

American Journal of  
**Food Sciences and Nutrition**  
(AJFSN)



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*Jackson Nkesiga, Joseph O. Anyango, and Peninah N. Ngoda*



## Protein Quality of Extruded Ready-to-Eat Baby Foods from Orange-Fleshed Sweet Potato, Amaranth Seeds, and Soybean Flour Blends

Jackson Nkesiga<sup>1\*</sup>, Joseph O. Anyango<sup>1</sup>, and Peninah N. Ngoda<sup>1</sup>

<sup>1</sup>Department of Dairy and Food Science and Technology, Faculty of Agriculture, Egerton University, Kenya.

\*Corresponding author: [nkesiga.1652318@student.egerton.ac.ke](mailto:nkesiga.1652318@student.egerton.ac.ke)

DOI: <https://doi.org/10.47672/ajfsn.1287>

### Abstract

**Purpose:** Protein quality refers to the total protein content, essential amino acid content, and digestibility of a protein. Source, bioavailability, food matrix, and processing conditions all have an impact on protein quality. Protein quality can be lost during food processing. This study was carried out to investigate the effect of extrusion cooking and blend proportions on the protein quality of extruded ready-to-eat baby foods.

**Methodology:** Different blends of orange-fleshed sweet potato, amaranth seeds, and soybean flour were used and analyzed for protein quality including *in vitro* protein digestibility (IVPD) of extruded ready-to-eat baby foods. In addition, nutrient damage due to heat or processing temperature was evaluated by analyzing available lysine in the end products to ensure the quality of extruded ready-to-eat baby foods. Extrusion cooking was carried out at a temperature of 90°C, screw speed of 400 rpm, and feed moisture content of 35%.

**Findings:** The results showed that IVPD ranged from 54.05 to 91.87%. The available lysine as a parameter to evaluate the nutritional damage due to thermal processing ranged from (1.69 to 2.79%). This research predicts the potential availability of highly digestible protein as well as the assurance of lysine availability once extrudates are consumed. Achieving high lysine retention during extrusion cooking depends on a number of factors, including low temperature, high screw speed, high feed moisture content, and high shear forces that lead to a short residence time.

**Recommendation:** It is important to conduct more research on how extrusion cooking affects the molecular and physical interactions between starches, proteins, lipids, and phenolic compounds.

**Keywords:** *Extrusion cooking, protein quality, ready-to-eat extrudates, orange-fleshed sweet potato, amaranth seeds, and soybean flour*

## INTRODUCTION

Proteins are enormous and complex containing nitrogen molecules created by amino acids that are essential for the structure, function, and control of body tissues. Proteins are required by the human body in several different ways, including antibodies, which attach to certain foreign particles to assist defend the body, enzymes, which perform nearly all of the chemical processes that occur in our cells, and structural components in muscle and other tissues (Hayes, 2019). Proteins play a variety of tasks and are essential to almost every aspect of all life processes in biological organisms. These functions can be divided into a few categories, such as the catalysis of metabolic processes, energy transfer, gene expression, solute transport across biological membranes, cellular communication, molecular recognition, defense, formation of intracellular and extracellular structures, and cell- and tissue-specific functions (Kessel & Ben-Tal, 2018).

Amino acids are important as both protein-building elements and metabolic intermediates. Twenty natural amino acids are contained within proteins, and their chemical characteristics dictate the biological functions of proteins (Hayes, 2019). Proteins are a common class of macromolecules that are used as nutrients in food compositions to confer nutritional, functional, and sensory qualities. The capacity of proteins to behave in differing ways is based on their distinct physicochemical qualities, which are in turn influenced by protein structure at different organizational levels (i.e., primary, secondary, tertiary, and quaternary) (Aryee *et al.*, 2018). Food proteins are a crucial nutrient and a dietary source of the amino acids required for the healthy growth and maintenance of the body. Additionally, a lot of food proteins display particular biological properties that can affect human health and fend against diseases. Proteins are essential for the synthesis and repair of body tissues as well as serving as structural and functional elements of the human body. Proteins have a significant impact on how bodily tissues grow and evolve (Gupta, 2020).

