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Comparative Studies on the Amino acid Content of Soft Cheese Produced from Cow and Goat Milk using Different Biocoagulants

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Abstract: ***A comparative study was carried out on the amino acid content of soft cheese produced from cow and goat milk; Extract of Calotropis procera and nonconventional coagulants such as Carica papaya extract, lemon juice and steep water from different grains (maize, sorghum, millet) were used. The raw milk was processed into soft cheese and amino acid profile of the cheese sample was carried out using standard methods. The revealed that, Leucine was the highest essential amino acid in cheese coagulated with Calotropis procera and methionine was the least amino acid in cheese coagulated with steep water from millet (9.98g/100g protein, 0.75g/100g protein). Leucine was also the highest essential amino acid in goat milk coagulated with lemon juice and tryptophan was the least amino acid in cheese coagulated with steep water from maize (11.60g/100g protein, 1.26g/100g protein). The highest non essential amino acid for cheese produced from both milk samples is glutamic acid (15.67g/100g protein, 14.00g/100g protein). The use of goat milk for the production of soft cheese appeared to be a very good source of both essential and non essential amino acids and coagulant such as lemon juice appears to be promising for the production of highly nutritious soft cheese.***

Keywords: Soft cheese, Cow milk, Goat milk, Amino acid

1. Introduction

In Nigeria, milk production is mainly done by the Fulani nomadic people, who are pastoralists involved in the rearing of cattle, moving from one location to another in search of green pasture. Due to lack of refrigeration facilities, the Fulani women process the surplus fresh milk into a soft, unripened soft cheese called "warankasi" or "wara" in short term. The soft cheese is widely consumed at home and sold in the market in South-western Nigeria. The cheese is usually stored in a mixture of whey at room temperature (28ºC). Under these storage conditions, soft cheese is highly perishable and has a shelf life of 2-3 days (Orhevba and Taiwo, 2016).

In preparing the soft cheese, *Calotropis procera* juice extract, a milk protein coagulant, is usually added to warmed milk. The milk is gently stirred and the temperature increased slowly until it reaches the boiling point. At this stage a visible separation of the curds from the whey is observed. The pieces of curds are then collected into small raffia baskets that define the shape of the product (Sulley *et.al*., 2013). Milk is an extremely nutritious food. It is an aqueous colloidal suspension of proteins, fats and carbohydrates that contains numerous vitamins and minerals (Oladipo and Jadesimi, 2012).

The principle of cheese processing is based on the coagulation of the protein in milk, during which about 90% of the milk fat is encapsulated. The coagulated mass is called curd; the remaining liquid is called whey. Curd consists mainly of milk proteins (casein) and milk fat; while whey mainly contains water, milk sugar (lactose), protein (serum proteins) and B vitamins (Pauline, 2006).

Cow's milk has been used for the production of soft cheese but recently the use of goat and sheep milk has proven very nutritious and of health benefit in the production of cheese. Goat has been referred as the "poor man's cow'' due to his great contribution to the health and nutrition of the landless and rural poor (Dresch, 1988). Goat milk differs from cow or human milk in having better digestibility, alkalinity and buffering capacity (Park, 1994). The aim of the present study was to assess and compare the amino acid profile of milk samples collected from cow and goat with the use of different biocoagulants.

2. Materials and Methodology

Collection of Samples: Fresh milk was collected aseptically in sterile bottles from cow and goat from a Fulani pastoralist in a small settlement in Ado Ekiti, Nigeria. The milk samples were thereafter transported to the Laboratory for further analysis. Prior to milk collection, the udder was washed with water and dried to avoid contamination. Following washing of the udder, the milk samples were collected directly in the bottle.

