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Research Paper

Influence of Different Coagulants on the Amino acid Content of Soft cheese (wara) Produced from Sheep milk

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Abstract: *Soft cheese, a coagulated product of raw milk is usually produced from cow milk using Calotropis procera, though it can be produced from other animals' milk using other coagulants. Influence of different coagulants (Calotropis procera, Carica papaya, lemon juice and steep water from cereals (maize, millet and sorghum)) was carried out on the amino acid content of soft cheese produced from sheep milk. Raw milk sample was collected from sheep and processed into soft cheese by these coagulants and the amino acid composition was carried out using standard methods. The result revealed that Calotropis procera coagulated soft cheese has the highest essential amino acid content Leucine (10.21g/100g), while steep water from millet coagulated soft cheese has the lowest essential amino acid content methionine (0.72g/100g). Lemon juice coagulated soft cheese has the highest non essential amino acid glutamic acid (16.27g/100g) and lowest glycine content was found in soft cheese coagulated with steep water from millet (1.38g/100g). In conclusion, this study revealed that highly nutritious soft cheese can also be gotten from sheep milk other than the commonly used cow milk and coagulants such as lemon juice can compete favorably well with Calotropis procera in production of highly nutritious soft cheese.*

Keywords: *Amino acids, biocoagulants, sheep milk, soft cheese*

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I. INTRODUCTION

Milk is considered as a source of food for infants due to its rich composition in order to meet their nutritional requirements. It is highly rich in both essential and non-essential amino acids which are needed for growth of both young and old. Due to the nutritional composition of milk, it can serve as means of developing a rural environment in order to have a healthy society. 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 (Ogunlade, 2019).

Soft cheese (wara) is an unripened cheese consumed in several parts of West Africa due to its various nutritional qualities. It is an excellent source of protein, fats and minerals such as calcium, iron and phosphorus, vitamins and essential amino acids, thus making it an important food in the diet of both old and young (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 (Yayota *et al.*, 2013). The coagulated mass is called curd while 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 and Karin, 2006).

Cow's milk is the major milk used for the production of cheese although milk can also be gotten from other animals such as bufallo, goat, sheep, camel e.t.c. It is usually coagulated with *Calotropis procera*, other coagulants suchs as *Carica papaya*, lemon juice and steep water from cereals (maize, millet and sorghum) can also be used. Cow's milk has long been considered a highly nutritious and valuable human food and is consumed by millions daily in variety of products (Heeschen, 1994). There are several animals from which milk can be gotten from although the milk differs in quality and quantity due to many factors. The quantity of the milk is usually determined by the size of the animal whereas the quality may be determined by either intrinsic or

extrinsic factors. Sheep milk is an excellent raw material for the milk processing industry especially in cheese production (Park *et al*., 2007). Sheep milk has higher specific gravity, viscosity, refractive index, titratable acidity, and lower freezing point than average cow milk (Haenlein and Wendorff, 2006).
Protein content of sheep's milk is higher than in women, cow, camel and goat's milk (Siddig, 2002).
Therefore the thrust of this study is to determine the influence of different coagulants on the amino acid content of soft cheese produced from sheep milk.

II. MATERIALS AND METHODS

Sample collection:

The raw milk sample was collected from sheep from Fulani pastoralists at a local farm settlement, Ado Ekiti. It was collected aseptically and transported to the laboratory for analysis.

Plant collection:

The leaves (*Carica papaya and Calotropis procera*) were collected from The Federal Polytechnic, Ado-Ekiti premises and the Authentications of the Plants were done at the Department of Plant Science and Biotechnology, Ekiti State University, Ado-Ekiti, Nigeria. The voucher specimens of UHAE 2018/022 for *Carica papaya* and UHAE 2018/023 for *Calotropis procera* have been deposited at the University herbarium.

Collection of coagulants:

Lemon fruits used were purchased from the market and steep water (effluent from pap produced from maize, sorghum, millet) were produced by steeping the grains in water for 3days after which it was milled and later steeped again for 2days. The steep water was then collected for use as coagulants.

Production of Wara

The milk was stirred gently during the heating process with a wooden spoon. About 4ml of the leaf extract of *Calotropis procera*, *Carica papaya*, Lemon juice and steep water were added to the warm milk and the mixture was heated the second time with intermittent stirring to about 45-50°C and was kept at this temperature until coagulation was achieved and the heating was stopped after the separation of curd and whey. The sign of coagulation was observed within the range of 10-15 min. It was transferred into a small raffia basket to facilitate whey drainage and characteristic shape, when the cheese was firm enough it was removed from the raffia basket and placed inside a covered plastic container for analysis.

