ajfand SCHOLARLY, PEER REVIEWED
AFRICAN JOURNAL OF FOOD, AGRICULTURE, NUTRITION AND DEVELOPMENT
Volume 21 No. 1
January 2021
PUBLISHED BY AFRICAN SCHOLARLY SCIENCE COMMUNICATIONS TRUST
ISSN 1684 5374

Afr. J. Food Agric. Nutr. Dev. 2021; 21(1): 17140-17157 https://doi.org/10.18697/ajfand.96.19300

DEVELOPMENT AND PACKAGING OF A LEUCINE-RICH COMPOSITE FLOUR FOR TREATMENT OF MODERATE WASTING IN CHILDREN AGED 6 – 24 MONTHS

Wamiti J[1]*, Kogi-Makau W[2], Ngala S[3] and FE Onyango[4]

Jeff Wamiti

*Corresponding author email: jwamiti@uonbi.ac.ke, jwamiti72@gmail.com

[1]PhD. Applied Human Nutrition, Tutorial Fellow, Department of Food Science, Nutrition and Technology, University of Nairobi, P.O. Box 29053-00625, Nairobi, Kenya

[2]PhD. Human Nutrition, Associate Professor, Department of Food Science, Nutrition and Technology, University of Nairobi, P.O. Box 29053-00625, Nairobi, Kenya

[3]PhD. Human Nutrition, Lecturer, Department of Food Science, Nutrition and Technology, University of Nairobi, P.O. Box 29053-00625, Nairobi, Kenya

[4]MBChBM.Med. (Paed) MPH). Associate Professor, Department of Paediatrics and Child Health, University of Nairobi, P.O. Box 19676-00202, Nairobi, Kenya

CC BY NC ND

ajfand SCHOLARLY, PEER REVIEWED
AFRICAN JOURNAL OF FOOD, AGRICULTURE, NUTRITION AND DEVELOPMENT
Volume 21 No. 1
January 2021
PUBLISHED BY AFRICAN SCHOLARLY SCIENCE COMMUNICATIONS TRUST
ISSN 1684 5374

ABSTRACT

Wasting, categorized as either severe or moderate, is a form of child malnutrition that manifests with a low weight-for-height Z-score. Previous treatment methods for moderate wasting, which affects approximately 300,000 children in Kenya, were ineffective as they lacked a mechanism to replace the accelerated loss of lean tissue. Supplementation with leucine, may be a safe and effective method for treating moderate wasting. At a high dosage, leucine activates the mammalian target of rapamycin within the muscles which enhances gain of lean tissue. Leucine supplements are currently inaccessible to populations affected by moderate wasting in Kenya. The objective of this study was, therefore, to formulate a leucine-rich composite flour (TheraPEM) from locally available foods for treatment of moderate wasting. Six composite flours were prepared using combinations of beans (*Phaseolus vulgaris*), groundnuts (*Voandzeia subterranea*), and foxtail millet (*Setaria italica*) selected for their high leucine content, local availability and relatively low cost. Nutrient composition analysis and sensory evaluation were conducted on each of the six flours. The three preferred flours in terms of sensory attributes were subjected to accelerated shelf-life evaluation to determine changes in peroxide value, fat acidity, moisture content and total viable count. Kraft paper, gunny bags and plastic containers were the packaging materials used. All six flours met the Codex Alimentarius food standards for minimum energy density (80 kcal/100g) and maximum fat content (27 %) in processed cereal-based foods used for complementary feeding of infants and young children. They all also met the required > 1050 mg leucine per 100 grams of flour. Formulations 2, 3 and 5 had the most preferred sensory attributes and were thus subjected to accelerated shelf-life evaluation. At the fifth month, fat acidity was least in the flours packaged in plastic containers. There was no peroxide formation in any of the three samples during the storage period. The study generated six formulations that meet the minimum requirement for leucine in treatment of moderate wasting but formulation 3, had the most preferred sensory attributes. It is recommended that formulation three be subjected to a study to further validate its effectiveness in the treatment of moderate wasting prior to release for up-scaled use.

Key words: Leucine supplementation, moderate wasting, therapeutic food, complementary feeding, diet optimization

BY NC ND

https://doi.org/10.18697/ajfand.96.19300

INTRODUCTION

Wasting is a form of malnutrition that is diagnosed in children when their weight for height z-score is below minus two standard deviations from the median of the reference population. The z-score is determined by plotting the child's weight against their height on the World Health Organization Child Growth Standards Chart. It is classified as either: moderate, when the weight for height z-score is greater than or equal to minus 3 but less than minus 2 ($-3 \leq$ z-score < -2), or severe where the z-score is less than minus 3 (z-score < -3) [1]. Wasting represents a depletion of two body compartments: the body's lean tissue and fat mass. Lean tissue is the largest body compartment and thus its rate of loss is the most significant determinant of total body weight in most cases of wasting [2]. Treatment of moderate wasting which affects more than 33 million children globally and approximately 300,000 children in Kenya varies across practice settings [3].

Current treatment methods for moderate wasting include use of lipid-based supplements and corn soy blend flour. Lipid-based supplements, however, do not reduce the risk of advancement to severe wasting or mortality [4]. Corn soy blend has no significant effect on the weight-for-height Z-score of moderately wasted children [5].

Treatment of moderate wasting should be built on a mechanism that accelerates muscle protein synthesis to make up for the significant losses incurred during the accelerated breakdown triggered by malnutrition. Current treatment methods fail to correct moderate wasting because of their emphasis on heavy calorie loading to enable gain in body fat, which is wrongfully assumed to contribute to overall gain in body weight [6].