The availability of the amino acids and their composition, which are influenced by the protein-containing food's capacity to be digested, are key factors in determining the quality of protein (Hayes, 2019). Disulfide bond breaking, unfolding, protein aggregation, dimerization, and the development of bigger oligomers are all possible outcomes of thermal treatment. In addition to the protein alterations that take place during thermal treatments, other chemical processes such glycation, Maillard reactions, oxidation, and deamidation (caused by high-temperature acid treatment or enzymatic treatment with deamidase or transglutaminases) may also take place (Aryee *et al.*, 2018).

When food is heated, the Maillard reaction occurs spontaneously, causing the reactive carbonyl groups of reducing sugars to combine with the nucleophilic amino group of amino acids, peptides, or proteins to produce a wide range of chemicals (Ruan *et al.*, 2018). Thermal processing, particularly when food is being processed, can facilitate the Maillard reaction, a complicated series of chemical reactions. One of the most significant chemical processes in food processing, the Maillard reaction (MR), has a significant impact on several food quality factors. Maillard reaction products (MRPs), which are important for various aspects of food quality, including texture, flavour, and colour, are formed in greater quantity as a result of MR (Giannetti *et al.*, 2021). The detrimental effects of the Maillard reaction, which are mostly reflected in public health issues, were revealed by Parisi *et al.* (2019). Different Maillard reaction products (MRPs), such as acrylamide and 5-hydroxymethylfurfural, are under attention due to their potential for being

mutagenic, cytotoxic, and carcinogenic. Advanced glycation end products (AGEs) can also exacerbate pre-existing diseases including diabetes and several cancers. Second, certain foods have many beneficial characteristics related to their textural, aromatic, and colourimetric look that may be unattractive or forbidden in other foods (Naik *et al.*, 2022; Parisi *et al.*, 2019).

Generally, some products may not want to have brownish colours or cooked flavours or odours. Last but not least, the Maillard reaction reduces the nutritional value. Ascorbic acid, a natural antioxidant that may also act as an inhibitor agent when added to food recipes, some metals like iron, calcium, or magnesium, and other minerals that are engaged are degraded or modified with loss of nutritious levels (Gancarz *et al.*, 2021; Parisi *et al.*, 2019). Nevertheless, melanoidins, brownish polymers that are produced during the Maillard reaction, have been found to have beneficial impacts on human health in the form of antioxidant and/or antibacterial capabilities. Additionally, in some situations, the textural, aromatic, and colourimetric qualities of particular foods are needed. The three chemical phases of the Maillard reaction—early, intermediate, and final stages—were succinctly described by Ruan *et al.* (2018). In the early stages of the Maillard reaction, sugar-amine condensation, the formation of a Schiff base, and the Amadori rearrangement products (ARPs) are all present. In the intermediate stage, sugar dehydration, fragmentation, and amino acid degradation are present, and in the final stage, reactive dicarbonyl and aldehyde intermediates are responsible for the formation of low- and high-molecular-weight heterocyclic compounds and polymers (Giannetti *et al.*, 2021; Naik *et al.*, 2022; Ruan *et al.*, 2018).

There may be the development of bioactive Maillard reaction products in the later phases of the Maillard reaction cascading, particularly the intermediate stage. The advanced stages of the process that result in AGEs are thought to be undesirable (Naik *et al.*, 2022). The products of the early stage of the reaction are condensed N-substituted glycosylamines, i.e. aldosamine or ketosamine, which undergo Amadori rearrangement to produce Amadori rearrangement (PPA) products. Cyclization, dehydration, and condensation are the three steps that make up the the final phase of the Maillard reaction. The macromolecular colour compounds melanoidins are created as a result of these processes. They also have an impact on food quality and are the cause of the brown colour of heat-treated food products (Gancarz *et al.*, 2021). Cooking extruded foods at high temperatures encourages the Maillard process, which alters the ultimate nutritional and organoleptic characteristics of food. The amino acid furosine, which is produced when Amadori compounds are hydrolyzed in acid and form during the first step of the Maillard reaction, has been widely utilized as a sign of heat damage (Giannetti *et al.*, 2021).

