

Amino Acid Composition of Cowpea Grains Preserved With Mixtures OF Neem (*Azadirachta indica*) and Moringa (*Moringa oleifera*) Seed Oils

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Abstract Dry cowpea grains are important in Africa but its storage has posed a serious challenge to farmers and researchers. Various bio-insecticides have been used for the preservation of cowpea grains in Africa, yet little effort has been made to ascertain their effects on the nutritional quality of the cowpea grains. The effect of mixture of neem and moringa seed oils used for the preservation of cowpea grains on its amino acid composition was investigated. The seed oils were extracted traditionally and applied on cowpea grain at concentrations of (2.5 µl/g and 5.0 µl/g) in ratio 1:2 and 1:3. The treated cowpea grain samples were stored for a period of 270 days. The amino acid composition of the treated cowpea grains was determined at the end of every 90 days. The result showed that the mixture of neem and moringa seed oils in ratio 1:3 and at a concentration of 5.0 µl/g significantly ($P<0.05$) improved the concentration of amino acids composition of the treated cowpea while no significant ($P<0.05$) difference in the concentration of the amino acid composition of cowpea samples treated with ratio 1:3/2.5 µl/g of the mixture of these oils. There was significant improvement in the concentrations of all the amino acids present as storage period progressed, especially at 180 days of storage. The study concludes that the mixtures of these oils especially ratios 1:3/2.5 µl/g and 1:3/5.0 µl/g may be nutritionally good for cowpea preservation.

Keywords: cowpea, preservation, storage, bio-insecticide, amino acid composition

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1. Introduction

Cowpea (*Vigna unguiculata* (L.) Walp) is a drought tolerant food crop, well adapted in varieties of climates and soils. This crop is widely cultivated throughout the tropics and subtropics, particularly in west and central Africa. Cowpea, one of the grains that suffer postharvest losses most, is a warm season, annual, herbaceous legume. It suffers heavily from insects, both in the field and when grains are stored after harvest. Yield reductions caused by insects can reach as high as 95%, depending on location, year, and cultivar [1].

Although insecticides are widely available, they require expensive equipment and training for their use. They are expensive, polluting, and potentially dangerous to users. Consequently, many cowpea growers in Africa do not use insecticides because they cannot obtain them; they cannot afford them; they do not have the necessary equipment or they are not taught how to apply them properly. That is why conventional insecticides are not the answer to the insect problems [1] Insecticides, especially the dust and gaseous forms are recommended for short-term storage. The product Actellic (2%) or Actellic super and Phostoxin gas are very helpful to the farmer, but they are expensive

and may not be available everywhere. Phostoxin is a fumigant that can kill humans and animals [2].

Botanical preparations have been used for the protection of stored produce by small scale farmers in oriental countries like India where the extracts of *Azadirachta indica* (Neem) have been used extensively. Neem oil with the main constituent of Azadirachtin is used as insect repellent, feeding inhibitors, egg laying deterrents, growth retardants and sterilants among others. It has both contact and systemic action on plants for controlling fungal diseases [3].

The successes in the use of this and other plant products by small scale farmers may serve as impetus for exploring the utilization of indigenous plant based extracts for small scale product protection and for possible industrial scale applications.

Moringa oleifera, (Moringaceae) commonly referred to as moringa is the most widely cultivated species of the genus *Moringa*. It belongs to the family Moringaceae. The *Moringa* seeds yield 38 to 40% edible oil. It is resistant to rancidity, which may be because it contains powerful antioxidants that act as natural preservatives.

The concept of using neem oil for preservation of cowpea is not new but the fact still remains that it lacks consumer acceptability especially in terms of taste, when used as preservative. The use of bio-insecticides for the

control of storage insect pests by small scale farmers is anchored on their availability, affordability, safety, quality and effectiveness. Neem as a botanical insecticide has been used traditionally in Asia and Africa for controlling pests of stored products [4]. Farmers usually mix neem leaves with grain before storage for several months. Neem leaves, oil or extracts act as repellent against several insects such as weevils, flour beetles, bean-seed beetles and potato moths [5].