In a clinical trial conducted on the effectiveness of leucine supplementation in the treatment of moderate wasting in children, it was shown that when administered at a dosage of 150mg/kg bodyweight/day it resulted in recovery (z-score ≥ -2) for majority (93 %) of the study children in the treatment group. Majority of those in the control group (60 %) remained wasted and leucine supplementation was thus proven to be effective in the treatment of moderate wasting [6]. When administered at a dosage of 150mg/kg bodyweight/day, leucine becomes a functional food by activating the mammalian target of rapamycin in muscles which triggers protein translation resulting in accelerated protein synthesis and growth [7]. This is what ultimately contributes to a significant gain in lean body mass and total body weight.

A functional food is defined as a natural, enriched or fortified food which provides therapeutic benefits to the health of the consumer beyond the provision of essential nutrients (for example: proteins, carbohydrates and minerals) when consumed regularly at an efficacious level as part of a diverse and healthy diet [8]. For leucine to act as a functional food by accelerating muscle protein responses, it is a prerequisite that its dosage is higher than that typically found even in high-quality protein food sources [7]. This is because primarily being an amino acid, it must be sufficient in the body to first satisfy all structural roles before it can then engage in signalling and metabolic roles.

As a result, the capacity of leucine to perform as a functional food is based on sufficient intracellular concentration [9]. Leucine supplements are currently used by strength-

CC BY NC ND

https://doi.org/10.18697/ajfand.96.19300

athletes to promote muscle growth and retention after strength training. They are made in either powder or capsule form both of which are expensive and inaccessible to the populations in Kenya that are most affected by wasting. As a result, it is necessary to locally develop a leucine-rich therapeutic food from foods that are rich in the amino acid yet locally available and accessible for use in treatment of moderate wasting. The objective of this study was, therefore, to formulate a leucine-rich composite flour (TheraPEM) from locally available foods for use in the treatment of moderate wasting and determine its nutritional composition, shelf life and sensory acceptability. In order to confer its therapeutic benefits, TheraPEM was required to deliver $\geq$ 1050 mg leucine/kg bodyweight/day to the consumer.

MATERIALS AND METHODS

Product development was carried out in four distinct but interrelated steps that included: nutrient optimization and formulation of composite flours, nutrient composition analysis, sensory evaluation and shelf life evaluation.

Food ingredients

The food ingredients that were selected for use in formulation of the leucine-rich composite flour included: bean (*Phaseolus vulgaris*), groundnut (*Voandzeia subterranea*), and foxtail millet (*Setaria italica*). These ingredients were selected because of their high availability and accessibility in Kenya food markets as they are produced locally by farmers. In addition to this, compared with other locally produced food crops, they have the highest leucine content and relatively lower cost as shown in Table 1.

The food ingredients were procured at Kangemi Market, Nairobi and stored at the Chemistry laboratory at the Department of Food Science, Nutrition and Technology, University of Nairobi.

Formulation and optimization

As shown in Figure 1, the beans were first sorted to remove foreign matter then washed under running water and soaked overnight to reduce the antinutritional factors [11]. Afterwards, the beans were boiled in water for two hours then dried in an air oven at 100^0C overnight after which they were ground to a fine powder using a laboratory hammer mill. The powder was sieved to remove large particles then stored in a clean dry container.

Afterwards, the millet was sorted and sieved to remove all foreign matter after which it was washed under running water. The millet grains were then placed in a tray lined with a moist muslin cloth and covered with another then stored for 28 hours so that they could germinate. Prior to removal from the muslin cloth, the sprouts of the grains were observed, to ensure they had reached a length approximately that of the grain. The grains were then placed in air oven at 100^0C overnight to stop the germination process and dry after which they were ground to a fine powder using a laboratory hammer mill. Germination of millet was done to increase the free amino acids within the grain and

CC BY NC ND

https://doi.org/10.18697/ajfand.96.19300

reduce antinutrients [12]. The powder was finally sieved then stored in a clean dry container.

On completion of this, the groundnuts were sorted to remove all foreign matter then washed under running water. Afterwards, the groundnuts were dried overnight in an air oven at 100^{0}C then roasted to enhance colour, flavour and aroma. The roasted groundnuts were again sorted to remove the burnt groundnuts after which they were ground together using a laboratory hammer mill with the other two ingredients (millet and beans) to a fine powder. This was done to prevent the groundnuts producing peanut butter which occurs when they are ground alone. The flours were stored in dry aseptic plastic containers to avoid contamination prior to mixing in the defined ratios.