On the other hand, it is widely known that some dietary proteins contain physiologically active peptides that can impact a favourable health response beyond their basic nutritional value, in addition to delivering energy and amino acids necessary for growth. Bioactive peptides have a wide range of effects, including anti-inflammatory, anti-cancer, anti-oxidant, anti-diabetic, and anti-hypertensive ones. They also play a significant role in the management of anxiety, type 2 diabetes, obesity, and hypertension, as well as in the regulation of the immune system, and blood pressure, or as signalling molecules (Ustunol, 2015; Yada, 2018). Extrusion cooking has mostly concentrated on the transitions that starches and proteins undergo during the process, but there is still untapped potential in terms of how starches, proteins, and other macro and micro-ingredients (phenolic chemicals) interact in the extruder (Shah *et al.*, 2021). The use of an extruder to operate a continuing reaction mechanism as heating, pressurizing, shearing, and mixing vessels can lead to the production of foods with distinct processing properties (Bhattacharya, 2020; Pichmony *et*

*al.*, 2020). Lipids interact with amylase and form amylose–lipid complexes, the asparagine-carbohydrates (formation of acrylamide), lipid-protein complex, and protein-carbohydrate complex in the Maillard reactions that take place during extrusion cooking (Shah *et al.*, 2021).

Fundamental issues in agri-food systems must be addressed through innovative food processing technology. Extrusion cooking technology, for example, can turn diverse resource-intensive food systems into more affordable, sustainable, and healthy food than traditional food processing methods. Food processing alters the structure and physicochemical characteristics of proteins, modulating functioning by enhancing heat stability, protein-protein interaction, unfolding and aggregation, and changing interaction strength and bond types, among other factors (Aryee *et al.*, 2018). Therefore, there is a need to understand the interaction of protein-carbohydrate that occur during extrusion cooking of extruded ready-to-eat baby foods. On the other hand, malnutrition leads to poor cognitive development and weak human body self-defense against diseases. This lack of certain nutrients may cause adverse health effects and even associated diseases. Children and babies, on the other hand, require little but sufficient amounts of food to fulfil their daily basic needs for energy, protein, and micronutrients. This study aims at developing and analyze the protein quality of extruded ready-to-eat baby foods from blends of orange-fleshed sweet potato, amaranth seeds, and soybean flour.

## **MATERIALS AND METHODS**

### **Materials and Reagents**

Orange-fleshed sweet potato, variety, Kenspot 5, and soybean of variety DPSB 19 were bought from Kenya Agricultural and Livestock Research Organization (KALRO), Njoro, Kenya, and amaranth seeds of variety Katumani Amaranth (KAM) 001 was procured from KALRO, Katumani, Machakos, Kenya. The Acid Orange 12 70% and the propionic anhydride were bought from Ipure Biology Co. Ltd, Jinhua, Zhejiang China, potassium dihydrogen phosphate anhydrous 98%, sodium acetate anhydrous 99%, and oxalic acid 99.0%, glacial acetic acid 99.7% were procured from LOBA Chemie PVT. Ltd, Mumbai, India.

### **Production of Flour from Orange-fleshed Sweet Potato, Amaranth Seeds, and Soybean**

Orange-fleshed sweet potato flour was processed according to Honi *et al.* (2017), amaranth seeds flour was produced based on the method described by Shevkani *et al.* (2014) while soybean flour was manufactured according to Shokunbi *et al.* (2011).