Amino acid analysis

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 (Na2SO4), copper sulphate (CuSO4) and selenium oxide (SeO2) 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.
Percentage Nitrogen=
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, 20012).

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 oneway Analysis Variance (ANOVA) using Duncan multiple range test (DMRT) ($p\leq 0.05$).

III. 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 sheep milk using different coagulants. 9 essential amino acids were analysed. The highest leucine content was found in soft cheese coagulated with *Calotropis procera* (10.21g/100g), while t he lowest methionine content was found in soft cheese coagulated with steep water from millet (0.72g/100g).

Table 1: Essential amino acid composition (g/100g)* of soft cheese produced from goat milk using different biocoagulant

Amino acids	SCPR	SCP	SLJ	SSO	SMI	SMA
Leucine	10.21 ± 0.01^a	9.98 ± 0.01^b	9.69 ± 0.01^c	9.51 ± 0.01^d	8.34 ± 0.01^f	8.58 ± 0.01^e
Lysine	4.88 ± 0.01^a	4.51 ± 0.01^b	4.35 ± 0.01^c	4.03 ± 0.01^d	3.60 ± 0.01^f	3.98 ± 0.01^e
Isoleucine	6.74 ± 0.01^a	6.68 ± 0.01^b	6.35 ± 0.01^c	5.83 ± 0.01^e	5.37 ± 0.01^f	6.02 ± 0.01^d
Phenylalanine	7.27 ± 0.01^a	7.09 ± 0.01^b	7.09 ± 0.00^b	6.56 ± 0.01^c	6.03 ± 0.01^d	6.03 ± 0.01^d
Tryptophan	1.52 ± 0.01^a	1.47 ± 0.01^b	1.31 ± 0.01^d	1.36 ± 0.01^c	1.20 ± 0.00^f	1.26 ± 0.01^e
Valine	7.19 ± 0.01^a	7.01 ± 0.01^b	6.84 ± 0.01^c	6.66 ± 0.01^d	6.02 ± 0.01^e	5.90 ± 0.01^f
Methionine	0.96 ± 0.01^a	0.96 ± 0.01^a	0.91 ± 0.01^b	0.85 ± 0.01^c	0.72 ± 0.01^e	0.80 ± 0.00^d
Histidine	4.15 ± 0.01^a	4.02 ± 0.01^b	3.90 ± 0.01^c	3.77 ± 0.01^d	3.13 ± 0.01^f	3.58 ± 0.01^e
Threonine	5.99 ± 0.01^a	5.77 ± 0.01^b	5.60 ± 0.01^d	5.69 ± 0.01^c	5.16 ± 0.01^f	5.42 ± 0.01^e

***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 2 shows the result of non-essential amino acids of soft cheese produced from sheep milk using different coagulants. 8 non-essential amino acids were analyzed. The result revealed that the highest value of glutamic acid content was found in soft cheese coagulated with lemon juice (16.27g/100g), while the lowest value of glycine content was found in soft cheese coagulated with steep water from millet (1.38g/100g).

Table 2: Non-Essential amino acid composition (g/100g)* of soft cheese produced from sheep milk using different biocoagulants

Amino acids	SCPR	SCP	SLJ	SSO	SMI	SMA
Alanine	8.19 ± 0.01^a	8.04 ± 0.01^b	7.81 ± 0.01^c	7.36 ± 0.01^d	6.37 ± 0.01^e	5.46 ± 0.01^f
Glutamic Acid	16.20 ± 0.01^b	16.05 ± 0.01^c	16.27 ± 0.01^a	15.29 ± 0.01^d	14.30 ± 0.01^f	14.99 ± 0.01^e
Glycine	1.95 ± 0.01^a	1.90 ± 0.01^b	1.76 ± 0.01^d	1.85 ± 0.01^c	1.38 ± 0.01^f	1.61 ± 0.01^e
Proline	5.28 ± 0.01^a	5.18 ± 0.01^b	4.87 ± 0.01^d	4.97 ± 0.01^c	4.47 ± 0.01^f	4.67 ± 0.01^e
Arginine	2.75 ± 0.01^a	2.75 ± 0.01^a	2.67 ± 0.01^b	2.58 ± 0.00^c	2.49 ± 0.01^d	2.49 ± 0.01^d
Tyrosine	5.50 ± 0.01^a	5.50 ± 0.00^a	4.99 ± 0.01^b	4.64 ± 0.01^c	4.04 ± 0.01^e	4.47 ± 0.01^d
Serine	4.97 ± 0.01^a	4.91 ± 0.01^b	4.59 ± 0.01^c	4.32 ± 0.02^d	3.73 ± 0.01^f	4.00 ± 0.01^e
Aspartic acid	9.24 ± 0.01^a	9.11 ± 0.01^b	8.80 ± 0.01^c	8.06 ± 0.01^d	7.81 ± 0.01^e	7.81 ± 0.01^e

