	Glu	Pro	Asp	Ser	Ala	His	Arg	Gly			
MC	1	26.67	20.15	20.91	16.22	12.23	11.22	19.07	10.48	82.66	57.02	18.70	14.67	8.91	7.27	8.18	3.71	136.95	338.07	40.51
	7	30.89	22.68	19.70	18.26	13.64	13.91	21.50	13.59	86.30	71.23	21.90	15.88	9.93	8.60	4.47	4.29	154.17	376.77	40.92
	15	25.47	19.73	16.80	14.98	11.79	10.50	14.42	11.49	74.63	61.51	18.81	13.77	8.20	7.15	3.84	3.56	125.18	316.65	39.53
	30	25.90	19.88	14.88	15.06	12.06	10.47	14.98	11.44	76.12	62.50	19.15	14.12	8.48	7.28	3.87	3.64	124.67	319.83	38.98
	45	31.46	21.54	27.67	18.41	13.13	14.81	25.46	14.61	88.64	55.23	19.47	15.94	10.23	6.71	8.75	3.99	167.09	376.05	44.43
	60	22.74	17.12	13.34	12.92	10.17	9.17	12.75	10.34	64.17	53.18	16.67	11.98	7.96	5.91	3.57	3.34	108.55	275.33	39.43
TC	1	26.75	20.51	20.92	16.24	12.12	11.24	18.37	10.86	83.10	60.06	19.26	14.69	9.13	7.37	8.32	3.76	137.01	342.70	39.98
	7	28.25	20.74	24.09	16.86	12.35	12.49	21.79	12.07	84.43	54.82	19.10	15.16	9.60	7.18	8.53	3.74	148.64	351.20	42.32
	15	31.95	23.53	20.50	18.98	14.03	14.40	22.12	14.38	89.62	73.57	22.58	16.47	10.67	8.91	4.67	4.45	159.89	390.83	40.91
	30	32.75	24.44	20.34	19.22	14.49	14.52	22.11	14.81	92.37	75.29	23.25	16.99	10.92	9.17	4.79	4.58	162.68	400.04	40.67
	45	35.17	24.39	35.05	20.78	14.87	16.26	28.26	15.90	100.54	62.08	21.88	18.02	11.74	7.86	9.96	4.53	190.68	427.29	44.63
	60	30.71	22.38	19.49	18.29	13.41	13.86	21.57	14.00	85.68	70.92	21.70	15.69	10.07	8.39	4.48	4.29	153.71	374.93	41.00
LC	1	18.87	14.42	14.48	11.49	8.68	7.90	13.33	7.15	58.71	42.39	13.20	10.25	6.05	5.21	5.70	2.54	96.32	241.65	40.07
	7	29.27	21.47	16.47	17.53	13.61	12.68	19.97	12.17	85.78	70.99	21.18	15.65	9.02	7.99	4.34	4.09	143.17	355.43	39.53
	15	24.77	19.05	16.43	14.69	11.52	10.09	14.38	10.79	72.65	60.09	17.92	13.20	7.42	6.87	3.68	3.36	121.72	310.58	39.66
	30	30.63	22.48	19.53	18.07	13.70	13.62	21.06	13.46	86.21	69.65	21.19	15.68	9.40	8.49	4.36	4.10	152.55	367.03	41.05
	45	29.55	22.25	26.17	19.22	13.70	14.41	26.37	13.20	86.78	68.07	20.81	15.76	10.34	7.43	8.98	4.09	164.87	375.74	42.59
	60	28.00	21.57	18.28	16.68	13.14	11.42	16.56	12.15	83.15	67.36	20.44	15.22	8.64	7.88	4.20	3.91	137.80	351.60	39.53
M1C	1	21.04	16.01	15.99	12.66	9.49	8.81	14.61	8.17	64.77	44.29	14.69	11.42	7.09	5.63	6.59	2.87	106.78	264.13	40.43
	7	23.18	17.58	15.68	13.35	10.54	9.43	13.19	10.39	67.22	57.08	17.16	12.42	7.51	6.26	3.50	3.29	113.34	287.78	39.38
	15	25.60	18.09	16.83	14.85	10.95	11.62	18.05	11.82	69.79	57.16	17.72	12.88	8.29	6.37	3.53	3.54	127.81	307.09	41.62
	30	25.88	19.00	17.13	14.83	11.04	11.69	16.46	11.76	70.80	59.25	18.08	12.95	8.36	6.65	3.66	3.55	127.79	311.09	41.08
	45	26.00	19.69	16.76	15.23	11.97	10.53	14.98	11.46	75.94	61.82	19.16	13.94	8.57	7.24	3.93	3.67	126.62	320.89	39.46
	60	27.99	20.19	18.43	16.28	12.16	12.63	19.56	12.62	77.50	63.57	19.59	14.35	9.08	7.46	4.02	3.89	139.86	339.32	41.22
M2C	1	26.91	20.03	21.47	16.57	12.16	12.01	19.54	10.96	82.17	53.64	18.80	14.72	9.33	6.82	8.51	3.76	139.65	337.40	41.39
	7	23.43	18.23	16.09	13.65	10.79	9.68	12.66	10.72	68.10	57.62	17.65	12.56	7.75	6.38	3.65	3.39	115.25	292.35	39.42
	15	25.49	18.51	17.18	14.97	10.97	11.85	17.76	11.98	69.38	58.62	18.03	12.67	8.38	6.54	3.73	3.60	128.71	309.66	41.56
	30	29.06	21.40	18.80	16.71	12.87	13.05	20.48	13.47	81.13	66.20	20.93	15.00	9.76	7.92	4.33	4.15	145.84	355.26	41.05
	45	29.79	21.98	19.38	17.13	13.28	13.34	20.77	13.57	83.54	68.89	21.53	15.47	10.15	8.16	4.32	4.28	149.24	365.58	40.82
	60	26.12	18.93	14.99	15.16	11.48	11.96	18.65	12.28	71.91	59.85	18.71	13.42	8.94	6.83	3.85	3.79	129.57	316.87	40.89