### **Blend Formulations and Extrusion Cooking Conditions**

The blends used in this work were identified from the pilot experiments and the available literature. The flour (100 g), sugar (15%), salt (1.5%), baking fat (1.5%), and vanilla essence (1%) were gently combined to produce a dough. Distilled water (35%) was progressively added while mixing until a well-textured, relatively hard dough was achieved. The dough was kneaded on a clean flat surface. A twin screw Extruder (PSHJ-20, Jiangsu Xinda Science and Technology Co.Ltd, China) was used for processing extruded ready-to-eat baby foods. The extruder was set at different conditions where, die temperature, screw speed, and feed moisture content were 90°C, 400 rpm, and 35%, respectively. Furthermore, the metering zone temperature was varied from 70 to 90°C while the feeding zone and compression zone temperatures were kept constant at 70°C. The firm dough was fed on the hopper of an extruder and extruded products were collected at the die nozzle section, dried at 55±5°C, cooled, and sealed in plastic polyethylene bags of 26.8 cm by 27.3 cm,

and stored at room temperature ( $24\pm 4^{\circ}\text{C}$ ) prior for *in vitro* protein digestibility, and available lysine analysis.

### Experimental Design

A Completely Randomized Design (CRD) in a Factorial Experimental Design with two variables (blend proportions at 5 levels and extrusion cooking temperature at two levels) was used in this study. The effect of blend proportions: C0 (control): 100:0:0; C1:50:25:25; C2:54.5:24:21.5; C3:50:30:20; and C4:54.5:26.5:19 for OFSP: Amaranth Seeds: Soybean flour, respectively, and extrusion cooking end barrel temperature ( $70\text{-}90^{\circ}\text{C}$ ) was investigated. The screw speed, feed moisture content, and die temperature used in this experiment were identified during the preliminary experiments.

$$Y_{ijk} = \mu + \alpha_i + \beta_j + (\alpha\beta)_{ij} + \epsilon_{ijk}$$

Where;  $Y_{ijk}$  = observation k in level i of factor A and level j of factor B,  $\mu$  = The grand mean

$\alpha_i$  = Effect due to blending,  $\beta_j$  = Effect due to extrusion temperature,

$(\alpha\beta)_{ij}$  = Interaction between blend proportions and temperature, and  $\epsilon_{ijk}$  = Random error

### Determination of *in vitro* Protein Digestibility in Extruded Ready-to-eat Baby Foods

The *in vitro* protein digestibility (IVPD) of the ready-to-eat baby foods was evaluated using the pepsin and trypsin sequential digestion model according to the method of Manus *et al.* (2021) with modifications. Briefly, 5 g of the formulated samples were weighed into 5 ml centrifuge tubes and suspended in 20 ml of 0.1 N HCl. and the pH was measured and adjusted using the base. Then 0.02 g of pepsin (CAS: 9001-75-6) of 0.8 Anson unit/mg was added and incubated in the water bath at  $37^{\circ}\text{C}$  for 3.5 hrs while shaking the tubes using Lab Rotator (DSR-2800P, S/No: 14030067, Digisystem Laboratory Instruments Inc., Taiwan) at intervals of 10 - 15 min as the digestion goes on. After the digestion by the first enzyme is done, the pH was adjusted to 8.0 with 1.0 N NaOH and a mixture of 0.02g trypsin (CAS: 9002-07-7, India), and 0.02 g of chymosin (CAS: 9001-98-3) was added into the tubes then incubated for a further 3.5 hours at  $37^{\circ}\text{C}$ , shaking the tubes at intervals of 10 - 15 min until the digestion is complete. The mixture was centrifuged using a centrifuge (Funke-Gerber, SuperVario-N, Germany) at  $3500\times g$  for 20 min and the liquid was decanted. The residue was dried at  $95^{\circ}\text{C}$  in the oven to a constant dry weight and then analyzed via Kjeldahl determination of the protein.

$$\text{Protein Digestibility (\%)} = \frac{(\text{Total sample } N - N \text{ in residue})}{\text{Total sample } N} \times 100$$

### Preparation of Buffer Solution for Available Lysine Analysis

A two-litre volumetric flask was filled with 40 g of oxalic acid dehydrate 99.0% (CAS: 6153-56-6) and 6.8 g of potassium dihydrogen phosphate anhydrous 98% (CAS: 7778-77-0). The powder was partly dissolved by the addition of distilled water in a small amount. After that, 120 ml of glacial acetic acid 99.7% (CAS: 64-19-7) was poured into the flask. The distilled water was used to make a final volume of 2l. The flask was set on a magnetic stirrer and stirred for roughly an hour to create a transparent, uniform solution. This produced 2l of buffer solution which was enough to create the dye solution. The buffer solution was prepared every day before the analysis.

