

Amino Acid Profile and Elemental Compositions of Some Commercially Produced Fermented Milk (Yoghurt) in Maiduguri Metropolis of Nigeria

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Abstract: Six fermented milk samples commonly called yoghurt produced in Maiduguri Metropolis and commonly consumed in University of Maiduguri, Borno State Nigeria were purchased from randomly selected commercial shops in the university and analyzed for essential and non-essential amino acid profile as well as cationic compositions. All the samples contained eight essential amino acids with the exception of tryptophan. Macroelements like calcium, sodium and potassium were detected to be present in most of them while the microelements manganese, iron, copper, zinc and magnesium were detected in most of the samples. Findings suggest that the yoghurts can serve as cheap sources of essential amino acids which can improve protein nutrition as well as play role in electrolyte balances in the body.

Keywords: Amino acids, elemental composition, fermented milk, Maiduguri

INTRODUCTION

Bovine based milk and milk products represent a good source of protein (Korhonen *et al.*, 1998; Clare and Swaisgood, 2000), lipids (Jensen and Newburg, 1995), amino acids (Nilson *et al.*, 2007), vitamins (Hayes *et al.*, 2001; Forssen *et al.*, 2000) and minerals (Brain, 2008). The nutritional benefits of milk have been well noted and extensively reviewed (Haug *et al.*, 2007). There is a decrease in the consumption of milk in some western societies (Brain, 2008). This can be partly explained by the assumed negative health effects that have been attributed to milk and milk products. There are evidences that milk fat contains a high fraction of saturated fatty acids assumed to contribute to heart diseases, weight gain and obesity (Mensink *et al.*, 2003). Milk is an important component of diets for all humans as it is high in essential amino acids and branched chain amino acids that are likely to be deficient in diets based on vegetable protein (Layman, 2003; Etzel, 2004). Although milk is a high-cost source of protein and fat relative to vegetable source, it is readily saleable particularly in the more affluent urban areas of developing countries. Improving milk products is, therefore, an important tool for improving the quality of life particularly for rural people in a developing country like Nigeria. Likewise, the consumption of other local natural products such as *Zonocerus variagatua*s, the giant grasshopper commonly known as

'fara' has been used as a novel source of proteins and other essential ingredients (Ladeji *et al.*, 2003). Resorting to local sources is part of the effort towards meeting the WHO standards for daily minimum protein intake stipulated and recommended since it is now difficult to afford animal proteins like beef among local inhabitants for survival in Maiduguri. In Maiduguri, North Eastern Nigeria, cattle rearing is a way of life and consumption of fresh and fermented milk (yogurt) products has become common in the recent past. The region where these samples were collected is still underdeveloped due to poverty and culture to a reasonable extent (Maduka *et al.*, 2011). This research was, therefore, designed to evaluate the nutritional quality of some commercially produced and consumed yogurts in Maiduguri typified by the samples sold and consumed in commercial shops in the University of Maiduguri in terms of their amino acid profile and elemental compositions.

MATERIALS AND METHODS

Sample collection: Six different samples of fermented milk (yogurt) that were commercially produced in Maiduguri, Borno State, Nigeria, were purchased from shops inside the University of Maiduguri. The samples were randomly labeled A-F according to the manufacturer and were subsequently stored in the refrigerator. The elemental analysis was conducted at

the Zoology Department of the University of Jos, Plateau State, Nigeria. All the analysis was conducted within three weeks from the day of collection.

Determination of amino acid profile: The amino acid profile of the six fermented milk samples was determined as described by Spackman and Moore (1958) using an amino acid analyzer (Technical TSM-1 model DWA 0209 Ireland). The samples were dried to constant weight, defatted, hydrolyzed and evaporated in a rotary evaporator and subsequently placed in a Technicon Sequential Multisampling amino acid analyzer (TSM).

Defatting of the samples: A known weight (100 g) of the dried sample was extracted with chloroform/methanol mixture as described by AOAC (1983). This lasted for 15 min.