^aLeu: Leucine; Lys: Lysine; Phe: Phenylalanine; Val: Valine; Thr: Threonine; Ile: Isoleucine; Met: Methionine; Tyr: Tyrosine; Glu: Glutamic acid; Pro: Proline; Asp: Aspartic acid; Ser: Serine; Ala: Alanine; His: Histidine; Arg: Arginine; Gly: Glycine.

^bMC = cheese made with M; TC = cheese made with T; LC = cheese made with *Lp*; M1C = cheese made with M1; M2C = cheese made with M2.

^cTEAA: Total essential amino acid; TAA: Total amino acid.

EAAAs are of vital importance to humans. However, they cannot be synthesized in the human body and must be taken up from dietary proteins. EAAs can be fully absorbed, and the nutritional value can be at the highest level only when they meet human needs. Therefore, it is more scientific and accurate to evaluate amino acid quality in food protein with a total amount of essential amino acids/total amino acids (Total essential amino acid [TEAA]/TAA). Table 2 shows that the amino acid TEAA/TAA in all cheese samples during the 60-day maturation ranged between 38.98%–44.63%, which was higher than the complete protein value (36%) recommended by the FAO/WHO/UNU [29] and reached the standard of high-quality protein [30]. Meanwhile, there was only a slight difference in TEAA/TAA of the cheese samples with different starter cultures or at different maturation times. Therefore, neither the kind of starter culture nor maturity time (within 60 days) influenced the goat cheese amino acid pattern. The TEAA values in the cheese samples during ripening exhibited an overall upward trend except for those of MC and M2C. TC had the highest TEAA value, especially at 45 days, which was 190.68 mg g⁻¹ sample. Leucine and glutamic

acid were the most abundant essential and non-EAAs, respectively, in the cheese samples at the end of the maturation, consistently with the results of Teter et al. [31]. In addition, we considered leucine and other branched-chain amino acids (isoleucine and valine), aromatic amino acids (phenylalanine and tyrosine), and methionine the main precursors of key aromatic compounds in cheese [32]. Leucine, isoleucine, and valine also exhibit several other special biological functions. While comparing these amino acid contents of the cheese samples, more precursor amino acids may have been transformed into aromatic compounds in MC and M2C at 60 days. The glutamic acid and proline contents among the non-EAAs at 60 days were higher in TC (85.68 and 70.92 mg g⁻¹ sample, respectively) and LC (83.15 and 67.36 mg g⁻¹ sample, respectively) than those of MC, M1C, and M2C. The differences of these amino acids in the cheese samples might be related to the type of starter culture. Garbowska & Pluta [33] described that selecting appropriate starter cultures in cheese making had an important role in converting proteins into peptides and amino acids during maturation, which was also the main reason for the specific sensory properties of cheeses.

Table 3. Essential amino acid content of 60-day-old goat cheeses with five starter cultures and their reference model (Expressed as mean; Unit: mg g⁻¹ protein)

Species of essential amino acids	MC	TC	LC	M1C	M2C	Whole egg protein (RP)*	WHO/FAO model*
Leucine	104.12	119.17	111.28	122.48	101.09	86.00	70.00
Lysine	78.39	86.85	85.73	88.35	73.26	70.00	55.00
Phenylalanine+Tyrosine	108.42	129.96	120.94	135.87	105.54	93.00	60.00
Valine	59.16	70.98	66.29	71.24	58.67	66.00	50.00
Threonine	46.57	52.04	52.22	53.21	44.43	47.00	40.00
Isoleucine	41.99	53.79	45.39	55.27	46.29	54.00	40.00
Methionine	58.38	83.71	65.82	85.59	72.18	57.00	35.00
Total essential amino acid content (TEAA)	497.03	596.50	547.67	612.01	501.46	473.00	350.00

*Values were from the WHO/FAO (1973) recommendations.

^aMC = cheese made with M; TC = cheese made with T; LC = cheese made with *Lp*; M1C = cheese made with M1; M2C = cheese made with M2.