Production of soft cheese: The West African soft cheese was prepared by coagulating fresh milk using vegetable rennet extract of the Sodom apple (*Calotropis procera*) which is found abundantly in the tropics and sub-tropics other coagulants used include, *Carica papaya* leaf extract, lemon juice and steep water from maize millet and sorghum. The plant; *Calotropis procera* contains the enzyme, Calotropin which curdles the milk (Belewu and Aina, 2000). The extract of the leaves were obtained by crushing the leaves and stems of *C. procera* and *Carica papaya* plant which was later rinsed in a calabash with the milk. Lemon juice extract was obtained

by washing the lemon fruit, cut into two and squeezed into a container and used as coagulants, the grains (maize, millet and sorghum) were washed and soaked in water for three days and milled into slurry then allowed to ferment for another three days, the steep water were also used as coagulants. The mixture of milk and the coagulants were strained into warm milk with constant stirring and heating. Coagulation starts within 15-20 min after the addition of the coagulants. The curd was boiled for 20 minutes to inactive the plant enzyme and facilitates whey expulsion. The curd was then strained through a sieve (a small conical raffia basket which facilitates whey drainage and gives a characteristic shape and size to the cheese).

Amino acid analysis
Four stages were involved in the determination of the amino acid content of cheese sample as follows:

Nitrogen determination
A small amount (200mg) of ground sample was weighed, wrapped in whatman filter paper (No.1) and put in the Kjeldhal digestion flask. Concentrated sulphuric acid (10mL) was added. Catalyst mixture (0.5g) containing sodium sulphate (Na_2SO_4), copper sulphate ($CuSO_4$) and selenium oxide (SeO_2) in the ratio of 10:5:1 was added into the flask to facilitate digestion. Four pieces of anti-bumping granules were added.

The flask was then put in Kjeldhal digestion apparatus for 3 hours until the liquid turned light green. The digested sample was cooled and diluted with distilled water to 100mL in standard volumetric flask. Aliquot (10mL) of the diluted solution with 10mL of 45% sodium hydroxide was put into the Markham distillation apparatus and distilled into 10mL of 2% boric acid containing 4 drops of bromocresol green/methyl red indicator until about 70mL of distillate was collected.

The distillate was then titrated with standardize 0.01 N hydrochloric acid to grey coloured end point.

$$\text{Percentage Nitrogen} = \frac{(a-b) \times 0.01 \times 14 \times V \times 100}{W \times C}$$

Where:
a. = Titre value of the digested sample
b. = Titre value of blank sample
v. = Volume after dilution (100ml)
W. = Weight of dried sample (mg)
C. = Aliquot of the sample used (10ml)

Defatting sample
The sample was defatted using chloroform/methanol mixture of ratio 2:1. About 4g of the sample was put in extraction thimble and extracted for 15 hours in soxhlet extraction apparatus (AOAC, 2006).

Hydrolysis of the sample
A known weight of the defatted sample was weighed into glass ampoule. 7mL of 6NHCL was added and oxygen was expelled by passing nitrogen into the ampoule (this is to avoid possible oxidation of some amino acids during hydrolysis e.g methionine and cystine). The glass ampoule was then sealed with Bunsen burner flame and put in an oven preset at $105^0C \pm 5^0C$ for 22 hours. The ampoule was allowed to cool before broken open at the tip and the content was filtered to remove the humins. The filtrate was then evaporated to dryness using rotary evaporator. The residue was dissolved with 5mL to acetate buffer (pH 2.0) and stored in plastic specimen bottles, which were kept in the freezer.

Loading of the hydrolysate into analyzer
The amount loaded was 60 microlitre. This was dispensed into the cartridge of the analyzer. The analyzer is designed to separate and analyze free acidic, neutral and basic amino acids of the hydrolysate.

Statistical Analysis
The statistical analyses were carried out using SPSS program (Statistical Package for social Sciences version 16). The significant difference between means were calculated by one-way Analysis Variance (ANOVA) using Duncan multiple range test (DMRT) ($p \leq 0.05$).

3. Results and Discussion

The results obtained are presented in the table below:

Table 1 shows the result of essential Amino acids of soft cheese produced from cow milk usimg different coagulants. 9 essential amino acids were analysed. The highest essential amino acid leucine was found in cow milk coagulated with *Calotropis procera*

Table 1: Essential Amino acids composition (g/100g)* of soft cheese produced from cow milk using different biocoagulants