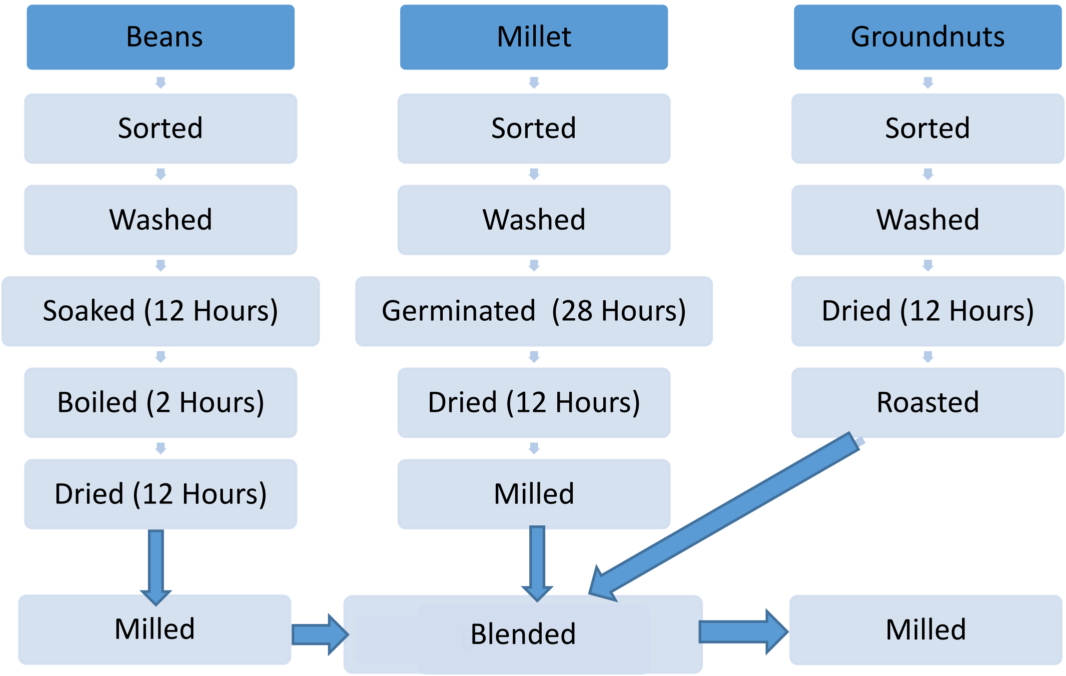

Figure 1: Process flow diagram for the development of the leucine-rich composite flours

A diet optimization model that uses linear programming was used to find unique combination of the three food ingredients (decision variables) that maximized the leucine content of the therapeutic food. The model provided six combinations (Table 2) that met this objective while fulfilling the constraint on the individual leucine content of the food ingredients. Three of these unique combinations were selected to be the leucine-rich therapeutic foods through sensory analysis.

Nutrient composition analysis

Proximate analysis was conducted on each of the six formulations using standard AOAC procedures [13]. Moisture content was determined through drying of the flour samples in an air oven at 105^{0}C whereas crude fat was determined through extraction in a Soxhlet extractor followed by evaporation in a rotary evaporator then drying in an air-oven for one hour at 105^{0}C. Crude protein was determined through boiling a mixture of the food sample and sulphuric acid in a Kjeldahl flask then distillation and back-titration with a sodium hydroxide solution. Total ash was determined by measuring the residue on ignition at 550^{0}C and crude fibre through digesting the food sample in sulphuric acid and sodium hydroxide solutions then ignition of the residue at 550^{0}C [13]. The results obtained were used to calculate the carbohydrates and energy in the formulations using the formula below.

% Carbohydrates = 100 – [% moisture content + % fibre + % ash + % protein + % fat]
Energy (Kcal/100g) = [fat (g/100g) × 9] + [protein (g/100g) × 4] + [carbohydrates (g/100g) × 4]

The branched-chain amino acid profiles of the formulations were determined using acid hydrolysis of the composite flour to release amino acids from the protein. This was followed by pre-column derivatization with omicron-phthaldialdehyde (OPA) for ease of analysis. omicron-phthaldialdehyde in the presence of mercaptan reacted rapidly with primary amino acids to form intensely fluorescent derivatives, which were then separated on a reverse-phase Ultra Performance Liquid Chromatography (UPLC) with fluorescent detection [14].

Sensory evaluation

Sensory attributes of the composite flours were evaluated by 10 trained panelists for: taste, flavour, colour, mouth feel, odour and general acceptability of a porridge made from each of the six flours. The training was specifically for this study to familiarize the panelists with the test procedures and to improve their individual sensitivity and memory. This would contribute to providing consistent, precise and reproducible sensory measurements. Ethical clearance to conduct the study was obtained from the Kenyatta National Hospital – University of Nairobi Ethical Review Committee (P 519/7/2016).
For each of the composite flours, 150 mg was mixed in 250 ml of cold water. The mixture was then poured into 1000 ml of boiling water in a cooking pot and cooked for 15 – 20 minutes, while constantly stirring then finally emptied into a storage jar. Different cooking utensils and storage jars were used to prepare and store each of the six porridges to avoid contamination.

The 10 trained panelists then scored the six sensory attributes of each of the porridges using a seven-point hedonic rating scale where 7 = like very much and 1 = dislike very much. For each porridge, a mean of each sensory attribute was computed and also used to calculate the overall score of the porridge. The composite flours were then ranked based on the number of sensory attributes in which they had the highest mean score with the top three flours being subjected to shelf-life evaluation.

Accelerated shelf-life evaluation

Accelerated shelf-life evaluation was conducted on the composite flours to determine the length of time the product would retain specific desired qualities including: acceptable microbial count, taste, appearance and odour. The formulations were stored in an air oven set at 55°C in three packages: Kraft paper bags, gunny bags and Polyethylene Terephthalate (PET) plastic containers for five days each day representing a month in storage. This accelerated aging time was based on a temperature coefficient of 2.0

cc BY NC ND

https://doi.org/10.18697/ajfand.96.19300

implying that the chemical reaction rate of the composite flours increased by a factor of 2.0 every time the temperature rose by 10°C. This resulted in an accelerated aging time of 37 days for every year of desired aging time. The foods were analysed pre-storage and monitored every day in storage for changes in: fat acidity, peroxide value, moisture content and growth of yeasts and moulds.

Fat acidity was measured as the milligrams of potassium hydroxide required to neutralize the free acid in a one-gram sample of the flour.

Peroxide value was determined by mixing a gram of the flour sample in a solution of potassium iodide and acetic acid followed by titration with a solution of sodium thiosulphate and starch. Moisture content was determined through drying in an air oven at 105^{0}C and weighing of the residue [13]. Yeasts and moulds were enumerated as the Total Viable Counts (TVCs) through the streak-plate technique. This involved serial dilutions of the food samples using a diluent (0.85% sodium chloride and distilled water) followed by streaking on potato dextrose agar in a petri dish [15].