Table 4. Protein evaluation nutritional values of 60-day-old goat cheeses with five starter cultures (Expressed as mean)

Index	Species of essential amino acids							
	Leucine	Lysine	Phenylalanine+Tyrosine	Valine	Threonine	Isoleucine	Methionine	
MC	AAS	1.49	1.43	1.81	1.18	1.16	1.05	1.67
	RC	1.06	1.02	1.29	0.85	0.83	0.75	1.19
	SRC (%)				81.53			
	EAAI (%)				101.61			
TC	AAS	1.70	1.58	2.17	1.42	1.30	1.34	2.39
	RC	1.00	0.93	1.27	0.84	0.77	0.79	1.41
	SRC (%)				76.90			
	EAAI (%)				122.69			
LC	AAS	1.59	1.56	2.02	1.33	1.31	1.13	1.88
	RC	1.03	1.01	1.31	0.86	0.85	0.74	1.22
	SRC (%)				80.84			
	EAAI (%)				112.16			
M1C	AAS	1.75	1.61	2.26	1.42	1.33	1.38	2.45
	RC	1.01	0.92	1.30	0.82	0.76	0.79	1.41
	SRC (%)				76.33			
	EAAI (%)				125.61			
M2C	AAS	1.44	1.33	1.76	1.17	1.11	1.16	2.06
	RC	1.01	0.93	1.23	0.82	0.78	0.81	1.44
	SRC (%)				77.02			
	EAAI (%)				103.51			

^aMC = cheese made with M; TC = cheese made with T; LC = cheese made with *Lp*; M1C = cheese made with M1; M2C = cheese made with M2.

3.3. Nutritional Evaluation

Human protein intake aims to obtain various suitable amino acids for the human body. However, the amino acid content and composition determine the nutritional quality of the proteins, and only the amino acid ratio in the ingested proteins is consistent with the proteins to be synthesized can those proteins be fully utilized. Therefore, it is important to evaluate the amino acid nutritional value in proteins. Table 3 and Table 4 show the results of the amino acid nutritional evaluation in 60-day-old goat cheeses. The TEAA in the five 60-day-old cheese samples was higher than the recommended amino acid content of the FAO/WHO model and the whole egg protein (RP) in Table 3 [12], indicating that goat cheese is a high-quality protein source. The TEAA of TC and M1C was 596.50 mg g⁻¹ protein and 612.01 mg g⁻¹ protein, respectively, whereas that of MC was the lowest (497.03 mg g⁻¹ protein). When the EAA contents in the cheese samples were converted through our calculation, all values in M2C, except for those of isoleucine and methionine, were closest to the ideal protein model recommended by the WHO/FAO [16]. Thus, the starter culture used in M2C might be more suitable for goat cheese production with high nutrition value.

RC represents the consistency of the content and ratio between amino acid components in food proteins and the model of amino acids. The closer RC is to 1, the closer the model is to the recommended model of amino acids. The RC of threonine was the lowest among TC (0.77), M1C (0.76), and M2C (0.78). Threonine was the first and isoleucine the second restricted amino acid in these three cheese samples (Table 4). In contrast, isoleucine was the first restricted amino acid in MC and LC with RC values of 0.75 and 0.74, respectively, whereas threonine was the second restricted amino acid. In addition, the score of RC (SRC) evaluates the protein quality by the dispersion of various amino acids from the amino acid pattern, which represents the consistency between the EAA composition ratio of the food proteins and the recommended pattern. Certain researchers argued that the closer SRC was to 100%, the more balanced the amino acid composition and the higher the quality and utilization of food protein was [34]. Therefore, the SRC of MC and LC, reaching beyond 80%, exhibited significant advantages over the cheese samples fermented by the other three starter cultures. The EAAI refers to the geometric mean of the EAA content ratio in the sample protein to those in the ideal model recommended by the FAO. Studies showed that when EAAI was ≥90%, the sample was a high-quality protein source [35]. The closer EAAI is to 100%, the closer the sample amino acid composition is to the ideal composition, and the higher the nutritional value is. In this study, the EAAI of MC (101.61%) and M2C (103.51%) were closer to 100% than that of the other three cheeses. However, EAAI ignores amino acid excess, which, based on Morales et al. [36], are not beneficial for absorption and utilization in humans. Therefore, M and M2 might be the ideal starter cultures for goat cheese production from the nutritional evaluation perspective.

4. Conclusion

The starter culture type exhibited a limited effect on the amino acid composition but a pronounced effect on protein degradation and nutritional evaluation in goat cheeses. The SDS-PAGE result showed that macromolecular proteins in M2C were degraded more rapidly into low-molecular-weight proteins and even peptides compared with the other four-cheese samples. The nutrition evaluation suggested that the MC nutritional value was the most balanced, whereas that of M2C was slightly lower. In conclusion, M2 was a suitable starter culture for goat cheese production with various molecular-weight proteins and high nutritional value. However, further research is necessary to investigate the roles of proteinase and peptidases with proteolytic effects produced by the starter cultures during goat cheese maturation.

Conflict of Interest

The authors declare that they have no conflict of interest.

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