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INFLUENCE OF FERMENTATION AND ROASTING ON THE AMINO ACID PROFILE OF INFANT FOOD CREATED FROM MIXTURES OF SORGHUM AND SOYBEAN

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SUMMARY

This study examined how fermentation and roasting alter the amino acid profile of infant food created from mixtures of sorghum and soybean. Sorghum and soybean were fermented, roasted and both fermented and roasted before milling into flours. Untreated sorghum and soybean flours were also produced to serve as the control. Except for the control samples, each blend had 5% malted sorghum flour added. Weaning foods were prepared with these blends, and their amino acid profiles were determined. The contents of essential amino acids, including histidine, isoleucine, leucine, lysine, methionine, phenylalanine, threonine, tryptophan, and valine, ranged from 2.03 to 2.48 g/100g, 3.39 to 5.22 g/100g, 8.16 to 11.21 g/100g, 4.18 to 6.38 g/100g, 1.45 to 2.41 g/100g, 4.22 to 6.34 g/100g, 3.41 to 5.86 g/100g, 2.98 to 4.30 g/100g, and 4.01 to 6.33 g/100g, respectively. The contents of non-essential amino acids, including alanine, arginine, aspartic acid, cystine, glutamic acid, glycine, proline, and serine, varied between 3.31-6.13 g/100g, 4.93-7.32 g/100g, 10.23-14.33 g/100g, 1.38-2.35 g/100g, 13.64-16.84 g/100g, 3.99-6.35 g/100g, 3.59-5.34 g/100g, and 2.94-4.88 g/100g, respectively. The fermentation and roasting processes were found to significantly enhance the amino acid content of weaning foods with inconsistent effects. Therefore, optimizing these conditions is recommended for weaning blends to maximize health and nutritional benefits for infants and young children.

Key words:
fermentation, roasting, amino acids profile, weaning food, sorghum, soybean

INTRODUCTION

Malnutrition remains a significant issue in many developing nations, affecting millions of individuals, particularly children, who experience inadequate consumption of both the quantity and quality of protein in their diets (Okorie et al., 2013). In children, especially in poorer nations, malnutrition stands as one of the leading causes of illness and death worldwide (Thapar & Sanderson, 2004). It is the leading cause of preventable illness in kids, accounting for roughly 300,000 annual fatalities (WHO, 2017). Over half of all infant and child deaths can be traced back to malnutrition in some way (Adejuwon et al., 2020). Recent research by Tariku et al. (2017) revealed that 21.9% of children under five experience stunted growth, 13.4% are underweight, and 7.3% suffer from wasting. Additionally, the World Health Organization estimates that 5.4 million children under five die each year, with 2.7 million deaths occurring in Africa, including Nigeria (WHO, 2017).

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Efforts to tackle childhood malnutrition often involve the development of supplementary diets alongside breastfeeding. However, in developing countries, poverty has posed a hindrance to this approach. As a result, research has concentrated on formulating complementary foods using locally available staple ingredients as an alternative solution. Abeshu et al. (2016) reported that some of the staple foods used to produce weaning foods in many developing countries lack a number of important nutrients required by the infants and young children to satisfy their nutritional requirements. Cereals typically have a low lysine content, but are abundant in sulfur-containing amino acids, such as methionine and cystine. Conversely, legumes tend to be rich in lysine, but lacking in sulfur-containing amino acids like methionine and cystine. Therefore, when cereals are supplemented with locally available legumes that are high in protein, it results in an augmentation of the protein content in cereal-legume blends (Simwaka et al., 2015).

Weaning is the process of gradually introducing solid foods to a baby who has been exclusively breastfed. Weaning foods are those introduced to a baby as they make the transition from nursing or bottle feeding to eating a more varied diet (Msheliza et al., 2018a). The sole purpose of the introduction of these foods is to supplement breast milk, eventually replace it, and help the child transition to a diet more typical of an adult. In many developing countries, traditional weaning foods like Nigeria's "Ogi" or "Akamu" (Abdulkadir & Danjuma, 2015), Kenya's "uji," and Ghana's "koko" (Onilude et al., 2004) are crafted from monocereal grains such as millet, sorghum, and maize, and typically exhibit a low nutrient density (Rombouts & Nouts, 1995). Protein energy malnutrition (PEM), which can lead to conditions like marasmus and kwashiorkor in infants, has been identified as a major issue during the weaning process (Ubesie & Ibeziakor, 2012). Weaning food that meets the infant's energy and nutritional needs can be created by combining the cereals readily available in the area with legumes, which are rich in protein (Msheliza et al., 2018a).