Hydrolysis of the samples: Fifty mg of the defatted samples were weighted into a glass ampoule and 7 mL of ammonium chloride was added. Nitrogen gas was passed into the ampoule to remove hydrogen and prevent possible oxidation of some of the amino acids during hydrolysis. The glass ampoule was then sealed with Bunsen burner flame and was placed in an oven preset at 105°C for 22 h. The ampoule was allowed to cool and was broken at the tip after which the content was filtered. The filtrate was then evaporated to dryness at 40°C under vacuum in a rotary evaporator. The residues was dissolved with 5 mL of acetate buffer (pH 2.0) and stored in a polemic specimen bottles inside a deep freezer.

Loading of the hydrolysate into the TSM analyzer: The amount loaded was between 5-10 µL which was then dispensed into the cartridge of the analyzer. The TSM analyzer separates the sample into free acidic,

neutral and basic amino acid of the hydrolysis. The period of analysis lasted for 76 min. The analysis was done with each of the six samples.

Calculation of the amino acid value from the chromatogram: The amount of each amino acid present in each sample was calculated in g/100 g as described by Spackman and Moore (1958).

Mineral elements determination: The samples were investigated for mineral composition by using Atomic Absorption Spectrophotometer (AAS) Perkin Elmer Model A 400, software WINLAB 32 for AAS. Appropriate working standard solutions were prepared for each of the minerals. Elemental quantitation and determination of the minerals were performed using nitric acid and hyperchloric acid (6:1) as a digestion mixture. The method as essentially used by Maduka and Okoye (2002) and Maduka *et al.* (2004) was adopted in this determination. The data were statistically analyzed by using fitting of straight line by the least square method.

Statistical analysis: Data were subjected to Analysis of Variance (ANOVA). In order to test whether or not significant differences exist between groups, we analyzed the mean values with the paired t-test. The analysis was carried out on SPSS windows version 13.0. The acceptable level of significance was $p < 0.05$.

RESULTS

The amino acid profile of the six samples of the commercially prepared fermented milk are as detailed in Table 1. As can be seen from the table, all the yoghurt samples analyzed contained eight essential amino acids namely lysine, Isoleucine, leucine, histidine, Valine, threonine, methionine and

Table 1: Amino acid profile of the six samples of the commercially produced fermented milks

Amino acid	Sample concentration in g/100 g protein						
	A	B	C	D	E	F	Standard
Lysine	3.22±0.41	4.35±0.03	3.70±0.12	3.70±0.30	4.06±0.10	3.49±0.14	12.92±4.10
Histidine	2.60±0.04	2.35±0.30	3.13±0.30	2.47±0.04	1.89±0.22	2.04±0.02	15.08±2.01
Arginine	4.00±0.31	4.51±0.01	2.55±0.02	4.17±0.44	3.70±0.27	3.49±0.02	9.10±0.38
Aspartic acid	8.63±1.01	9.72±0.01	7.79±1.00	12.09±1.22	8.07±0.07	7.59±2.01	8.57±2.21
Threonine	2.64±0.01	3.05±0.39	2.61±1.66	4.49±1.23	3.10±0.03	2.55±0.12	7.63±2.81
Serine	3.02±1.01	3.53±0.01	3.16±1.00	3.97±1.22	2.67±0.07	3.05±0.05	9.91±0.01
Glutamic acid	0.09±0.34	10.43±0.21	11.32±0.35	15.04±0.09	9.35±0.17	9.69±0.01	11.95±1.69
Proline	2.55±0.38	2.12±0.04	2.76±0.05	3.01±0.18	3.15±0.01	2.19±0.02	14.80±0.21
Glycine	3.65±0.02	4.55±0.07	3.70±0.49	3.79±0.05	3.36±0.01	4.01±0.26	5.85±3.40
Alanine	4.13±0.31	4.79±0.01	3.86±0.02	4.48±0.44	3.66±0.27	3.44±0.02	9.29±6.12
Cysteine	1.19±0.13	1.73±0.12	1.06±0.18	1.06±1.21	0.92±1.23	0.93±0.33	9.10±1.89
Valine	4.01±0.30	4.79±0.02	3.78±0.31	5.78±0.28	4.10±0.19	3.63±0.02	9.32±0.08
Methionine	1.15±0.10	1.30±0.15	1.25±0.09	2.68±0.21	1.13±0.01	0.78±0.11	10.03±2.75
Isoleucine	2.56±0.30	3.06±0.02	2.78±0.31	6.31±0.28	2.81±0.19	2.98±0.02	8.68±1.12
Leucine	7.56±0.34	7.33±0.21	6.23±0.35	8.95±0.09	6.59±0.17	5.85±0.01	7.55±0.09
Tyrosine	2.76±0.02	3.54±0.09	3.22±0.24	4.35±0.28	2.86±0.02	3.06±0.04	15.50±3.81
Phenyl alanine	3.97±0.47	4.23±0.01	3.89±0.15	4.82±0.15	3.59±0.13	3.72±0.16	10.17±1.01