### **Preparation of the Dye Solution**

The buffer solution was used to dilute the Acid Orange 12 dye (CAS: 1934-20-9) (0.27 g) to the desired final volume before being added to a 200 ml volumetric flask. This offered enough dye for the experiment.

### **Preparation of Sodium Acetate Solution**

On a magnetic stirrer, 16.4 g of sodium acetate anhydrous 99% (CAS: 127-09-3) was dissolved with 100 g of distilled water (w/w) and mixed for around 30 mins. It was also made every day and is known as the solvent.

### **Determination of Available Lysine in Extruded Ready-to-eat Baby Foods**

The method of Aalaei *et al.* (2016) was used with slight modification. A sample of 0.2 g was weighed using an analytical balance and put into 100 ml Erlenmeyer glass flasks. The flask was then filled with 2 ml of sodium acetate solution as the solvent. All flasks were covered with Parafilm and shaken vigorously on an orbital shaker at 300 rpm for about 20 mins to dissolve the powder. The flask was then filled with 0.2 ml propionic anhydride (the blocking agent) and shaken for another 20 mins. The dye solution (3.89 mM) was added to all samples, covered with Parafilm, and shaken vigorously on an orbital shaker for 2 h at 300 rpm. During this time, the protein in the sample binds to the dye and forms a complex. Centrifugation was used to isolate the dye complex. The solution of 10 ml was poured into plastic tubes and centrifuged at 5000×g for 10 mins, thoroughly separating the complex from the supernatant. The samples were diluted 100 times before the spectrophotometer could measure their absorbance. The oxalic acid–acetic acid phosphate buffer solution was used to dilute 1 ml of the supernatant to a final volume of 100 ml. After that, the absorbance was measured at 475 nm using UV-Visible Spectrophotometer (UV-1800, Shimadzu Corporation., Kyoto, Japan) with a buffer solution as a blank. The concentration of available lysine in the samples may be determined using the equation generated from the standard curve.

### **Data Analysis**

The data for *in vitro* protein digestibility and available lysine were statistically analyzed using SAS version 9.4 TS Level 1M7 (SAS Institute Inc.). Basic statistical measures and goodness-of-Fit tests were conducted for normality distribution of data using PROC UNIVARIATE while the Levene's Test was carried out for standard homogeneity of variance using HOVTEST=LEVENE. The analysis of variance (ANOVA) was executed to examine the effect of blend proportions and extrusion cooking temperature on the protein quality of extruded ready-to-eat baby foods while mean separations were tested using Tukey's Studentized Range (HSD) Test at 5% confidence level.

## **RESULTS AND DISCUSSION**

The *in vitro* protein digestibility in extruded ready-to-eat baby foods varied from 54.05 to 91.87% and a significant difference ( $p < 0.05$ ) was noted among food samples (Table 6.1). The results fall within the values (80.46-86.44%) reported by Edima-Nyah *et al.* (2020) for breakfast cereals produced with yellow maize, soybean, and banana blends. Furthermore, the results are lower than the values (79-96%) reported by Kanu *et al.* (2009) for breakfast cereal-based porridge mixed with sesame and pigeon peas for adults but confirm well with the range (56.1-79.3%) reported by Elkonin *et al.* (2013) for grain sorghum but the results are in agreement with the values (88.53-

92.97) reported by Wafula *et al.* (2020) for extrudates from rice, sorghum and bamboo shoots flour blends. The hydrolysis treatment of proteins can produce peptides with antihypertensive and antioxidant functions (Manus *et al.*, 2021).