There is an increasing world demand of less expensive proteins with good nutritional and functional properties, particularly in developing and under-developed countries where the supply of food of animal origin is limited due to non-availability and high cost [6]. This situation has resulted from constant increase of the human population and growing interest for protein by industries for application in food and non-food markets.

Cowpeas are an important source of protein in developing countries, especially in West Africa where they are eaten in a variety of ways. Variability in protein content of cowpea grains has been reported to be in the range of 23 to 30% and is influenced by genotypes as well as environmental factors [5]. Like other legumes, cowpeas contribute to the level of dietary protein in starchy tuber-based diets through their relatively high protein content and to the quality of dietary protein by forming complementary mixtures with staple cereals. Lysine content is relatively high making cowpea an excellent improver of the protein quality of cereal grains [7]. Legumes are considered as poor man's meat. However, the protein in cowpea, just like other food legumes, is low in sulfur-containing amino acids. This deficiency is definitely important when the diet is based on root crops or starch foods, and diets based on cereal grains. Increase in sulfur – containing amino acids of cowpea protein would be nutritionally beneficial [8]. Addition of methionine to cowpea protein increases the protein quality significantly, demonstrating the nutritional value of increasing sulfur amino acid content in cowpea protein [9]. The addition of a small amount of cowpea ensures the nutritional balance of the diet and enhances the protein quality by the synergetic effect of high protein and lysine from cowpea and high methionine and energy from the cereals [7]. This nutritious and balanced food ensures good health and enables the body to resist infectious diseases and slows down their development [10]. The high protein content represents a major advantage in the use of cowpea in nutritional products, for infants and children's food, and to compensate for the large proportion of carbohydrates often ingested in African diets [1]. Cowpea is especially rich in lysine, but is low in sulfur amino acids. Compared to other legumes, methionine and tryptophan levels in cowpea grains are high [11,12]. Under storage conditions, there are tendencies for nutritive values and indeed the bio-chemical composition of the stored grains to change over time conditions [13]. This may be as a result of respiration, attack by insects or some storage conditions [13]. It is not clear what effect mixtures of neem-moringa seed oils will have on the amino acid composition of cowpea grains treated with mixtures of the oils and stored for up to 270 days. Therefore, the objective of this study was to assess the effect of the mixture of neem and moringa seed oils treatment on the amino acid profile of cowpea grains within the storage period.

2. Materials and Methods

The materials required for this study were: cowpea (*Vigna unguiculata* (L.) Walp.) grains; neem (*Azadirachter indica* A. Juss) seeds; Moringa (*Moringa oleifera*) seeds. The neem seeds used in this study were hand-picked from the premises of Modibbo Adama University of Technology (MAUTECH) Yola, Adamawa State. The moringa seeds were also obtained partly from MAUTECH, Yola premises and also from Kaltungo (Kaltungo Local Government area of Gombe State, Nigeria) where it is commonly found and sold. The cowpea sample (Ife brown) used for this study was obtained from a farm within the University.

2.1. Experimental Design

The treatment comprised the mixture of neem and moringa seed oils in ratios of 1:2 and 1:3 at a concentration of 2.5µl/g and 5.0µl/g of cowpea grains. These were arranged in a 1 x 2 x 3 factorial in Completely Randomized Design (RCD) and were replicated 3 times. The untreated cowpea sample was used as control.

Neem and Moringa seeds oils were extracted traditionally according to the methods of Ilesanmi and Gungula [14] and cowpea (Ife Brown) grains samples were treated with two concentrations (2.5 µl/g and 5.0 µl/g) of mixture of neem and moringa seed oils in ratios 1:2 and 1:3. The treated cowpea samples were stored for a period of 270 days. During this period the amino acid composition of the treated cowpea samples were determined at the end of every 90 days of storage period.

2.2. Sample Preparation

After every 90 days of storage, treated cowpea samples were milled into flour using Hammer Mill and packaged in polythene bags for the determination of amino acid profile.