Statistical analysis

Proximate analyses, branched-chain amino acid profiling and shelf-life evaluation were carried out in triplicate ($n = 3$). All values were entered in Microsoft Excel® and uploaded onto GenStat 15th Edition SP1 (32 bit) for analysis to obtain means and standard deviations. A two-way ANOVA was used to analyse the significance of differences ($p < 0.05$) between the six formulations.

Sensory evaluation data ($n = 10$) was entered into Statistical Package for Social Sciences® (SPSS®) Version 20 for analysis where the means and standard deviations were computed for each sensory attribute. An overall mean score for all six of the sensory attributes was also computed.

RESULTS AND DISCUSSION

Proximate composition

As shown in Table 3, there were significant differences ($p < 0.05$) in the fat, crude protein, crude fibre and energy of the six formulations. There were, however, no significant differences in the moisture content and total ash in all six formulations. The moisture content of all six formulations was below the maximum moisture content recommended for composite flours (13.5 %) [16]. Formulation 1, (beans : groundnuts : millet = 5 : 2 : 3) however, had the highest moisture content (5.5 %) as well as the highest ash content (3.0 %) while formulation 4 (beans : groundnuts : millet = 11 : 3 : 6) had the lowest moisture content (4.6 %) but the highest crude fibre (6.6 %). The lower the moisture content of a flour, the higher its shelf life and microbial stability [17].

The Codex Alimentarius International food standards require that processed cereal-based foods for complementary feeding of infants and young children have an energy density of no less than 0.8 kcal/g (80 kcal/100g) and maximum fat content of 27 % [18]. All six formulations met this criterion. Formulation 2 (beans : groundnuts : millet = 3 : 2 : 5), which comprised of 20 % groundnuts, had the highest fat (13.0 %) and energy content

CC BY NC ND

(418Kcal/100g), while formulation 6 which had the lowest fat (5.0 %) and energy content (37.2 kcal/100g) consisted of the least amount of groundnuts (10 %). This is because a 100 g serving of groundnuts contains 49.2 g of total fat which provides 165 % of the Recommended Daily Allowance (RDA) and 567 kcal of energy (29 % of RDA).

Formulation 4, with the highest amount of beans (55 %), had the highest crude protein (17.95 g/100g), while formulation 6 (beans : groundnuts : millet = 3 : 1 : 6), with the lowest amount of beans (30 %) and groundnuts (10 %), had the lowest amount of crude protein (13.4 g/100 g). Groundnuts contain 25.8 % crude protein [19] while the crude protein in beans varies between 15 – 30 % both on dry matter basis [11]. Millet has the lowest amount of protein (12.3 %) [20] and, therefore, groundnuts and beans were ultimately the greatest contributors of crude protein in the formulations.

Branched-chain amino acid profile

All six formulations contained above 1.1 g of leucine (the minimum requirement in moderate wasting) in 100 g of the product. As shown in Table 4, there was no significant difference ($p < 0.05$) in the leucine, isoleucine and valine content of the six formulations. Formulation 6, however, had the highest amount of leucine (1.4 g/100g) and the lowest amount of isoleucine (1.1 g/100g) and valine (0.8 g/100g). This was because it had the highest amount of millet (60 %) and millet was the ingredient used richest in leucine (1.8 g/100g). Formulation 4, which had only 30 % millet had the lowest amount of leucine (1.2 g/100g), but the highest amount of isoleucine (1.4 g/100g) and valine (0.9 g/100g).

It is a requirement that all three branched-chain amino acids (BCAAs) are consumed concurrently since all three compete for cell transport and metabolism. Ingesting leucine alone may lead to depletion of plasma valine and isoleucine nonetheless leucine should be the dominant BCAA [21].

Sensory Evaluation

As shown in Table 5, there were significant differences ($p < 0.05$) in the perceived taste, flavour, colour, mouth feel and general acceptability of the six porridges made from each of the six formulations. The only exception was in the odour of the porridges, where there was no significant difference among all six. Formulation 3, which had a ratio of beans: groundnuts: millet of 2:1:2, had the most preferred taste (5.2 ± 0.9), flavour (5.0 ± 0.9), colour (5.9 ± 0.7), mouth feel (4.6 ± 1.3) and general acceptability (5.4 ± 1.0). It also had the highest overall mean score among all six sensory attributes (5.3).

Formulation 5 (beans : groundnuts : millet = 6 : 3 : 11), which had the highest amount of millet (55 %), had the most preferred odour (5.6 ± 1.2). Formulation 4 had the highest amount of beans (55 %) compared to the other formulations and was the least preferred in taste (2.1 ± 0.9), flavour (3.0 ± 0.9), colour (4.5 ± 1.9), general acceptability (3.8 ± 1.5) and ultimately it had the lowest mean score (3.7 ± 1.0). Formulation 1, with the second highest amount of beans (50 %) had the least preferred mouth feel (2.5 ± 1.6) and odour (4.7 ± 1.6). A high amount of bean flour was, therefore, related to a low acceptability of the sensory attributes of the composite flour while a high amount of millet flour was associated with a desirable odour. Beans contain unsaturated lipids that are susceptible to oxidative degradation and cause the development of off flavours [22].

CC BY NC ND

https://doi.org/10.18697/ajfand.96.19300

A high amount of beans may also cause an interaction of products of this degradation with carbohydrates and proteins present in the flour and this may ultimately affect flavour characteristics of the flour.

A ratio of beans: millet of 1:1, however, gives the composite flour the most desirable sensory qualities. Formulations 2, 3 and 5 were the only ones subjected to shelf-life evaluation since they were the most preferred of the six.