Protein availability for intestinal absorption is significantly influenced by protein digestibility. The widely used IVPD assay can be used to calculate many aspects of protein digestibility. By utilizing various proteolytic enzymes, the IVPD assay simulates conditions comparable to those of the human digestive tract (Kumar *et al.*, 2021). It was discovered that thermal treatment denatured native protein structure, altered the structure of protease inhibitors and storage proteins from legumes, and caused protein aggregation, rendering the proteins more vulnerable to digestive proteases during unfolding. Wet heating improves structural changes even more as gelatinization and crosslinking between proteins and starch take place. Thermal treatment boosts structural changes. Thermal treatment, however, has a considerable impact on non-polar interactions and intramolecular hydrogen bonds, changing the native structure (Ohanenye *et al.*, 2022).

Extrusion cooking temperature and shear forces promote *in vitro* protein digestibility by causing protein denaturation by exposing the protein configuration to enzyme activities. The elimination of anti-nutrients may also contribute to an improvement *in vitro* protein digestibility (Gulati, 2018). Food protein digestibility exposes the proteolysis vulnerability of the protein, which is dependent on factors such as the structure and amino acid composition of the protein, the pH and temperature of processing conditions, and the presence of certain secondary molecules such as anti-nutritional factors and emulsifiers (Jingyu *et al.*, 2022).

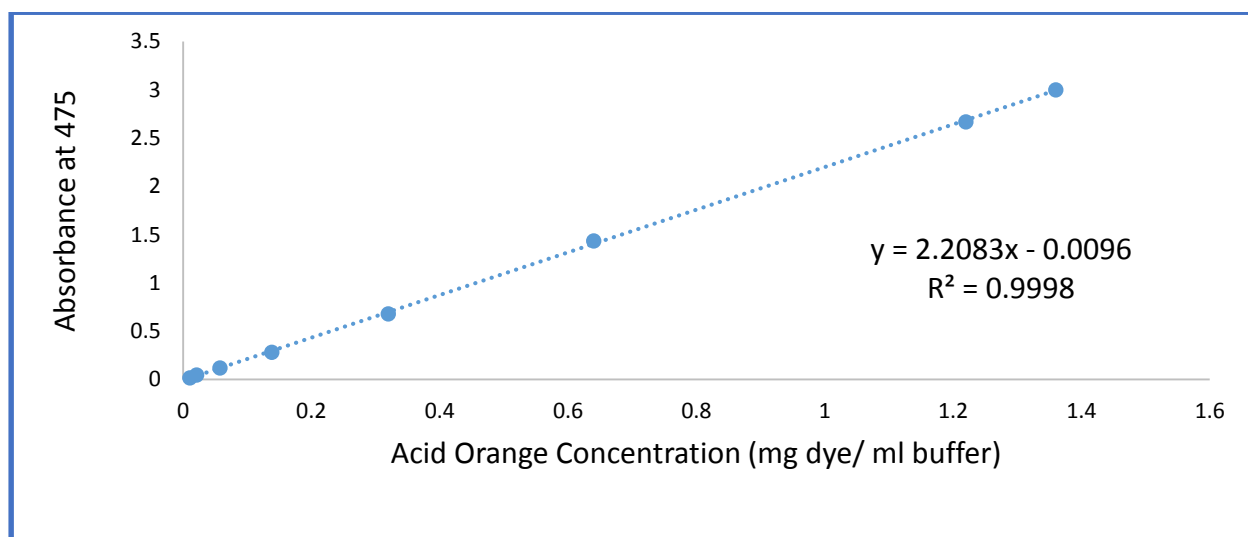
The available lysine (Table 1) in extruded ready-to-eat baby foods varied from 1.69 to 2.79% and the standard curve used during the experiment is shown in Figure 1. The results are above the values (1.22-1.63%) reported by Li *et al.* (2019) in diets from channel catfish, *Ictalurus punctatus*, but in agreement with the values 2.86%, 1.34%, 0.72%, 0.07%, and 0.52% in soybean meal, cottonseed meal, corn germ meal, corn grain, and wheat middlings, respectively reported by Li *et al.* (2019). The results are slightly above the values (0.95-1.28%) reported by Aalaei *et al.* (2019) in infant formulas but below the range (2.33-3.31%) reported by Aalaei *et al.* (2016) for skim milk powders. These high values may be due to the high protein content of skim milk powder compared to the low protein content present in extrudates from OFSP, amaranth seeds, and soybean flour blends. Amaranth seed is a nutrient-rich pseudocereal that contains roughly the same amount of essential amino acids in its grains as is recommended by the FAO and the WHO standard. The available lysine of the extrudates was positively affected probably due to the low temperature used during extrusion cooking. Similar observations were reported by Bjorck *et al.* (1983), and Brestenský *et al.* (2014) where the available lysine content did not change up to a process temperature of 144°C but decreased 20-30% at temperatures around 150°C.