Proteins are the structural backbone of the human body, and amino acids are the building blocks that make up these proteins. Protein synthesis, hormone production, and neurotransmitter release all depend on amino acids. According to Lopez & Mohiuddin (2023), there are 20 essential amino acids that are beneficial for human development and proper physiological function, but only 9 of them are considered essential because the human body cannot synthesize them, due to a lack of corresponding metabolic pathways in the human body. Therefore, these amino acids must be obtained from an exogenous diet. These essential amino acids are histidine, isoleucine, leucine, lysine, methionine, phenylalanine, threonine, tryptophan, and valine. Animal proteins like meat, eggs, and poultry are excellent sources of essential amino acids. However, there are drawbacks to using animal proteins as a source of amino acids. They are often quite high in cholesterol and saturated fat, two factors that have been linked to an increased risk of cardiovascular disease and other persistent illnesses (Mozaffarian & Wu, 2011). Furthermore, there are ethical concerns that may arise from issues of animal welfare and factory farming associated with the production of animal proteins.

Plant-based proteins can provide all the necessary amino acids by combining different plant sources, making it unnecessary to rely solely on animal proteins as a complete source. The health benefits of plant-based diets are often greater than those of diets based on animal products, and vice versa. Therefore, the purpose of this research was to examine how blending sorghum and soybean affected the amino acid profile of weaning food. Proteins in sorghum and soybeans can be made more easily digestible through fermentation and roasting. These procedures simplify protein structures, making it easier for the body to absorb and utilize them (Adejuwon et al., 2020). The improved digestibility of these plant sources ensures that the body can effectively utilize the essential amino acids they contain. Anti-nutrients such as phytic acid and trypsin inhibitors, which hinder protein digestion and absorption, are reduced or deactivated through the processes of roasting and fermentation (Vagadia et al., 2017; Rodríguez-España et al., 2022). Malting is another crucial weaning stage that enhances the nutritional content and suitability of grains for infants and young children in a variety of ways. It facilitates the easy digestion of proteins and complex carbohydrates by breaking them down. Malted sorghum has many benefits when used to make weaning foods, including higher digestibility, better protein quality, higher energy density, better nutritional availability, and improved acceptance and taste. It is a healthy and appropriate ingredient for baby and early child feeding, showing promise as a substitute for conventional foods. The implementation of these methods to incorporate sustainable and locally accessible protein sources into traditional cuisine not only enhances food security but also preserves cultural traditions.

MATERIAL AND METHODS

Materials and their preparation

Sorghum and soybean kernels were sourced from Gombe Main Market, Nigeria, whereas analytical chemicals and reagents were obtained from Modibbo Adama University, Yola, and Federal College of Education. The sorghum and

soybean kernels were meticulously hand-cleaned individually to remove sand, foreign seeds, damaged and infected seeds, as well as any other dirt or impurities.

Fermentation of sorghum
The fermentation process was conducted as described by Msheliza et al. (2018a). A kilogram of sorghum was dehulled, winnowed, and washed to remove germs and hulls. The grains were soaked at 32°C for 72 hours, and then dried in a hot air oven at 60°C for 10 hours. The fermented sorghum flour was processed, sealed in a sterile plastic bag, and ready for further use.

Fermentation of soybean
The fermentation process was carried out as described by Msheliza et al. (2018a). A kilogram of soybeans was immersed in clean water for 2 hours to saturate and moisten the seed coat, aiding in dehulling. After dehulling, the soybeans were washed to remove the seed coat. They were then placed in a plastic container for 72 hours of natural fermentation. The water was removed, and the grains were dried in a hot air oven at 60°C for 10 hours. The fermented soybean flour was processed into flour and packed in a polyethylene bag for preservation.

Roasting of sorghum
The roasting process was conducted as described by Msheliza et al. (2018a). A commercial grain huller (at Gombe Main Market) was used to dehull one kilogram of whole sorghum grains. The grains were partially dried in the sun after being winnowed, washed, and drained, following the dehulling process. The sorghum grains were roasted in a traditional manner, using a thick aluminium pot and an open flame. A commercial grinder was used to process the grains into flour. To achieve a finer particle size, the resulting flour was sifted through a locally built sieve with a diameter of approximately 1 mm. This finely milled flour was then stored in a plastic container with a tight seal.

Roasting of soybean
Whole soybean grains were immersed in clean water for 2 hours to soften the seed coat, facilitating dehulling. After dehulling, the soybeans were washed, drained, and partially sun-dried. They were roasted in an aluminium pot and subsequently ground into finely textured flour using a commercial grinding machine. The flour was sieved and packaged in a plastic container for preservation. The resulting flour was then used for food production.

Fermenting and roasting sorghum flour and soybean flour
A 1-kg batch of sorghum grains was fermented and roasted, followed by milling into flour and storing in a plastic container until needed. Separately, a 1-kilogram batch of soybean grains was also fermented and roasted, milled into flour, and stored in a plastic container until ready for use, following the previously mentioned procedures.