Results are mean±S.D.; Values are taken as triplicates of three readings

Table 2: Elemental composition of the six samples of the commercially produced fermented milks

Elemental composition	Sample concentration (mg/L)					
	A	B	C	D	E	F
Sodium	612.80±0.17	737.30±0.01	888.00±0.15	349.60±0.13 ^a	979.20±0.16 ^b	785.60±2.60
Potassium	129.80±0.02 ^a	98.60±0.24 ^a	454.00±0.28	886.00±0.04 ^b	794.80±0.05 ^b	131.90±0.03 ^a
Calcium	66.70±0.39	77.57±3.10	98.75±1.66	128.90±0.03	98.70±0.12	76.64±0.39
Magnesium	193.70±0.07	79.30±1.22	114.60±1.33	145.80±3.10	120.90±0.04	112.70±0.17
Zinc	0.10±0.03	ND	0.20±0.01	ND	0.10±0.11	0.30±1.04
Copper	0.01±0.15	0.22±1.33	ND	ND	0.01±0.11	ND
Iron	0.60±0.18	0.70±0.22	0.70±0.12	ND	0.70±0.41	0.70±0.05
Manganese	0.04±1.00	0.01±0.07	0.02±0.05	ND	0.02±0.14	0.02±0.15

ND: Not detected; Results are mean±S.D.; Values are taken as triplicates of three readings; Values with different superscript in a row are statistically significant ($p<0.05$)

phenylalanine while the non-essential amino acids detected were arginine, aspartic acid, serine, glutamic acid, proline, glycine, alanine, cysteine and tyrosine. The results show that the seventeen amino acids tested were present in all the samples in various amounts. For all the amino acids tested, sample D recorded the highest aspartic and glutamic acid values which were higher than the concentrations of the standard ($p>0.05$). The results suggest that yoghurt can serve as a good source of essential and non-essential amino acids required in metabolism.

The elemental concentrations (mg/L) of the six samples of yoghurts are shown in Table 2. As can be seen from the table, samples A and E contained all the cations analyzed namely Na, K, Ca, Mg, Zn, Cu, Fe and Mn. Copper was not detected in samples C, D and F. The results show that Zn, Cu, Fe and Mn were absent in sample D. Samples B and C also contained all the elements analyzed except Zn and Cu respectively. Sample E had the highest concentration of sodium (979.2±0.16 mg/L) which was significantly higher compared with sample D ($p<0.05$). For potassium, samples D and E recorded significantly higher values ($p<0.05$) with respect to samples A, B and F. Sample D showed the highest concentration of magnesium ions but was not significant relative to others ($p>0.05$). The results suggest that the yoghurt samples can serve in electrolyte balance reactions mediated by the elements detected.

DISCUSSION

Milk, the first nutrient of the new born, provides adequate amounts of all nutrients needed by infants (Yamamuchi, 1993; Cervato *et al.*, 1999). Bovine milk has the nutrients needed for growth and proper development of the calf and is a good source of proteins, amino acids, vitamins, minerals and lipids. It contains growth factors, enzymes, some bioactive peptides, cytokinines and nucleotides (Haug *et al.*, 2007). In addition to supply of amino acids, milk proteins are also important for their biological properties; in fact some peptides derived from milk digestion have possible regulatory effects on nutrient uptake, postprandial hormone secretion, immune defence and neuroendocrine information transfer (Schlimme and Melsel, 1993).