Amino Acids	CCPR	CCP	CLJ	CSO	CMI	CMA
Leucine	9.98±0.01^a	9.81±0.01^c	9.86±0.01^b	8.89±0.01^e	8.11±0.01^f	9.51±0.01^d
Lysine	3.10±0.01^a	3.02±0.01^c	3.08±0.01^b	2.97±0.01^d	2.92±0.01^e	3.02±0.01^c
Isoleucine	7.92±0.01^a	7.59±0.01^b	7.79±0.01^a	6.81±0.01^d	6.22±0.24^f	7.00±0.06^c
Phenylalanine	7.18±0.01^a	6.92±0.01^c	7.01±0.01^b	6.21±0.01^e	5.85±0.01^e	6.56±0.01^d
Tryptophan	0.99±0.01^a	0.94±0.01^b	0.89±0.01^c	0.79±0.01^e	0.84±0.01^d	0.89±0.01^c
Valine	9.36±0.01^a	8.89±0.01^c	9.24±0.01^b	8.01±0.01^e	7.66±0.01^f	8.53±0.01^d
Methionine	0.96±0.01^a	0.85±0.01^c	0.91±0.01^b	0.80±0.01^e	0.75±0.01^f	0.80±0.01^d
Histidine	3.90±0.01^a	3.58±0.01^c	3.77±0.01^b	3.00±0.01^e	2.94±0.01^f	3.32±0.01^d
Threonine	8.10±0.01^a	7.66±0.01 ^{c}	7.83±0.01^b	6.66±0.01^e	6.00±0.01^f	6.99±0.01^d

*Values are Means of replicate (n=3); Means with different letters within a row are significantly different by Duncan's New Multiple Range Test. (P<0.05).

Key: **CSO –** cow milk coagulated with steep water from sorghum**, CMA -** cow milk coagulated with steep water from maize, **CMI -** cow milk coagulated with steep water from millet,**CLJ -** cow milk coagulated with steep water from lemon juice,**CCPR -** cow milk coagulated with *Calotropis procera*,**CCP -** cow milk coagulated with *Carica papaya*

Table 2 shows the result of non essential amino acids of soft cheese produced from cow milk and 8 amino acids were analysed. The highest non essential amino acid glutamic acid was observed in cow milk coagulated with *Calotropis procera* while the least value was observed in glycine from cow milk coagulated with steep water from maize

Table 2: Non essential Amino Acids composition (g/100g)* of soft cheese produced from cow milk using different biocoagulants

AMINO ACIDS	CCPR	CCP	CLJ	CSO	CMI	CMA
Alanine	6.52±0.01^{a}	5.99±0.01^{c}	6.22±0.01^{b}	5.01±0.01^{e}	4.78±0.01^{f}	5.54±0.01^{d}
Glutamic Acid	15.67±0.01^{a}	14.61±0.01^{c}	15.14±0.01^{b}	14.00±0.00^{e}	13.48±0.01^{f}	14.08±0.01^{d}
Glycine	1.99±0.01^{b}	1.99±0.00 ^{b}	1.99±0.01^{b}	2.04±0.01^{a}	1.99±0.01^{b}	1.96±0.01^{c}
Proline	5.58±0.01^{a}	5.18±0.01^{c}	5.38±0.01^{b}	4.67±0.01^{e}	4.47±0.01^{f}	4.87±0.01^{d}
Arginine	2.67±0.01^{a}	2.58±0.01^{b}	2.58±0.01^{b}	2.32±0.01^{c}	2.24±0.01^{d}	2.32±0.01^{c}
Tyrosine	4.30±0.01^{a}	3.96±0.01^{c}	4.21±0.01^{b}	3.70±0.01^{d}	3.61±0.01^{e}	3.96±0.01^{c}
Serine	4.92±0.01^{a}	4.48±0.01^{b}	4.92±0.01^{a}	3.89±0.01^{d}	3.67±0.01^{e}	4.00±0.01^{c}
Aspartic Acid	8.00±0.01^{a}	7.94±0.01^{b}	8.00±0.00^{a}	7.57±0.01^{d}	7.26±0.01^{e}	7.87±0.01^{c}

*Values are Means of replicate (n=3); Means with different letters within a row are significantly different by Duncan's New Multiple Range Test. (P<0.05).

Table 3 shows the result of essential amino acids of soft cheese produced from goat milk with different coagulants. 9 essential amino acids were analysed. The highest essential amino acid leucine was found in goat milk coagulated with lemon juice while the least was observed in goat milk coagulated with steep water from maize.