Malting of sorghum
The malted sorghum was prepared following the procedure outlined by Badau et al. (2006). The sorghum grains were steeped at 32°C for 12 hours, with the steeping liquor changed every 6 hours. After the steeping, the grains were submerged in a 0.1% solution of commercial bleach, specifically 3.5% sodium hypochlorite. After sterilisation, the grains were wrapped in a damp cotton cloth and placed on a wet jute bag. The grains were allowed to germinate at 32°C for 72 hours. During germination, water was sprayed onto the grains, and agitated by a wooden rod. The grains were moistened twice daily for 48 hours, then sprayed and turned over in the afternoon. After germination, the grains were dried in an oven at 50°C for 24 hours to achieve a moisture content of 5.45 ± 0.48%. The dried grains were then polished to remove shoots and rootlets, which were separated from the kernels by rubbing the mixture between palms over a mesh sieve. The polished malt was processed into fine flour using a commercial milling machine and sieved. The resulting malted sorghum flour was packaged in an airtight plastic container and stored until use.

Formulation of weaning foods
The ratios for formulating the weaning foods were established according to the specifications provided in Table 1.

Table 1. Formulation of the Weaning Foods

Samples	sorghum (%)	malted sorghum (%)	soybean (%)
F1, R1 & FR1	55.00	5.00	40.00
F2, R2 & FR2	65.00	5.00	30.00
F3, R2 & FR3	75.00	5.00	20.00

U1	60.00	0.00	40.00
U2	70.00	0.00	30.00
U3	80.00	0.00	20.00

Legend: F1, F2 and F3 - Fermented samples; R1, R2 and R3 - Roasted samples; FR1, FR2 and FR3 - Fermented and roasted samples; U1, U2 and U3 - Untreated samples

Nitrogen determination

Nitrogen was determined by the micro Kjedhal method described by Amaechi et al. (2017). A 200 mg sample was measured, enveloped in Whatman filter paper, and placed in a Kjeldahl digestion flask. Concentrated sulfuric acid was introduced, and a catalyst mixture of sodium sulphate, copper sulphate, and selenium oxide was added. Four anti-bumping granules were included. The flask was placed in a Kjeldahl digestion apparatus for 3 hours until the solution turned light green. After cooling, the samples were diluted with distilled water to reach 100 ml. A portion of the diluted solution was combined with 45% sodium hydroxide in a Markham distillation apparatus, resulting in a solution containing 2% boric acid and 4 drops of bromocresol methyl red indicator. The distillation process continued until around 70 ml of distillate was collected.

The distillate was then titrated with standardized 0.01 N hydrochloric acid until a gray color appeared.

$$Percentage\ Nitrogen = \frac{(a-b) \times 0.01 \times 14 \times V}{W \times C} \times 100 \quad (1)$$

where:

a - titre value of the digested sample
b - titre value of blank sample
V - volume after dilution (100 ml)
W - weight of dried sample (mg)
C - aliquot of the sample used (10ml)
14 - nitrogen constant.

Amino Acids Analysis

The amino acid profile of the sample was assessed using the techniques outlined by Benitez (1989). The samples were dried, defatted, hydrolyzed, and evaporated using a rotary evaporator. The resulting material was then analysed using the Technicon Sequential Multi-Sample Amino Acid Analyzer (TSM-1 Technicon Instrument Basingstoke, Model 6890 N, USA).

Sample Defatting

The sample was defatted using a mixture of chloroform and methanol in a 2:1 ratio. Approximately 4 g of the sample were placed within an extraction thimble and extracted for 15 hours using a soxhlet extraction apparatus, in keeping with the procedure outlined in AOAC (2016).

Sample Hydrolysis

A defatted sample was placed in a glass ampoule, containing 7 ml of 6N hydrochloric acid and nitrogen to prevent oxidation of certain amino acids. The ampoule was sealed and placed in an oven at 105°C ± 5°C for 22 hours. The contents were filtered, and the 6N hydrochloric acid degraded tryptophan during hydrolysis. The filtrate was evaporated under vacuum at 40°C, and residue was dissolved with 5 ml of acetate buffer (a pH 2.0). The resulting solution was transferred into plastic specimen bottles and stored in a freezer.

Loading of the hydrolysate into a TSM analyzer

The TSM analyzer was used to separate and evaluate the amino acids within the hydrolysate, with each analysis session lasting 75 minutes, involving placing 5-10 µl of the sample into the cartridge.