The results from this study showed that the amino acid contents of the fermented milk samples varied comparatively. It was also observed that the fermented milk samples contained the essential amino acids tested. From the experimental results, it can be seen that glutamic and aspartic acids were the most abundant amino acids followed by leucine. Glutamine and asparagine are the storage forms of nitrogen in addition to being the starting compounds from which the backbones of amino acids are made. During acid hydrolysis steps glutamic acid and asparagine are converted to aspartic acid respectively with the liberation of ammonium ions (Onwuliri and Anekwe, 1993b). This could explain the high concentrations of these two amino acids. An obligatory dietary requirement exists for tryptophan, leucine, isoleucine, valine, phenylalanine, methionine, lysine, threonine and histidine. The last three of this indispensable group of amino acids cannot be transaminated and so must be supplied in the diet as such (Young, 2001). There were moderate concentrations of isoleucine, valine, phenylalanine, lysine, threonine and histidine from the result. Milk is essentially rich in essential amino acids and branched chain amino acids. Evidence shows that these amino acids have unique roles in human metabolism; in addition to providing substrates for protein synthesis, suppressing protein catabolism and serving as substrates for gluconeogenesis, they also trigger muscle protein synthesis (Haug *et al.*, 2007; Layman, 2003; Etzel, 2004; Wolfe, 2002).

Eight elements were tested in the study and they showed various degree of variation in the test samples. Sodium, potassium, calcium and magnesium were present in all the samples while sample D lacked zinc, copper, iron and manganese. The calcium concentration in bovine milk is about 1 g/L (Haug *et al.*, 2007). The major source of concentrated calcium from food in many parts of the world is dairy products (Weaver, 2001). Calcium is the most abundant mineral in the body and essentially all body processes require calcium. Inadequate dietary calcium has long been associated with osteoporosis (Weaver, 2001). Getting adequate calcium in the diet gives healthy bones and teeth and it may also help prevent hypertension, decrease odds of getting colon or breast cancer, improve weight control and reduce the risk of developing kidney stones. Magnesium has been shown to be essential dietary

component for humans (Fleet and Cashman, 2001). Magnesium is ubiquitous in foods and milk while the recommended daily intake is 400 mg/day (Haug *et al.*, 2007). Our result shows that the test samples on the average have above 100 mg/L. Magnesium concentration except for sample B which had 79.30±1.22 mg/L. Magnesium has many functions participating in more than 300 enzyme reaction processes (Mildvan, 1987).

Zinc, copper and manganese are micro elements required in the body metabolism. They were detected in some of the samples except for samples D and then B and C which were deficient in Zn and Cu respectively. This may be suggesting that samples B, C and D may show reduced role of cytoprotection in oxidative stressed conditions. The catalytic role of zinc is required for the biological function of more than 300 enzymes covering all six classes of enzymes and from different species of all phyla (McCall *et al.*, 2000; Valle and Auld, 1990). It is also involved in DNA repair, cell growth and replication, gene expression, immune function, hormone activity, protein and lipid metabolism (Haug *et al.*, 2007). Milk is a good source, containing about 4 mg/L. Our data show that the amount of zinc in the samples were very much less than the required concentration. The bioactivity of zinc is better from milk than from vegetable food (Haug *et al.*, 2007) and inclusion of milk in the diet may improve total bioavailability of zinc (Hansen *et al.*, 1996). Although manganese was present in some of the samples it was detected in a very low concentration. Low blood and tissue manganese has been associated with osteoporosis, diabetes, epilepsy, atherosclerosis, impaired wound healing and cataracts (Nielsen, 2001). Manganese is considered essential nutrient for humans because it is known to function as an enzyme activator and to be a constituent of several metalloenzymes (Leach and Harris, 1997).

CONCLUSION

We observed from our results that there were variations in the mineral elements and amino acid constituents of the six fermented milk commonly called yoghurt samples studied. This could be attributed to factors such as stage of lactation, age, breed, nutrition, energy balance, health status of the udder and environmental factors. These fermented milk products could serve as alternative sources of essential amino acids as a way of improving protein nutrition especially with these arrays of essential amino acids detected in them. These results also tend to support earlier reports that fermentation releases bound essential ingredients of foods.

Competing interest: The authors declare that they have no competing interests.

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