2.3. Determination of Amino Acids Profile of Treated and Untreated Cowpea

The Amino acid profile of the samples was determined using a well-known standard procedure [15]. The samples were dried to constant weight, defatted, hydrolyzed, evaporated in a rotary evaporator and loaded into the Technicon sequential Multi-Sample Amino Acid Analyzer (TSM) which is designed to separate, detect and quantify amino acids by the development of chromatogram. The area under each peak was calculated, the concentration of each amino acid was expressed as g/16 gN to the equivalent of g/100 g protein on dry weight basis.

2.4. Determination of Nutritional Parameters

Biological value of cowpea seed was determined on the basis of its amino acid profile. Chemical score was calculated according to Block and Mitchell [16]. The content of each essential amino acid in test protein was expressed as a percentage of the content of the same amino acid in a standard protein. Amino acid was calculated using the FAO/WHO [17,18] reference pattern. The amino acid showing the lowest percentage was called the "limiting amino acid" representing the chemical score.

Essential amino acid index (EAAI) was calculated according to Oser [19] using the amino acid composition of the whole egg protein [20].

Protein efficiency ratio (PER) was estimated according to the regression equation reported by Alsmeyer *et al.* [21].

Predicted protein efficiency ratio (P-PER) was computed as follows:

$$P - PER = -0.468 + 0.454 (\text{Leucine}) - 0.105(\text{Tyrosine})$$

The net protein value (NPV) was calculated by multiplying the lowest amino acid score by the percent of protein divided by 100 using the formula:

$$NPV = (\text{The lowest amino acid score} \times \% \text{ protein}) / 100$$

2.5. Statistical Analysis

Analysis of variance was used to ascertain the significant differences between means. Least significant differences (LSD) test was used to compare means that were significantly different at $p < 0.05$ using generalized linear model procedure of the SAS/STAT[®] software Release 9.2 [22].

3. Results

3.1. Effect of Mixture of Neem and Moringa Seed Oils on the Amino Acid Composition of Stored Cowpea Grains

The effect of treatment of cowpea grains with various levels of mixtures of neem and moringa seed oils on its amino acid composition is presented in Table 1. The concentration of alanine in grains treated with ratios 1:2/2.5 and 1:3/5.0 $\mu\text{l/g}$ neem–moringa seed oils significantly ($p < 0.05$) increased. At ratio 1:3/5.0 $\mu\text{l/g}$ neem–moringa seed oils had the highest concentrations of all the amino acids present in the sample followed by 1:2/2.5 $\mu\text{l/g}$ while the cowpea grains treated with ratio 1:2/5.0 $\mu\text{l/g}$ neem–moringa oils had the least concentration of most of the amino acids present in the samples. Generally, amino acids concentrations in the treated samples were significantly higher than the control samples. From results, glutamic acid content was no significant differences ($p < 0.05$) of the treated grains and the control.

Table 1. Amino acid profile of cowpea grains as affected by various concentrations of neem and moringa seed oil after 270 days of storage

Treatment	Amino acids and their concentrations (g/100g protein on dry weight basis)								
	Alanine	Aspartic acid	Arginine	Cystine	Glutamic acid	Glycine	Histidine	Isoleucine	
Control	3.74	9.18	7.04	1.65	13.44	3.53	2.70	3.82	
1:2/2.5 $\mu\text{l/g}$	4.22	9.30	6.96	1.63	13.85	3.70	3.17	3.74	
1:2/5.0 $\mu\text{l/g}$	3.87	8.93	6.58	1.57	12.96	3.40	3.11	3.52	
1:3/2.5 $\mu\text{l/g}$	3.67	8.97	6.62	1.56	13.08	3.33	2.67	3.53	
1:3/5.0 $\mu\text{l/g}$	4.33	9.73	7.33	1.75	13.71	3.89	3.62	3.92	
Mean	3.97	9.22	6.91	1.63	13.41	3.57	3.05	3.71	
LSD at 0.05	0.23	0.14	0.27	0.07	0.94	0.17	0.09	0.20	
Prob. of F	<0.001	<0.001	<0.001	<0.001	0.26	<0.001	<0.001	<0.001	