Calculation of amino acid contents

The height of each peak on the TSM chart recorder, representing individual amino acids, was measured, and the half-height and width of the peak at half-height were meticulously documented. The area under each peak was calculated by multiplying its height by the width at half-height. The norleucine equivalent (*NE*) for each amino acid in the standard mixture was computed using the provided formula as described by Amaechi et al. (2017).

$$NE = \frac{Area\ of\ norcleucine\ peak}{Area\ of\ each\ amino\ acid} \quad (2)$$

A constant S_{std} was calculated for each amino acid in the standard mixture according to Equation 3:

$$S_{std} = NE_{std} \times Molecular\ weight \times \mu AA_{std} \quad (3)$$

Finally, the amount of each amino acid present in the sample was calculated in g/16, g/N or g/100g protein using the following formulas:

$$Concentration\ (\frac{\text{g}}{100\text{g}} protein) = NH \times W@NH/2 \times S_{std} \times C \quad (4)$$

$$C = \frac{Dilution \times 16}{Sample\ W_t(g) \times N\% \times 10 \times Vol.loaded \times NH \times W(nleu)} \quad (5)$$

where:
NH - Net height
W@NH/2 - Width at half height
nleu - Norleucine

Statistical Analysis
The experiments were conducted three times independently (in triplicate), and the results were presented as means along with their corresponding standard errors (SE). To ascertain significant variations in measurements, an analysis of variance (ANOVA) was performed. The analysis was conducted using the SPSS statistical software, with a 95% confidence level. To identify noteworthy distinctions among the means, the Duncan's Multiple Range Test was employed. Statistically significant differences were acknowledged when the p-value was less than 0.05, as stipulated by Msheliza et al. (2018b).

RESULTS AND DISCUSSION

Essential Amino Acid of the Weaning Foods Prepared from the Blends of Sorghum and Soybean
The results obtained for the essential amino acid composition of the weaning foods prepared from the blends of sorghum and soybean are shown in Table 2. The sample histidine concentrations ranged from 2.03 to 2.48 g/100 g, with the fermented/roasted sample FR75:20:5 showing the lowest value and the roasted sample R55:40:5 displaying the highest. These figures exceed the histidine requirement estimated by FAO, WHO, and UNU for infants aged 3 to 4 months, which is 0.2 g/100g per day (Omeire, 2012). The results showed that both the fermentation and roasting processes had a positive impact on the histidine content of the weaning products. However, these improvements were not statistically significant ($p > 0.05$). This suggests that the effects of fermentation and roasting on the histidine content of weaning food made from sorghum and soybean blends are notable but not highly pronounced. Fermentation has the capability to increase the accessibility of histidine by deconstructing protein structures and reducing anti-nutritional factors. Conversely, roasting has the potential to result in the breakdown of certain amino acids, including histidine, due to exposure to heat.
The concentration of isoleucine in the samples ranged from 3.39 to 5.22 g/100g, with the fermented/roasted sample FR75:20:5 showing the lowest concentration and the fermented sample F65:30:5 displaying the highest. The leucine concentrations in the samples ranged from 8.16 to 11.21 g/100g, with the fermented and roasted sample FR75:20:5 having the lowest value and the roasted sample R55:40:5 showing the highest. Leucine exhibited the highest values among all the essential amino acids, according to the findings. This demonstrates that there is a strong positive link between leucine and protein. Similar observations were reported by Awadalkareem et al. (2008) for weaning food produced from blends of sorghum and soybean protein concentrate. The results also showed that as the level of inclusions of soybean decreases in the blends, the values of isoleucine and leucine contents of the weaning food also

decrease. This indicates that the addition of soybean flour to sorghum flour significantly increases the levels of isoleucine and leucine in the weaning products.

The introduction of fermentation to the supplements led to a minor decline in the content of essential amino acids, reaching their highest values of 5.22 g/100g protein for isoleucine and 11.21 g/100g protein for leucine. Conversely, both roasting and the combined process of fermentation and roasting were observed to considerably decrease the content of essential amino acids. This reduction in essential amino acids can be attributed to the alteration of protein structure during the application of heat. A similar observation was documented by Fadlallah et al. (2010) in their study on weaning food produced from sorghum flour supplemented with chickpea flour. The predominant characteristic of sorghum protein, which naturally has a substantially greater ratio of leucine, may also contribute to the raised leucine concentration, surpassing other essential amino acids (Adeyeye, 2010). In order to prevent the development of pellagra, it is therefore advisable to moderate any potential amino acid imbalances caused by an abundance of leucine.

The range recorded for lysine was from 4.18 to 6.38 g/100g, with the fermented/roasted sample FR75:20:5 showing the lowest value and the fermented sample F65:30:5 displaying the highest. It was shown that the fermented samples were more valuable than the roasted, fermented/roasted, and untreated samples. This resulted from the fermentation process, which raised the relative nutritional value of both the protein quality and the concentration of lysine. Asma et al. (2006) reported a comparable finding. They claimed that combining lysine-deficient sorghum with high-lysine legumes enhanced the amino acid profile, particularly for lysine, but at the expense of sulfur-containing amino acids.