**Table 1: Available lysine and IVPD (%) of extruded ready-to-eat baby foods**

BP	Temperature (°C)	IVPD	Available lysine
C0	70	54.05±0.80 <sup>h</sup>	1.69±0.09 <sup>f</sup>
C0	90	67.09±0.72 <sup>g</sup>	1.87±0.02 <sup>ef</sup>
C1	70	76.55±0.30 <sup>e</sup>	1.91±0.07 <sup>e</sup>
C1	90	89.37±0.35 <sup>c</sup>	2.67±0.18 <sup>bc</sup>
C2	70	71.60±0.15 <sup>f</sup>	2.29±0.05 <sup>d</sup>
C2	90	90.66±0.58 <sup>ab</sup>	2.79±0.05 <sup>a</sup>
C3	70	87.44±0.28 <sup>d</sup>	2.35±0.05 <sup>cd</sup>
C3	90	91.87±0.09 <sup>a</sup>	2.56±0.05 <sup>abc</sup>
C4	70	72.61±0.17 <sup>f</sup>	2.02±0.05 <sup>e</sup>
C4	90	90.15±0.14 <sup>bc</sup>	2.47±0.05 <sup>bcd</sup>

Data are indicated in triplicate values as the mean ± standard deviation. Mean values with different superscript letters in the same column are significantly different ( $p \leq 0.05$ ). Where; BP is the blend proportions.



**Figure 1: Standard curve of available lysine**

High screw speed and reduction in die diameter can substantially improve lysine retention during extrusion by reducing thermal exposure inside the barrel (Gulati *et al.*, 2020). As the screw speed increases, shear increases, resulting in a more drastic breakdown of protein structure; the

accompanying reduction in residence time (as a result of the increase in screw speed) reduces the duration of heat treatment, probably leading to high lysine retention. Additionally, a higher amount of sweet potato could enhance lysine retention, perhaps because of the lower levels of lysine in the sweet potato raw material. Losses are more pronounced at higher levels of soy component, given that it has a relatively higher lysine content (Gamlath *et al.*, 2007).

Furthermore, It is well-known that high nutrient digestibility is generally associated with its high availability. This notion supports the observation in the current study, where an increase in an *in vitro* protein digestibility had a corresponding increase in the available lysine of extruded ready-to-eat baby foods. It is generally understood that the primary reason for losses in available lysine can be associated with Maillard-type reactions upon high extrusion temperature and low moisture content (Gulati *et al.*, 2020). Therefore, some researchers such as Gamlath *et al.* (2007), suggest that to keep lysine losses within an acceptable range, it is necessary to avoid extrusion cooking above 180°C at water contents below 15%, and/or avoid the presence of a higher amount of reducing sugars during the extrusion process. Extrusion cooking is a technique of high temperatures processing that can affect the amino acid composition and enhance the nutritional and quality attributes of legume seed proteins, as evidenced by the observed rise in the phenylalanine content of kidney beans (Drulyte & Orlie, 2019).

In a study carried out by Ohanenye *et al.* (2022), it was found that after extrusion at 142°C, the tryptophan content dropped while the contents of valine, phenylalanine, and lysine dropped in peas. In a reversible reaction known as the early Maillard reaction, extrusion at high temperatures can lead to the condensation of the free amino groups of amino acids (or proteins/peptides) with the carbonyl group of reducing carbohydrates. The advanced Maillard reaction is irreversible, though, once the condensation has progressed to cyclization and the aldose produced in the early stages is converted to ketone in an irreversible reaction (Ohanenye *et al.*, 2022).