Treatment	Amino acids and their concentrations (g/100g protein on dry weight basis)								
	Leucine	Lysine	Methionine	Phenyl alanine	Proline	Serine	Threonine	Tyrosine	Valine
Control	7.24	6.19	1.41	5.31	3.16	3.28	3.47	3.08	5.14
1:2/2.5 $\mu\text{l/g}$	7.35	6.20	1.45	5.09	3.26	3.43	4.00	3.29	5.16
1:2/5.0 $\mu\text{l/g}$	7.01	6.24	1.34	4.92	3.02	3.19	3.56	2.90	4.86
1:3/2.5 $\mu\text{l/g}$	6.94	5.91	1.36	4.98	3.02	3.17	3.32	2.86	4.92
1:3/5.0 $\mu\text{l/g}$	7.67	6.75	1.53	5.49	3.47	3.53	4.30	3.36	5.29
Mean	7.24	6.26	1.42	5.15	3.19	3.32	3.73	3.10	5.08
LSD at 0.05	0.18	0.12	0.07	0.13	0.13	0.13	0.21	0.12	0.17
Prob. of F	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001

All values are means of triplicates.

Table 2. Mean Effect of Storage Period on the Amino Acid Profile of Cowpea Grains Treated with a Mixtures of Neem and Moringa Seed Oils

Period in days	Amino acids and their concentrations (g/100g protein on dry weight basis)								
	Alanine	Aspartic acid	Arginine	Cystine	Glutamic acid	Glycine	Histidine	Isoleucine	
90	3.62	8.81	6.56	1.45	13.07	3.21	2.70	3.37	
180	4.18	9.43	7.08	1.73	13.58	3.75	3.22	3.87	
270	4.10	9.37	6.70	1.58	13.64	3.71	3.13	3.78	
Mean	3.97	9.20	6.88	1.58	13.43	3.56	3.02	3.67	
LSD at 0.05	0.06	0.12	0.19	0.06	0.74	0.13	0.08	0.16	
Prob. of F	<0.001	<0.001	<0.001	<0.001	0.236	<0.001	<0.001	<0.001	

Period in days	Amino acids and their concentrations (g/100g protein on dry weight basis)								
	Leucine	Lysine	Methionine	Phenyl alanine	Proline	Serine	Threonine	Tyrosine	Valine
90	6.81	5.77	1.24	4.80	2.89	3.05	3.28	2.79	4.71
180	7.46	6.51	1.51	5.32	3.34	3.46	3.96	3.25	5.26
270	7.48	6.38	1.47	5.01	3.26	3.32	3.64	3.25	5.21
Mean	7.25	6.22	1.41	5.04	3.16	0.10	3.63	3.10	5.06
LSD at 0.05	0.13	0.09	0.05	0.09	0.11	0.118	0.14	0.23	0.15
Prob. of F	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001

All values are means of triplicates.

3.2. Effect of Storage Period on the Amino Acid Composition of Cowpea Treated with Mixture of Neem and Moringa Seed Oils

The effect of storage period on the amino acid composition of cowpea treated with mixture of neem and moringa seed oils and untreated cowpea (control) is presented in Table 2. There were significant increases in

the concentration of the amino acids of grain samples as storage period progressed. The amino acids concentrations were however found to be highest in all samples at the end of 180 days of storage and then, tended to decline at the end of 270 days although there were no significant differences between the values obtained at 180 days and that of 270 days.