The methionine levels recorded were 1.45 to 2.41 g/100g, with the fermented/roasted sample FR75:20:5 showing the lowest value and the roasted sample R55:40:5 displaying the greatest. The findings show that methionine had the lowest values of all the necessary amino acids. This demonstrates that there is a markedly negative association between protein and methionine. Awadalkareem et al. (2008) observed similar findings for weaning diet made from mixtures of sorghum and soybean protein concentrate. The concentration of phenylalanine in the samples ranged from 4.22 to 6.34 g/100g, with the fermented and roasted sample FR75:20:5 showing the lowest value and the fermented sample F65:30:5 displaying the highest. This indicates that fermentation has beneficial impacts on both the amount of phenylalanine present and its absorbability. Research indicates that the microbial processes occurring during fermentation can break down proteins into smaller peptides and amino acids, potentially enhancing the accessibility of phenylalanine and other essential nutrients (Nkhata et al., 2018).

Nkhata et al. (2018) found that the effect of fermentation on the amino acid composition has led to different results. This is likely because the experiments were set up differently, the length of the studies was different, and the protein content of the foods being studied was different at the start. Although a number of studies have reported an increase in specific amino acids during fermentation (Chaven & Kadam, 1989; El-Hag et al., 2002; Doudu et al., 2003; Pranoto et al., 2013), others have argued the opposite (Osman, 2011; Pranoto et al., 2013). These effects seem to be predominantly relative rather than actual changes, stemming from the loss of dry matter due to microorganisms breaking down carbohydrates and fats for energy. Following fermentation, lysine, glycine, and arginine were reduced (Osman, 2011), whereas methionine showed an increase (Chaven & Kadam, 1989). The escalation in protein content could partly be linked to the loss of dry matter during fermentation. Bacterial fermentation has been shown to increase the amount of lysine in fermented grains (Nkhata et al., 2018). This could be because microorganisms break down complex proteins, releasing peptides and amino acids in the process (Pranoto et al., 2013). Nonetheless, it is noteworthy that fermenting microorganisms also utilise amino acids, which might lower the protein content and quality of some fermented foods (Osman, 2011; Pranoto et al., 2013). Furthermore, in specific instances, particular bacterial strains or yeast may metabolise phenylalanine, which could lead to a reduction in its presence in the final product.

The threonine values recorded ranged from 3.41 to 5.86 g/100g, with the untreated sample U80:20 showing the lowest value and the fermented and roasted sample FR65:30:5 displaying the greatest. The impact of fermentation on the threonine content, like that of other amino acids, can be diverse. Certain research suggests that the activity of microorganisms during fermentation could lead to the breakdown of proteins into smaller peptides and amino acids, potentially influencing the threonine content (Sharma et al., 2020). However, the degree of this alteration can vary. In specific instances, fermentation could enhance the availability of threonine and other amino acids by breaking down proteins into forms that are more easily digested. Conversely, some studies have indicated reductions in particular amino acids, including threonine, during fermentation (Nkhata et al., 2018). This phenomenon might be attributed to the microbial utilisation or other fermentation-specific processes. However, further research is warranted to fully understand the mechanisms behind these reductions.

Table 2. Essential Amino Acid of the Weaning Foods Prepared from the Blends of Sorghum and Soybean (g/100g)