Total Viable Count (TVC)

Growth of yeasts and moulds progressively increased with length of storage. As shown in Table 6, by the third day of storage, the TVCs of all six formulations were significantly different ($p < 0.05$) from their pre-storage value. On the fifth day, formulation 2 packaged in a Kraft paper had the highest number of coliforms (3.2×10^8 CFU/gram). In comparison, formulations 2, 3 and 5 packaged in plastic containers had the lowest number of coliforms compared to the other two packaging materials on the fifth day (1.5×10^8, 7.6×10^7, and 2.8×10^7 CFU/gram, respectively). The gradual but varied increase in the TVC in all three formulations indicated a high nutrient availability. In addition to this, the increase in TVC in storage also indicated favourable environmental conditions such as humidity as well as atmospheric gases including carbon dioxide and oxygen [23]. It is, therefore, necessary to control the permeating of environmental conditions to slow the increase in TVCs, which present food safety concerns of the composite flour. The lower microbial proliferation in the samples stored in the PET plastic container implied a lower permeability to these environmental conditions compared to the other packaging materials.

Fat acidity

As shown in Table 7, after one day of storage, there were significant changes ($p < 0.05$) in the acid value of formulation 2 packaged in a gunny bag and plastic container as well as formulations 3 and 5 packaged in the Kraft paper. Formulations 3 and 5, both packaged in gunny bags only, had significant changes in acid value after three days of storage. In addition, after three days of storage all three formulations except formulation 5 in the Kraft paper still met the minimum acceptable acid value (50 mg KOH/100g) as specified in the East African Community standards for composite flours [16].

On the fifth day of storage, all samples in all the three packaging materials had exceeded the minimum acceptable acid value. Formulation 5 had the highest increase in fat acidity with an increase of +61.4 mg/100g in the gunny bag, +60.9 mg/100g in the Kraft paper and +54.7 mg/100g in the plastic container. Formulation 3 had the lowest increase with an increase of +30.2 mg/100g in the plastic container, +31.7 mg/100g in the gunny bag and +33.1 mg/100g in the Kraft paper. Formulations 2, 3 and 5 stored in the plastic container had the lowest final fat acidity compared to those stored in the other packaging materials (74.6, 57.9 and 83.0 mg/100g, respectively). Fat acidity value increases with increased decomposition of glycerides in the formulations by the action of the enzyme lipase. This process is accelerated during storage due to the presence of heat and light and is an indicator of the condition and edibility of the food [24].

CC BY NC ND

Peroxide value

The peroxide value was used to estimate the overall oxidation status of the fats in the formulations. It measures the hydroperoxides and lipid peroxides formed in the primary phase of oxidation (induction period) [24]. There was no peroxide formation in any of the three samples during the storage period. This was anticipated because peroxide formation during storage is slow at first during the incubation period, which ranges from a few weeks to several months depending on the oils in the food as well as storage temperature. High peroxide value early in storage negatively impacts on the storage stability of the food [24].

Moisture content

Moisture content of the three formulations steadily declined in all packaging materials during storage as seen in Table 8. Formulations 2 and 3, stored in plastic containers, only had significant changes ($p < 0.05$) in moisture content from the pre-storage value on the fifth day. In comparison, formulations 3 and 2, stored in the gunny bag and Kraft paper had significant changes in moisture by the third and fourth days, respectively. Formulation 5 stored in the plastic container had a significant change in moisture content on the fourth day, while that in the gunny bag and Kraft paper significantly changed on the third day. This indicates that the gunny bags and Kraft paper permit a higher rate of moisture content loss in storage compared to the plastic containers. Formulation 5 had the lowest moisture content at day 5, with the samples stored in the gunny bag, plastic container and Kraft paper having 0.0 %, 0.1 % and 0.1 %, respectively. It was also the formulation with the largest decrease in moisture content during storage, with the sample stored in the gunny bag having a change of -7.3 %, that in the plastic container, -7.2 % and that in the Kraft paper, -7.2 %.

Formulation 2 stored in the gunny bag had the smallest change in moisture content (3.4 %) and consequently the highest moisture content on the fifth day (3.0 %). These changes in moisture content reflect the permeability of the packaging materials to environmental conditions in storage, including temperature, which cause evaporative loss of moisture from the flours. The rate of moisture loss would vary in different environmental conditions of storage reflective of the differing climatic conditions in separate regions.

CONCLUSION

All six formulations meet the minimum leucine requirement (1050 g/100 g serving). Each contains sufficient amounts of isoleucine and valine to permit a ratio of the three branched-chain amino acids that would prevent the plasma depletion of either of the three. The formulations meet the minimum energy density and maximum fat content standards for processed cereal-based foods for infants and young children as prescribed by the Codex Alimentarius International Food Standards. Formulation 3 was the most preferred based on sensory attributes.

It is recommended that a feeding trial be conducted to determine the effectiveness of formulation three in the management of moderate wasting in children aged 6 – 24 months.. To slow down the decomposition of the fats by the action of lipase and to slow down microbial (yeasts and moulds) growth, PET plastic containers are recommended

cc BY NC ND

as the most effective packaging material for the composite flours. The study recommends that microbial analysis be conducted on formulation 3 prior to studying its effectiveness in treating moderate wasting. To improve the shelf-stability of the formulations a chemical preservation method should be considered to control growth of yeasts and moulds that is expected beyond the third month of storage.