Table 3. Interaction between Storage Period and Mixtures of Neem and Moringa Seed Oils on the Amino Acid Profile of Stored Cowpea Grains

Period in days	Control	1:2/2.5 μ l/g	1:2/5.0 μ l/g	1:3/2.5 μ l/g	1:3/5.0 μ l/g	Mean	LSD at 0.05	Prob. of F
Alanine								
90	3.86	3.01	3.24	3.63	4.35			
180	3.91	4.77	3.89	3.85	4.48			
270	3.44	4.89	4.49	3.54	4.15	3.97	0.36	<.001
Aspartic Acid								
90	9.16	8.00	8.37	8.81	9.70			
180	9.18	9.95	9.21	9.05	9.75			
270	8.97	10.52	9.63	8.46	9.26	9.20	0.26	<0.001
Arginine								
90	6.92	5.73	6.19	6.70	7.26			
180	7.10	7.58	6.78	6.58	7.37			
270	6.76	7.82	7.05	6.62	6.74	6.88	0.43	<0.001
Cystine								
90	1.59	1.10	1.27	1.50	1.76			
180	1.69	1.90	1.72	1.59	1.74			
270	1.34	1.99	1.77	1.37	1.41	1.58	0.14	<0.001
Glycine								
90	3.49	2.59	2.97	3.14	3.87			
180	3.55	4.25	3.61	3.42	3.90			
270	3.35	4.52	3.80	3.15	3.74	3.56	0.29	<0.001
Histidine								
90	2.66	2.07	2.34	2.72	3.72			
180	2.69	3.72	3.50	2.64	3.57			
270	2.53	3.77	3.63	2.38	3.33	3.02	0.17	<0.001
Isoleucine								
90	3.88	2.82	2.74	3.35	4.04			
180	3.77	4.20	3.90	3.63	3.86			
270	3.33	4.51	3.93	3.49	3.62	3.68	0.35	<0.001
Leucine								
90	7.15	6.04	6.48	6.75	7.63			
180	7.30	8.01	7.28	7.03	7.70			
270	6.94	8.55	7.68	7.01	7.21	7.25	0.30	<0.001
Lysine								
90	6.12	5.01	5.35	5.71	6.67			
180	6.25	6.80	6.68	6.01	6.79			
270	5.90	7.08	6.82	5.85	6.26	6.22	0.20	<0.001
Methionine								
90	1.41	0.92	1.06	1.25	1.56			
180	1.41	1.72	1.48	1.41	1.51			
270	1.25	1.86	1.61	1.28	1.36	1.41	0.11	<0.001
Phenyl alanine								
90	5.24	4.00	4.40	4.84	5.52			
180	5.33	5.63	5.18	5.04	5.41			
270	4.92	5.51	5.06	4.72	4.83	5.04	0.20	<0.001
Proline								
90	3.18	2.34	2.55	2.90	3.47			
180	3.16	3.72	3.26	3.08	3.47			
270	3.02	3.84	3.49	2.88	3.09	3.16	0.24	<0.001
Serine								
90	3.35	2.52	2.77	3.11	3.47			
180	3.24	3.89	3.39	3.20	3.56			
270	3.06	4.30	3.56	2.96	3.39	3.32	0.23	<0.001
Threonine								
90	3.50	2.77	2.96	3.22	3.92			
180	3.48	4.61	3.86	3.37	4.50			
270	3.13	4.83	3.91	3.07	3.26	3.63	0.30	<0.001
Tyrosine								
90	2.90	2.36	2.58	2.79	3.33			
180	3.16	3.76	3.05	2.90	3.38			
270	2.86	3.81	3.54	3.02	3.02	3.10	0.23	<0.001
Valine								
90	5.11	3.91	4.32	4.84	5.36			
180	5.15	5.78	5.13	4.97	5.26			
270	4.83	5.89	5.51	4.79	5.04	5.06	0.32	<0.001

All values are means of triplicates.