Sample	Histidine	Isoleucine	Leucine	Lysine	Methionine	Phenylalanine	Threonine	Tryptophan	Valine
F55:40:5	2.16 ± 0.08^{e}	5.02 ± 0.04^{b}	11.21 ± 0.06^{a}	6.12 ± 0.09^{ab}	2.36 ± 0.06^{a}	5.98 ± 0.03^{b}	4.32 ± 0.03^{d}	4.30 ± 0.02^{a}	6.33 ± 0.03^{a}
F65:30:5	2.35 ± 0.04^{bc}	5.22 ± 0.02^{a}	10.92 ± 0.04^{b}	6.38 ± 0.02^{a}	2.31 ± 0.04^{ab}	6.34 ± 0.04^{a}	5.00 ± 0.08^{b}	4.30 ± 0.04^{a}	6.20 ± 0.03^{b}
F75:20:5	2.22 ± 0.03^{de}	4.17 ± 0.07^{e}	9.98 ± 0.04^{d}	5.17 ± 0.03^{cd}	1.93 ± 0.03^{d}	5.46 ± 0.02^{e}	3.98 ± 0.06^{e}	4.14 ± 0.13^{b}	6.02 ± 0.02^{c}
R55:40:5	2.48 ± 0.04^{a}	4.57 ± 0.03^{c}	10.92 ± 0.02^{b}	6.32 ± 0.05^{a}	2.41 ± 0.03^{a}	5.81 ± 0.03^{c}	4.88 ± 0.03^{b}	3.97 ± 0.03^{c}	5.78 ± 0.05^{d}
R65:30:5	2.35 ± 0.02^{bc}	3.85 ± 0.03^{g}	8.86 ± 0.04^{f}	4.84 ± 0.06^{e}	1.77 ± 0.04^{f}	4.84 ± 0.04^{g}	3.92 ± 0.01^{e}	3.88 ± 0.04^{c}	4.99 ± 0.08^{f}
R75:20:5	2.28 ± 0.02^{cde}	3.98 ± 0.05^{f}	9.51 ± 0.02^{e}	5.17 ± 0.02^{cd}	1.79 ± 0.02^{ef}	5.10 ± 0.06^{f}	3.92 ± 0.04^{e}	3.64 ± 0.03^{d}	4.01 ± 0.01^{i}
FR55:40:5	2.41 ± 0.02^{ab}	4.37 ± 0.03^{d}	10.56 ± 0.04^{c}	5.28 ± 0.03^{c}	2.04 ± 0.03^{c}	5.63 ± 0.06^{d}	4.60 ± 0.02^{c}	3.97 ± 0.04^{c}	5.84 ± 0.03^{d}
FR65:30:5	2.29 ± 0.02^{bcd}	3.98 ± 0.06^{f}	8.98 ± 0.06^{f}	5.00 ± 0.03^{cde}	1.88 ± 0.03^{de}	5.10 ± 0.06^{f}	5.86 ± 0.05^{a}	3.81 ± 0.08^{c}	5.54 ± 0.04^{e}
FR75:20:5	2.03 ± 0.03^{f}	3.39 ± 0.02^{i}	8.16 ± 0.03^{h}	4.18 ± 0.02^{f}	1.45 ± 0.05^{h}	4.22 ± 0.04^{i}	3.69 ± 0.03^{f}	3.64 ± 0.02^{d}	4.68 ± 0.03^{g}
U60:40	2.29 ± 0.05^{bcd}	4.57 ± 0.03^{c}	10.80 ± 0.05^{b}	5.99 ± 0.05^{b}	2.25 ± 0.04^{b}	5.81 ± 0.02^{c}	4.32 ± 0.03^{d}	3.47 ± 0.03^{e}	5.54 ± 0.04^{e}
U70:30	2.16 ± 0.04^{e}	3.91 ± 0.03^{fg}	8.86 ± 0.04^{f}	4.89 ± 0.05^{de}	1.72 ± 0.03^{f}	4.93 ± 0.02^{g}	3.98 ± 0.05^{e}	3.31 ± 0.02^{f}	4.93 ± 0.02^{f}
U80:20	2.22 ± 0.03^{de}	3.52 ± 0.02^{h}	8.51 ± 0.03^{g}	4.95 ± 0.32^{de}	1.61 ± 0.01^{g}	4.66 ± 0.02^{h}	3.41 ± 0.03^{j}	2.98 ± 0.03^{g}	4.32 ± 0.02^{h}

Legend: F - Fermented; R - Roasted; FR - Combined fermented and roasted; U - Untreated; Means in the same column bearing different superscript are significantly different at $p < 0.05$.

Table 3. Non-essential Amino Acid of the Weaning Foods Prepared from the Blends of Sorghum and Soybean (g/100g)