CC BY NC ND

Table 1: Leucine content and market price of selected foods locally available in Kenya [10]

	Ingredients	Leucine content* (g/kg)	Price (KES/kg)	Price (USD/kg)**
1	foxtail millet (*Setaria italica*)	17.6 ± 6.96	90	0.83
2	Bean (*Phaseolus vulgaris*)	16.9 ± 4.06	90	0.83
3	Groundnut (*Voandzeia subterranea*) whole	13.9 ± 1.98	110	1.02
4	Maize (*Zea mays*) grain or whole meal	11.9 ± 4.60	49	0.45
5	Millet (*Pennisetum spp.*) grain	9.27 ± 4.72	90	0.83
6	Wheat (*Triticum spp.)* whole grain	8.71 ± 2.23	90	0.83
7	Rice (*Oryza spp.*) brown or husked	6.48 ± 1.37	158	1.46
8	Potato (*Solanum tuberosum*)	1.21 ± 0.01	40	0.37
9	Sweet potato (*Ipomoea batatas*)	0.71 ± 0.06	38	0.35
10	Cassava (*Manihot esculenta*) meal	0.64 ± 0.18	26	0.24

*Dry matter basis
** 1 USD = 108.30 KES

Table 2: Ratios of beans, groundnuts and millet used in each formulation

Formulation	Beans (g)	Groundnuts (g)	Millet (g)	Total (g)
1	50	20	30	100
2	30	20	50	100
3	40	20	40	100
4	55	15	30	100
5	30	15	55	100
6	30	10	60	100

cc BY NC ND

Table 3: Proximate composition of the formulations

Formulation	(g in /100g sample)						Energy (Kcal/100g)
	Moisture content	Fat	Crude Protein	Total Ash	Crude Fibre	Carbohydrates	
1	5.5^a ±0.04	10.7^a ±0.05	17.4^a ±0.13	3.0^a ±0.12	3.9^a ±0.03	59.6^a ±0.13	404^a ±0.06
2	5.4^a ±0.13	13.0^b ±0.01	14.5^b ±0.04	2.8^a ±0.09	3.4^a ±0.06	60.9^a ±0.07	419^b ±0.10
3	5.4^a ±0.08	12.1^b ±0.08	16.7^a ±0.12	2.9^a ±0.03	4.5^a ±0.08	58.4^a ±0.15	409^a ±0.15
4	4.6^a ±0.02	9.0^a ±0.07	18.0^a ±0.13	3.0 ±0.15	6.6^b ±0.13	59.0^a ±0.06	388^c ±0.02
5	5.2^a ±0.08	10.8^b ±0.06	17.8^a ±0.13	2.7^a ±0.02	4.8^a ±0.15	58.7^a ±0.04	404^a ±0.12
6	5.1^a ±0.05	5.0^c ±0.02	13.4^b ±0.15	2.8^a ±0.02	5.3^a ±0.15	68.3^b ±0.02	372^c ±0.13

[a] Values in the same column with different lowercase superscript letters are significantly different ($p < 0.05$)

Table 4: Branched-chain amino acid profile of the formulations

Formulation	g/100g product		
	Leucine	Isoleucine	Valine
1	1.2^a ±0.07	1.4^a ±0.02	0.9^a ±0.01
2	1.3^a ±0.04	1.2^a ±0.02	0.9^a ±0.02
3	1.2^a ±0.02	1.3^a ±0.04	0.9^a ±0.01
4	1.2^a ±0.02	1.4^a ±0.07	0.9^a ±0.01
5	1.4^a ±0.04	1.2^a ±0.05	0.9^a ±0.02
6	1.4^a ±0.04	1.1^a ±0.03	0.8^a ±0.04

[a] Values in the same column with different lowercase superscript letters are significantly different ($p < 0.05$)

Table 5: Sensory attributes of the formulations

Formulation	Sensory Attributes (1 – 7)						
	Taste	**Flavour**	**Colour**	**Mouth feel**	**Odour**	**General acceptability**	**Mean score**
1	3.1 ± 1.87^{a}	3.4 ± 1.39^{a}	5.3 ± 1.10^{a}	2.5 ± 1.62^{a}	4.7 ± 1.55^{a}	4.2 ± 1.60^{b}	3.9 ± 1.07^{a}
2	4.3 ± 1.31^{b}	4.7 ± 1.41^{b}	5.3 ± 1.32^{a}	4.1 ± 1.43^{b}	5.2 ± 1.01^{a}	5.1 ± 1.19^{a}	4.8 ± 0.48^{b}
3	5.2 ± 0.94^{b}	5.0 ± 0.88^{b}	5.9 ± 0.68^{a}	4.6 ± 1.28^{b}	5.4 ± 1.48^{a}	5.4 ± 1.03^{a}	5.3 ± 0.42^{b}
4	2.1 ± 0.92^{c}	3.0 ± 0.90^{a}	4.5 ± 1.86^{b}	3.8 ± 1.41^{b}	5.0 ± 1.08^{a}	3.8 ± 1.46^{b}	3.7 ± 1.02^{a}
5	4.2 ± 1.58^{b}	4.3 ± 1.18^{b}	5.5 ± 1.31^{a}	4.5 ± 1.57^{b}	5.6 ± 1.21^{a}	5.1 ± 1.02^{a}	4.9 ± 0.62^{b}
6	3.6 ± 1.46^{a}	4.2 ± 0.93^{b}	5.2 ± 1.59^{a}	3.3 ± 1.83^{a}	4.7 ± 1.62^{a}	4.5 ± 1.36^{a}	4.3 ± 0.68^{a}

^{a} Values in the same column with different lowercase superscript letters are significantly different ($p < 0.05$)