Table 3: Essential amino acid composition (g/100g)* of soft cheese produced from goat milk using different biocoagulants

Amino Acids	GCPR	GCP	GLJ	GSO	GMI	GMA
Leucine	9.98±0.01^{c}	10.97±0.01^{ab}	11.60±0.59^{a}	11.32±0.01^{a}	10.62±0.01^{b}	9.63±0.01^{c}
Lysine	4.14±0.01^{e}	4.56±0.01^{b}	4.67±0.01^{a}	4.51±0.01^{c}	4.35±0.01^{d}	3.98±0.01^{f}
Isoleucine	2.29±0.01^{d}	2.62±0.01^{c}	2.81±0.01^{b}	2.82±0.01^{b}	2.92±0.01^{a}	2.29±0.01^{d}
Phenylalanine	4.97±0.01^{e}	5.85±0.01^{c}	6.21±0.01^{a}	6.12±0.01^{b}	5.50±0.01^{d}	4.52±0.01^{f}
Tryptophan	1.31±0.01^{d}	1.31±0.01^{d}	1.39±0.01^{b}	1.42±0.01^{a}	1.36±0.01^{c}	1.26±0.01^{e}
Valine	7.31±0.01^{e}	8.01±0.01^{c}	8.36±0.01^{a}	8.19±0.01^{b}	7.60±0.01^{d}	6.49±0.01^{f}
Methionine	2.56±0.01^{e}	2.94±0.01^{c}	3.53±0.01^{a}	3.15±0.01^{b}	2.78±0.01^{d}	2.40±0.01^{f}
Histidine	2.87±0.01^{e}	3.00±0.01^{c}	3.13±0.01^{a}	3.07±0.01^{b}	2.94±0.01^{d}	2.75±0.01^{f}
Threonine	5.94±0.01^{e}	6.71±0.01^{c}	6.88±0.01^{a}	6.83±0.01^{b}	6.27±0.01^{d}	5.49±0.01^{f}

*Values are Means of replicate (n=3); Means with different letters within a row are significantly different by Duncan's New Multiple Range Test. (P<0.05). Key: GSO – goat milk coagulated with steep water from sorghum, GMA - goat milk coagulated with steep water from maize, GMI - goat milk coagulated with steep water from millet,GLJ - goat milk coagulated with steep water from lemon juice,GCPR - goat milk coagulated with Calotropis procera,GCP - goat milk coagulated with Carica papaya

Table 4 shows the result of non essential amino acids of soft cheese produced from goat milk with different coagulants. 8 non essential amino acids were analysed. The highest non essential amino acid glutamic acid was found in goat milk coagulated with lemon juice while glycine had the least value from goat milk coagulated with steep water from maize

Table 4: Non essential amino acid composition (g/100g)* of soft cheese produced from goat milk using different biocoagulants

AMINO ACIDS	GCPR	GCP	GLJ	GSO	GMI	GMA
Alanine	5.01±0.01^{e}	5.84±0.01^{c}	6.22±0.01^{a}	6.07±0.01^{b}	5.46±0.01^{d}	4.47±0.01^{f}
Glutamic Acid	12.87±0.01^{d}	13.32±0.01^{f}	14.00±0.00^{a}	13.63±0.01^{b}	13.02±0.01^{c}	12.49±0.01^{e}
Glycine	2.23±0.01^{c}	2.33±0.01^{a}	2.33±0.00^{a}	2.28±0.01^{b}	2.28±0.01^{b}	2.14±0.01^{d}
Proline	3.96±0.01^{e}	4.57±0.01^{c}	4.87±0.01^{a}	4.67±0.01^{b}	4.26±0.01^{d}	3.86±0.01^{f}
Arginine	2.75±0.01^{e}	3.01±0.01^{c}	3.35±0.01^{a}	3.27±0.01^{b}	2.92±0.01^{d}	2.75±0.01^{e}
Tyrosine	3.01±0.01^{d}	3.18±0.01^{c}	3.78±0.01^{a}	3.44±0.01^{b}	3.01±0.01^{d}	3.01±0.01^{d}
Serine	3.89±0.01^{e}	4.91±0.01^{c}	5.08±0.01^{a}	4.97±0.01^{b}	4.75±0.01^{d}	3.51±0.01^{f}
Aspartic Acid	7.19±0.01^{d}	7.56±0.01^{b}	7.94±0.01^{a}	7.57±0.01^{b}	7.57±0.01^{b}	7.32±0.01^{c}

*Values are Means of replicate (n=3); Means with different letters within a row are significantly different by Duncan's New Multiple Range Test. (P<0.05).