***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).**

IV. DISCUSSION

The results of essential and non-essential amino acids of soft cheese (wara) produced from sheep milk using different coagulants shows that sheep milk coagulated with *Calotropis procera* has the highest total amino acids, total essential amino acids and total non essential amino acids content, It has been reported that if one or more essential amino acid is in inadequate supply, the rate at which protein synthesis occurs will be reduced by the same ratio (Crisan and Sands, 1978). The present investigation revealed that leucine was the major amino acid in soft cheese produced while phenylalanine was second among all essential amino acids as shown in Table 1. In current study, high concentration of essential amino acid leucine was found in soft cheese coagulated with *Calotropis procera* (10.21g/100g). A substantial quantity of phenylalanine, valine, isoleucine and threonine was also observed in other soft cheese samples. Leucine plays a distinct role in protein metabolism and the translation initiation pathway of muscle protein synthesis. It is also involved in reversible phosphorylation of proteins that control mRNA binding to the 40S ribosomal subunit (Anthony *et al*., 2001). It helps in regulating the blood sugar level, promotes the growth and the recovery of muscle and bone tissues and also helps in production of growth hormones.

The high leucine content observed in this study is similar to the work done by Sabahelkheir and Hassan (2012) who also recorded high leucine content in sheep milk when compared with other animal's milk. A similar work done by Rafiq *et al*., (2016) also revealed high content of leucine in sheep milk and substantial amount of valine, isoleucine, threonine and phenylalanine. Previous investigation on the amino profile of sheep milk (Claeys *et al.*, 2014) depicted that leucine and glutamic acid were the major amino acid in sheep milk, while methionine and glycine were present in traces. The possible reason for the high content of leucine observed in the soft cheese sample might be due to the fact that leucine content is abundantly present in sheep milk.

Soft cheese coagulated with steep water from millet has the lowest methionine content (0.72g/100g). The lowest value obtained as methionine is in agreement with the findings of El- Agamy, (2006) who reported that human, sheep, goat, camel and cow milk are very rich in all essential amino acids except methionine and this is also similar to the report of Sabahelkheir and Hassan (2012) in their work on Amino Acid Composition of Human and Animal's Milk (Camel, Cow, Sheep and Goat). Similarly, Stancheva *et al*. (2011) reported the highest percentage for leucine (10.09%) among the essential amino acids and the lowest content was determined for methionine in sheep milk. Sulfur containing amino acids (methionine, cysteine) boost up the immune functions through intracellular conversion to glutathione, thereby serves as antioxidants (Hall *et al*., 2003). These proteins are the subject of great attention for specific dietary manipulations that aim to enhance host defenses.

Among the non essential amino acids of soft cheese produced from sheep milk, glutamic acid content was the highest in soft cheese coagulated with lemon juice (16.27g/100g). glutamic acid appears to be the highest non essential amino acid in all animal milk. Steep water from millet coagulated soft cheese also had the lowest non-essential amino acid content glycine (1.38g/100g). Importantly, the amino acid profile differences in dietary proteins also influence their utilization in the body. Moreover, proteins are an important factor affecting the quality of dairy products as the reduction in proteins and casein (α- and β-casein) contents results in poor cheese making properties (Bernabucci *et al*., 2002).

V. CONCLUSION

In conclusion, this study revealed that highly nutritious soft cheese can also be gotten from sheep milk other than the commonly used cow milk. Coagulants such as lemon juice can compete favorably well with *Calotropis procera* in production soft cheese with high nutritive value. Moreover, favorable balance of all the essential amino acids, especially, branched-chain amino acids (luecine, isoleucine, and valine) were found in all the cheese samples. The present investigation would be useful for the dairy processing industries to formulate nutritionally enhanced soft cheese from sheep milk based on functional products for vulnerable segment of population, even from the milk of non-bovine species.

It is therefore recommended that soft cheese produced from sheep milk coagulated by lemon juice should be incorporated into daily diet due to its highly nutritional content.

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