Table 6: Total viable count of formulations 2, 3 and 5 during storage

Sample	Packaging	CFU/gram					
		Day 0	**Day 1**	**Day 2**	**Day 3**	**Day 4**	**Day 5**
2	Gunny bag	1.4×10^{3a}	2.6×10^{3a}	5.7×10^{4a}	7.1×10^{5b}	9.2×10^{7c}	2.1×10^{8c}
	Plastic container	1.4×10^{3a}	2.3×10^{4a}	1.1×10^{4a}	7.9×10^{5b}	1.7×10^{7c}	1.5×10^{8c}
	Kraft paper	1.4×10^{3a}	2.6×10^{3a}	5.0×10^{4a}	1.1×10^{6b}	1.2×10^{7b}	3.2×10^{8c}
3	Gunny bag	1.8×10^{3a}	3.3×10^{3a}	1.1×10^{4a}	8.1×10^{5b}	1.2×10^{7c}	2.2×10^{8c}
	Plastic container	1.8×10^{3a}	6.9×10^{3a}	8.3×10^{3a}	7.2×10^{5b}	6.0×10^{6b}	7.6×10^{7c}
	Kraft paper	1.8×10^{3a}	6.9×10^{3a}	8.8×10^{3a}	4.2×10^{5b}	4.6×10^{6b}	8.9×10^{7c}
5	Gunny bag	2.5×10^{3a}	3.2×10^{3a}	3.9×10^{4a}	8.8×10^{6b}	1.2×10^{7b}	9.3×10^{7b}
	Plastic container	2.5×10^{3a}	2.5×10^{4a}	3.0×10^{4a}	1.2×10^{6b}	1.2×10^{6b}	2.8×10^{7b}
	Kraft paper	2.5×10^{3a}	1.2×10^{4a}	3.5×10^{4a}	3.9×10^{6b}	6.4×10^{6b}	5.2×10^{7b}

^{a} Values in the same row with different lowercase superscript letters are significantly different from the Day 0 value ($p < 0.05$)

Table 7: Free acidity of samples 2, 3 and 5 during storage

Sample	Packaging	Fat acidity (mg KOH/100g)					
		Day 0	**Day 1**	**Day 2**	**Day 3**	**Day 4**	**Day 5**
2	Gunny bag	28^{a} ±1.50	41^{b} ±0.74	43^{b} ±0.77	45^{b} ±0.66	48^{b} ±1.85	81^{b} ±0.75
	Plastic container	28^{a} ±1.51	36^{b} ±1.71	38^{b} ±1.56	42^{b} ±1.78	52^{b} ±1.90	75^{b} ±0.96
	Kraft paper	28^{a} ±1.44	32^{a} ±0.70	35^{b} ±1.78	40^{b} ±1.02	66^{b} ±0.31	76^{b} ±0.28
3	Gunny bag	28^{a} ±0.77	31^{a} ±0.46	34^{a} ±0.80	35^{b} ±0.47	53^{b} ±1.64	59^{b} ±0.78
	Plastic container	28^{a} ±0.36	34^{a} ±0.63	38^{b} ±0.34	45^{b} ±0.99	50^{b} ±0.36	58^{b} ±0.32
	Kraft paper	28^{a} ±1.24	38^{b} ±0.78	43^{b} ±1.66	44^{b} ±0.53	45^{b} ±1.47	61^{b} ±0.30
5	Gunny bag	28^{a} ±0.33	29^{a} ±0.18	33^{a} ±0.56	39^{b} ±0.21	72^{b} ±1.62	90^{b} ±0.84
	Plastic container	28^{a} ±0.45	34^{a} ±0.81	47^{b} ±0.34	48^{b} ±1.74	50^{b} ±1.17	83^{b} ±0.97
	Kraft paper	28^{a} ±1.20	37^{b} ±1.22	40^{b} ±0.28	51^{b} ±0.24	56^{b} ±1.52	89^{b} ±0.98

[a] Values in the same row with different lowercase superscript letters are significantly different from the Day 0 value ($p < 0.05$)

Table 8: Moisture content of Formulation 2, 3 and 5 during storage

		Moisture Content (%)					
Formulations	**Packaging**	**Day 0**	**Day 1**	**Day 2**	**Day 3**	**Day 4**	**Day 5**
2	Gunny bag	6.4^{a}	5.0^{a}	4.7^{a}	3.5^{a}	3.4^{b}	3.0^{b}
	Plastic container	6.4^{a}	5.2^{a}	4.6^{a}	4.5^{a}	4.0^{a}	1.0^{b}
	Kraft paper	6.4^{a}	5.7^{a}	5.2^{a}	4.2^{a}	2.7^{b}	1.1^{b}
3	Gunny bag	6.4^{a}	4.8^{a}	4.7^{a}	3.4^{b}	1.5^{b}	0.5^{b}
	Plastic container	6.4^{a}	4.6^{a}	4.5^{a}	4.2^{a}	4.1^{a}	0.3^{b}
	Kraft paper	6.4^{a}	4.5^{a}	4.2^{a}	3.1^{b}	1.5^{b}	0.8^{b}
5	Gunny bag	7.3^{a}	5.8^{a}	5.3^{a}	2.6^{b}	1.9^{b}	0.0^{b}
	Plastic container	7.3^{a}	6.1^{a}	5.1^{a}	4.8^{a}	3.3^{b}	0.1^{b}
	Kraft paper	7.3^{a}	5.3^{a}	4.5^{a}	4.3^{b}	2.5^{b}	0.1^{b}

[a] Values in the same row with different lowercase superscript letters are significantly different from the Day 0 value ($p < 0.05$)

CC BY NC ND

REFERENCES

1. **WHO.** World Health Organization. Global Nutrition Targets 2025: Policy Brief Series. WHO, Geneva, 2014.