4. Discussion

African soft cheese is produced from animal milk such as cow, sheep, goat, and camel. It can be coagulated with different biocoagulants among which are the commonly used *Calotropis procera*, *Carica papaya* e.t.c.

In this study, the local cheese was gotten from cow and goat milk and it was coagulated with six different coagulants which are *Calotropis procera* leaf extract, *Carica papaya* extract, lemon juice extract and steep water from grains (maize, millet and sorghum). Amino acid profile of the local cheese was carried out and it was observed that the local cheese protein was rich in both essential and nonessential amino acids.

From the tables, the essential amino acid analysed were leucine, lysine isoleucine, phenylalanine, methionine histidine, threonine, valine and tryptophan. While the non essential amino acids analysed were alanine, glutamic acid, glycine, proline, arginine, tyrosine, serine and aspartic acid. The addition of the coagulants increased the concentration of all the amino acids. The amino acid profile of soft cheese investigated in this study suggests that soft cheese produced is an excellent source of essential amino acids notably leucine, valine and phenylalanine. This is similar to the work of Nweke *et al.* (2011) who reported high essential amino acids leucine, valine and phenylalanine in sesame seed. Satish and Shrivastava (2011) also reported higher levels of these essential amino acids. In table 1, Leucine which is an essential amino acid is found in the local cheese and the value ranged from 8.11-9.98g/100g protein and the highest value was found in soft cheese coagulated with *Calotropis procera* while the least essential amino acid methionine is found in soft cheese coagulated with steep water from millet with values ranging from 0.75-0.96g/100g protein. The least methionine observed is smilar to the findings of El- Agamy (2006) in his work on camel milk; he also recorded lowest methionine content in camel milk samples. The highest leucine content recorded is similar to the findings of Adeela *et al*. (2013) who also recorded highest leucine content in cheese whey. The amount of methionine present is adequate and adequate methionine prevents disorder of hair, skin, and nail; reduces liver fat and protect the kidney (Satish and Shrivastava 2011). The result of non essential amino acid presented in Table 2 showed that glutamic acid is the highest non essential amino acid and is found in cow milk coagulated with *Calotropis procera* while glycine had the least value in cow milk coagulated with steep water from maize.

In Table 3 and 4, the results of essential and non essential amino acid of goat milk coagulated with different coagulants were presented. Leucine is the major amino analysed and is found in goat milk coagulated with lemon juice with value of 11.60g/100g protein followed by valine and threonine. This work agrees with the work of Adetunji *et al*. (2008) who worked on the use of lemon juice for production of soft cheese and suggest lemon juice as a better alternative to the commonly used *Calotropis procera.* The highest non essential amino acid glutamic acid is observed in goat milk coagulated with lemon juice with the value of 14.00g/100g protein. Highest glutamic acid observed is similar to the findings of Sabahelkheir *et al*. (2012) who worked on amino acid composition of Human and Animal's milk (camel, cow, sheep and goat) and Siddig (2002) in his work on Milk products and management project in Sudan. The least non essential amino acid glycine is observed in goat milk coagulated with steep water from maize (2.14g/100g protein). Glutamic acid appears to be the highest amino acid in this study and this might be due to the fact that the glutamic content of goat milk is generally high (Siddig, 2002).

5. Conclusion

This study reveals that soft cheese coagulated with different biocoagulants is a good source of both essential and non essential amino acids. Goat milk can also be used for the production of local cheese since it is a very rich source of amino acid content. Soft cheese coagulated with lemon juice extract appears to be the best among all the coagulants used and this suggest that lemon juice appears to be a promising coagulant and a better alternative for the production of local cheese.

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