When food is heated, the Maillard reaction occurs spontaneously, forming a wide range of chemicals when the reactive carbonyl groups of reducing sugars combine with the nucleophilic amino group of amino acids, peptides, or proteins (Ruan *et al.*, 2018). It is common to link the significance of Maillard reaction products to food and beverage processing as well as to related changes in foods, such as colours, flavours, and scent (Perisi *et al.*, 2019). Nevertheless, the Maillard reactions, which result in the formation of protein-saccharide complexes that are unavailable to organisms, are the best-recognized interactions between lysine and reducing sugars. The  $\epsilon$ -amino group of lysine attaches to molecules of reducing sugars during this reaction, lowering the availability of lysine and ileal digestibility. The only form of lysine that is present in living things is reactive lysine, which is not related to reducing sugars (Brestenský *et al.*, 2014).

The availability and quantity of important amino acids determine the quality of the protein. A decline in protein quality results from numerous protein changes that occur during food processing, including cross-linking, racemization, breakdown, and the creation of complexes with sugars (Lalitha, & Singh, 2020). Lysine shortage is an issue, especially among cereals, because of Maillard-type reactions that happen during food preparation and cause a loss of lysine that is readily available (Aggarwal & Bains, 2020). Lysine is an essential amino acid that is heat-labile. In heat-processed foods, the  $\epsilon$ -amino group attaches to other groups, such as reducing sugars, and rendering it nutritionally unavailable (Lalitha & Singh, 2020).

It may be of interest to note also that, amaranth seed is a nutrient-rich pseudocereal that contains roughly the same amount of essential amino acids in its grains as is recommended by the FAO and the WHO standard. Furthermore, amaranth seed is a good source of polyphenols (phenolic acids and flavonoids) and fatty acids (Procopet, & Oroian, 2022). On the other hand, the recommended daily allowance (RDA) of lysine for children aged 1-3 years is 58 mg/kg/day, 51 mg/kg/day for pregnant, and 52 mg/kg/day for lactating women (Institute of Medicine, 2006). The nine amino acids leucine, isoleucine, valine, phenylalanine, threonine, tryptophan, methionine, histidine, and lysine are considered to be essential. The body depends heavily on essential amino acids for boosting protein synthesis, human metabolism, regulation of several biological functions, body weight regulation, and energy balance (Drummen *et al.*, 2018; Tashiro *et al.*, 2020; Xiao & Guo, 2021).

## CONCLUSION

The extrusion cooking temperature and blend proportions affect significantly ( $p < 0.05$ ) the protein quality of extruded ready-to-eat baby foods. The findings from this study suggest that the nutritional quality of extrudates was observed, controlled, and maintained, and hence, the products could be used as the potential source of protein by children as well as adult people in case of inadequate protein intake. Compositing soybean and amaranth seeds with OFSP makes the extrudates a valuable source of nutrients and may be utilized in the preparation of many foods, particularly as fundamental components in enhancing other foods such as infant and baby foods and any other foods. The blend proportions and extrusion cooking have significant ( $p < 0.05$ ) positive effects on *in vitro* protein digestibility as well as available lysine in extruded ready-to-eat baby foods probably due to the relatively low temperatures and feed moisture content used during the experiment. Therefore, extrusion cooking at low temperatures and high feed moisture content can help to achieve high-quality protein foods. The study found that extrudates have a high protein digestibility of 91.87% and available lysine of 2.79%, implying that high-quality protein is available in the body once extrudates are consumed. It is important to conduct more research on how extrusion cooking affects the molecular and physical interactions between starches, proteins, lipids, and phenolic compounds.

## Acknowledgements

The authors are grateful to the Centre of Excellence in Sustainable Agriculture and Agribusiness Management (CESAAM) at Egerton University in Kenya, for funding the research.

## Conflicts of Interest

The authors declare no conflicts of interest

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