Sample	Alanine	Arginine	Aspartic acid	Cysteine	Glutamic acid	Glycine	Proline	Serine
F55:40:5	5.55 ± 0.08^{c}	6.30 ± 0.04^{d}	13.19 ± 0.11^{d}	2.35 ± 0.06^{a}	16.49 ± 0.05^{b}	5.99 ± 0.06^{b}	4.52 ± 0.03^{d}	4.29 ± 0.02^{b}
F65:30:5	4.14 ± 0.02^{ef}	7.15 ± 0.04^{b}	12.31 ± 0.06^{e}	2.35 ± 0.04^{a}	16.06 ± 0.05^{d}	5.78 ± 0.04^{c}	5.34 ± 0.04^{a}	4.88 ± 0.09^{b}
F75:20:5	4.06 ± 0.03^{fg}	5.95 ± 0.04^{f}	11.74 ± 0.03^{f}	2.35 ± 0.03^{a}	15.07 ± 0.07^{h}	5.58 ± 0.03^{d}	4.99 ± 0.06^{b}	3.41 ± 0.03^{fg}
R55:40:5	5.88 ± 0.04^{b}	7.32 ± 0.02^{a}	13.19 ± 0.05^{d}	2.21 ± 0.02^{b}	15.92 ± 0.03^{e}	6.35 ± 0.04^{a}	3.94 ± 0.06^{g}	4.11 ± 0.02^{c}
R65:30:5	6.13 ± 0.03^{a}	6.55 ± 0.02^{c}	11.30 ± 0.04^{g}	1.66 ± 0.01^{e}	15.07 ± 0.08^{h}	6.09 ± 0.11^{b}	3.94 ± 0.03^{g}	3.35 ± 0.04^{g}
R75:20:5	5.22 ± 0.02^{d}	6.21 ± 0.02^{e}	11.30 ± 0.03^{g}	1.66 ± 0.02^{e}	15.63 ± 0.03^{f}	5.58 ± 0.03^{d}	3.94 ± 0.06^{g}	3.35 ± 0.04^{g}
FR55:40:5	4.23 ± 0.02^{e}	6.30 ± 0.03^{d}	14.33 ± 0.03^{a}	1.94 ± 0.03^{c}	16.84 ± 0.02^{a}	5.79 ± 0.02^{c}	4.76 ± 0.03^{c}	3.70 ± 0.02^{e}
FR65:30:5	3.89 ± 0.04^{hi}	5.79 ± 0.02^{g}	13.89 ± 0.05^{b}	1.80 ± 0.03^{d}	16.49 ± 0.05^{b}	5.02 ± 0.03^{e}	4.29 ± 0.06^{e}	3.52 ± 0.02^{f}
FR75:20:5	3.65 ± 0.03^{j}	5.53 ± 0.03^{h}	13.83 ± 0.03^{c}	1.59 ± 0.01^{e}	16.34 ± 0.04^{c}	4.50 ± 0.01^{h}	3.83 ± 0.03^{g}	3.00 ± 0.03^{h}
U60:40	3.98 ± 0.05^{gh}	5.27 ± 0.03^{i}	10.23 ± 0.03^{j}	1.66 ± 0.04^{e}	15.35 ± 0.03^{g}	4.71 ± 0.02^{g}	4.29 ± 0.03^{e}	3.99 ± 0.03^{d}
U70:30	3.81 ± 0.04^{i}	5.02 ± 0.02^{j}	10.98 ± 0.02^{h}	1.38 ± 0.04^{f}	14.00 ± 0.03^{i}	4.86 ± 0.05^{f}	3.94 ± 0.02^{g}	3.41 ± 0.02^{fg}
U80:20	3.31 ± 0.03^{k}	4.93 ± 0.04^{k}	10.78 ± 0.04^{i}	1.38 ± 0.04^{f}	13.64 ± 0.04^{j}	3.99 ± 0.04^{i}	3.59 ± 0.04^{h}	2.94 ± 0.03^{h}

Legend: F - Fermented; R - Roasted; FR - Combined fermented and roasted; U = Untreated; Means in the same column bearing different superscript are significantly different at $p<0.05$.

The tryptophan concentrations recorded varied from 2.98 to 4.30 g/100 g, with the untreated sample U80:20 exhibiting the lowest value and the fermented samples F55:40:5 and F65:30:5 showing the highest. The tryptophan levels in the fermented samples were noted elevated in comparison to other processing techniques. This indicates that the process of fermentation enhances the accessibility of tryptophan when contrasted with both the roasted and untreated samples. According to similar findings reported by Fagbemi (2007) and Asma et al. (2006), fermentation increases the availability and digestibility of nutrients, whereas roasting can result in some nutrient reduction due to the breakdown of heat-sensitive compounds. Nevertheless, the degree of tryptophan depletion during roasting might hinge on factors like temperature, duration, and the moisture content.

Valine was found in the roasted and fermented samples, ranging from 4.01 to 6.33 g/100g for the roasted sample R75:20:5 and the fermented sample F55:40:5, respectively. The levels of valine were observed to be higher in the fermented samples than those achieved by other processing methods. This suggests that the fermentation process improves the availability of valine when compared to both the roasted and untreated samples. The rise in certain essential amino acids within the weaning blends could be attributed to the actions of microorganisms that convert nutrients in the food into amino acids for their use during the fermentation period. Conversely, the reduction in some essential amino acids due to fermentation could be attributed to the utilisation of these amino acids by the fermenting seeds. This corresponds with the similar findings of Oyarekua & Eleyinmi (2004), who noted that the increase in amino acids during fermentation might be due to the breakdown of other nutrients, such as carbohydrates, for the synthesis of amino acids required by fermenting seeds for their biochemical activities and growth. The decrease in amino acids during roasting might be attributed to the alteration of protein content through denaturation caused by heat during roasting (Chamba et al., 2021).

Non-Essential Amino Acid of the Weaning Foods Prepared from the Blends of Sorghum and Soybean

The findings for the non-essential amino acid composition of the weaning diets made from sorghum and soybean blends are presented in Table 3. Similar to the essential amino acids, the non-essential amino acids are important for growth and development. For instance, alanine contributes to glucose and organic acid metabolism, whereas arginine helps maintain nitrogen balance and facilitates the elimination of excess nitrogen. The alanine content recorded varied between 3.31 and 6.13 g/100g among different samples. The untreated sample U80:20 showed the lowest amount, whereas the roasted sample R65:30:5 displayed the highest. In terms of arginine, its content ranged from 4.93 to 7.32 g/100g. The untreated sample U80:20 showed the lowest content, whereas the roasted sample R55:40:5 displayed the highest. Notably, all the treated samples exhibited elevated arginine levels, which are crucial for children's growth, according to Uchegbu et al. (2019). This study revealed that both the alanine and arginine content in the treated samples surpassed that of the untreated, with the roasted samples exhibiting the highest levels, followed by the fermented and fermented/roasted samples.