2. **Porth CM, Gaspard KJ and KA Noble** Disorders of Nutritional Status. **In:** Essentials of Pathophysiology. Philadelphia, PA: Lippincott Williams and Wilkins, 2011: 228 – 232.

3. **FAO, IFAD, UNICEF, WFP and WHO**. United Nation's Food and Agriculture Organization, International Fund for Agricultural Development, United Nations Children's Fund, World Food Programme and World Health Organization. The State of Food Security and Nutrition in the World 2017. FAO, Rome, 2017.

4. **Lazzerini M, Rubert L and P Pani** Specially Formulated Foods for Treating Children with Moderate Acute Malnutrition in Low- and Middle-Income Countries. *Cochrane Database Syst. Rev*. 2013; **6**: 4 – 6.

5. **Thakwalakwa C, Ashorn P, Phuka J, Cheung YB, Briend A, Puumalainen T and K Maleta** A Lipid-Based Nutrient Supplement but not Corn-Soy Blend Modestly Increases Weight Gain among 6 to 18 Month Old Moderately Underweight Children in Rural Malawi. *Nutr. J*. 2010; **140**(**11**): 2008 – 2013.

6. **Wamiti J, Kogi-Makau W, Ngala S and FE Onyango** Effectiveness of Leucine Supplementation in the Management of Moderate Wasting in Children. *JFND*. 2018; **4**(**1**): 1023 – 1029.

7. **Glynn EL, Fry CS, Drummond MJ, Timmerman KL, Dhanani S, Volpi E and BB Rasmussen** Excess Leucine Intake Enhances Muscle Anabolic Signalling but not Net Protein Anabolism in Young Men and Women. *Nutr. J*. 2010; **140**(**11**): 1970–1976.

8. **Tur JA and MM Bibiloni** Functional Foods. *Encyclopaedia of Food and Health*. **2016:** 157- 161.

9. **Churchward-Venne TA, Burd NA, Mitchell CJ, West DW, Philp A, Marcotte GR, Baker SK, Baar K and SM Phillips** Supplementation of Suboptimal Protein Dose with Leucine or Essential Amino Acids; Effects on Myofibrillar Protein Synthesis at Rest and Following Resistance Exercise in Men. *J. Physiol*. 2012; **590**(**11**): 2751 – 2765.

10. **FAO.** United Nation's Food and Agriculture Organization. Amino-Acid Content of Foods and Biological Data On Proteins. FAO, Rome, 1981.

CC BY NC ND

https://doi.org/10.18697/ajfand.96.19300

11. **Martino HS, Bigonha SM, Cardoso LM, Rosa CO, Costa NM, Cárdenas LL and SM Ribeiro** Nutritional and Bioactive Compounds of Bean: Benefits to Human Health. **In**: Tunick MH and de Mejia EG (Eds). Hispanic Foods: Chemistry and Bioactive Compounds. Washington DC, USA: Oxford University Press, 2012: 233 – 258.

12. **Ahmed SM, Qing Z, Jing C and S Qun** Millet Grains: Nutritional Quality, Processing, and Potential Health Benefits. *Compr. Rev. Food Sci. Food Saf.* 2013; **12**(**3**): 284 – 285.

13. **AOAC.** Association of Official Analytical Chemists. Official Methods of Analysis of the Association of Official Analytical Chemists. AOAC, Maryland, 2005.

14. **FAO.** United Nation’s Food and Agriculture Organization. Food Energy – Methods of Analysis and Conversion Factors. FAO, Rome, 2003.

15. **ISO.** International Organization for Standardization. Horizontal Method for the Enumeration of Yeasts and Molds—Colony Count Technique. ISO, Geneva, 2001.

16. **EAC.** East African Community. East African Standard: Composite Flour – Specification. EAC, Arusha, 2012.

17. **Chukwu O and H Abdullahi** Effects of Moisture Content and Storage Period on Proximate Composition, Microbial Counts and Total Carotenoids of Cassava Flour. *Int. J. Innov. Sci. Eng. Technol.* 2015; **2**(**11**): 753 – 763.

18. **Joint FAO/WHO Codex Alimentarius Commission**. United Nation’s Food and Agriculture Organization/World Health Organization. Codex Alimentarius: Standard for Processed Cereal-Based Foods for Infants and Young Children. FAO/WHO, Rome, 2017.

19. **Arya SS, Akshata RS and S Chauhan** Peanuts as Functional Food; A Review. *J. Food Sci. Technol.* 2016; **53**(**1**): 31 – 41.

20. **Sharma N and K Niranjan** Foxtail Millet: Properties, Processing, Health Benefits and Uses. *Food Rev. Int.* 2017; **34**(**4**): 329 – 363.

21. **Cynober L, Bier DM and M Kadowaki** A Proposal For an Upper Limit of Leucine Safe Intake in Healthy Adults. *Nutr. J.* 2012*;* **142**: 2249-2250.

22. **Sessa DJ and JJ Rackis** Lipid-Derived Flavors of Legume Protein Products. *J. Am. Oil. Chem. Soc.* 1977; **54**: 468 – 473.

23. **Adejumo BA and AO Raji** Microbiological Safety and Sensory Attributes of Garri in Selected Packaging Materials. *Acad. res. Int.* 2012; **3**: 2– 8.

cc BY NC ND

24. **Eldin AK** Methods to Determine the Extent of Lipid Oxidation in Foods. **In**: Decker EA, Elias RJ and McClements DJ (Eds). Oxidation in Foods and Beverages and Antioxidant Applications: Understanding Mechanisms of Oxidation and Antioxidant Activity. Sawston, UK: Woodhead publishing, 2010: 181 – 195.

BY NC ND