The range of aspartic acid content was 10.23 to 14.33 g/100 g, with the sample U60:40 displaying the lowest value and the fermented and roasted sample FR55:40:5 showing the greatest value. The cystine content ranged from 1.38 to 2.35 g/100 g, with the lowest values found in the untreated samples U70:30 and U80:20, and the highest values found in the fermented samples F55:40:5, F65:30:5, and F75:20:5. The glutamic acid content recorded ranged from 13.64 to 16.84 g/100g, with the lowest value determined in the untreated sample U80:20 and the highest in the fermented and roasted sample FR55:40:5. Among these amino acids, glutamic acid was found to have the highest range, followed, in descending order, by aspartic acid and serine, which showed the lowest range. This result is consistent with the findings of Tesby Mohamed et al. (2019) in the context of weaning food derived from rice and soybean blends. Ng'ong'ola-Manani et al. (2014) also reported that glutamic acid was the most abundant amino acid, followed by aspartic acid, whereas cysteine and methionine were the limiting amino acids in the blends of fermented soybean and maize.

Similar observations were found in this study on weaning foods produced from blends of sorghum and soybean, whereas the lowest range was found in the cysteine and methionine contents. The lowest value range observed in the cysteine and methionine contents could possibly be due to differences in processing methods and blend composition. These fluctuations might also reflect the transformation of peptides into free amino acids and their subsequent utilisation, as pointed out by Ng'ong'ola-Manani et al. (2014). Since amino acids participate in Maillard reactions and Strecker degradation, they contribute significantly to the formation of aroma and flavour in food (Park et al., 2010; Dajanta et al., 2011). For instance, roasted blends tend to have higher concentrations of sweet amino acids like serine and alanine, whereas fermented blends tend to have higher concentrations of glycine, alanine, and lysine. The higher levels of aspartic acid and glutamic acid in fermented and roasted blends are likely to make the umami taste stronger, whereas the higher levels of amino acids that are linked to sweetness in roasted samples are likely to make the sweetness taste stronger.

The weaning food samples exhibited glycine contents ranging from 3.99 to 6.35 g/100g. The roasted sample R55:40:5 displayed the highest value, whereas the untreated sample U80:20 displayed the lowest. The proline content ranged from 3.59 to 5.34 g/100g, with the untreated sample U80:20 showing the lowest value and the fermented sample F65:30:5 displaying the highest. The serine content recorded ranged from 2.94 to 4.88 g/100g, with the untreated sample U80:20 showing the lowest value and the fermented sample F65:30:5 displaying the highest. In general, the results obtained indicate that the effects of fermentation and roasting on non-essential amino acid levels, similar to those of essential amino acids, were diverse. Fermentation positively affected the cysteine and proline contents, whereas roasting had a positive impact on the alanine, arginine, glycine, and serine contents. Additionally, the combined fermentation and roasting processes had a positive effect on the aspartic acid and glutamate contents.

The increase in amino acid content observed during fermentation could be attributed to the hydrolytic breakdown of nutrient components, whereas the improvement in amino acids during roasting might result from the destruction of antinutrients through the applied heat. These findings align with the findings of Abd El-Hady & Habiba (2003), who suggested that increased amino acid levels could be due to the removal of antinutrients and the deactivation of enzyme inhibitors through heating. Furthermore, fermentation is recognised for enhancing mineral and amino acid levels in food samples. Nkhata et al. (2018) reported that fermentation could increase individual amino acids, although some might decrease during the process. This decrease is attributed to microbes utilising amino acids for growth, whereas the increase stems from the microbes' ability to synthesise certain amino acids.

CONCLUSION

This study examined how fermentation and roasting affected the amino acid profile of weaning diets made from mixtures of sorghum and soybean. Prior to milling into flours, sorghum and soybean were subjected to fermentation, roasting, and combined fermentation and roasting, with untreated sorghum and soybean used as the control. The blends were prepared in various ratios, and 5% malted sorghum flour was added to each blend with the exception of the control samples. Weaning foods were prepared with these blends, and their amino acid profiles were determined. The results obtained indicate that fermentation and roasting have a noteworthy impact on improving the content of amino acid profiles in the formulated weaning foods. In contrast to other research, the effects of fermentation and roasting on the amino acid content of weaning foods were found inconsistent. This is likely due to differences in experimental setups, study durations, and initial protein contents of the foods. As a result, it is necessary to identify the most suitable fermentation and roasting conditions for each weaning blend to enhance the health and nutritional advantages of these procedures. Accordingly, the benefits of the weaning blends for infants and young children could be maximized.

Conflict of interest: The authors declare that they have no conflict of interest.

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