3.3. Interaction between Storage Periods and Mixture of Neem and Moringa Seed Oils on the Amino Acid Composition of Stored Cowpea

The interaction between the period of storage and ratios of neem–moringa seed oil treated cowpea grains on amino acid composition of stored cowpea grains is presented in Table 3. At 90 days of storage, ratio 1:3/5.0 $\mu\text{l/g}$ treated grains recorded the highest concentrations of all the amino acid profile investigated followed by untreated grains and the least values present were from treated grains with ratio 1:2/2.5 $\mu\text{l/g}$ neem-moringa seed oil. At 180 days of storage, ratio 1:2/2.5 $\mu\text{l/g}$ neem-moringa seed oil treated grains recorded the highest concentrations of all the amino acid present in the sample followed by samples treated with ratio 1:3/5.0 $\mu\text{l/g}$. The least values were recorded from samples treated with ratio 1:3/2.5 $\mu\text{l/g}$. At 270 days in storage, however, ratio 1:2/2.5 $\mu\text{l/g}$ treated grains had the highest alanine content (4.89 g/100 g) followed by ratio 1:2/5.0 $\mu\text{l/g}$ treated grains (4.49 g/100 g). The least alanine content (3.54 g/100 g) at the end of 270 days in storage was from ratio 1:3/2.5 $\mu\text{l/g}$ treated cowpea grains.

The same trend observed at 90 days of storage was observed for Aspartic acid, Arginine, Cystine, Glycine, Histidine, Isoleucine and Leucine content of the stored cowpea grains, as the interaction between the 90 days period of storage and ratio 1:3/5.0 $\mu\text{l/g}$ treated grains recorded the highest values for Aspartic acid (9.70 g/100 g), Arginine (7.26 g/100 g), cysteine (1.76 g/100 g), Glycine (3.87 g/100 g), Histidine (3.72 g/100 g), Isoleucine (4.04 g/100 g) and Leucine (7.63 g/100 g). A similar trend was also observed for Lysine, Methionine,

Phenylalanine, Proline, Serine, Threonine, Tyrosine and Valine at 90 days of storage period as the interaction between period of storage and ratio 1:3/5.0 $\mu\text{l/g}$ treated cowpea grains recorded the highest values for each of the aforementioned amino acids.

At 180 and 270 days in storage, ratio 1:2/2.5 $\mu\text{l/g}$ treated cowpea grains recorded the highest content of Aspartic acid, Arginine, Cystine, Glycine, Histidine, Isoleucine and Leucine. Conversely, ratio 1:3/2.5 $\mu\text{l/g}$ treated cowpea grains within the same period recorded the lowest values for Aspartic acid, Arginine, Cystine, Glycine, Histidine, Isoleucine and Leucine.

Similarly, ratio 1:2/2.5 $\mu\text{l/g}$ treated cowpea grains followed the same trend as earlier presented for 180 and 270 days of storage as it recorded the highest content of Lysine, Methionine, Phenylalanine, Proline, Serine, Theronine, Tyrosine and Valine. However the lowest values at these periods for the aforementioned amino acids were from ratio 1:3/2.5 $\mu\text{l/g}$ treated cowpea grains samples.

3.4. Effect of Preserving Cowpea with Mixture of Neem and Moringa Seed Oils on Its Nutritional Quality

The effect of preserving cowpea grains with mixtures of neem and moringa seed oils on its nutritional quality is presented in Table 4. The cowpea preserved with ratio 1:3/5.0 $\mu\text{l/g}$ of the mixture of these oils had the highest 97.74%, 2.65, 2.66, 73.04%, 18.29 and 92.73%. Essential Amino acid Index (EAAI) Protein Efficiency Ratio (PER₁, PER₂) Biological Value (BV), Net Protein Value (NPV) and chemical score respectively. The least is from cowpea preserved with ratio 1:3/2.5 $\mu\text{l/g}$.

Table 4. Nutritional Parameters of Cowpea Preserved with Mixtures of Neem and Moringa Seed Oils

Treatment	Chemical Score (%)	EAAI (%)	PER		BV (%)	NPV
			PER ₁	PER ₂		
Control	88.18	71.27	2.47	2.50	65.98	17.38
1:2/2.5 $\mu\text{l/g}$	90.90	73.18	2.51	2.52	68.07	24.64
1:2/5.0 $\mu\text{l/g}$	91.82	69.74	2.37	2.41	64.74	20.21
1:3/2.5 $\mu\text{l/g}$	91.82	67.93	2.34	2.38	62.34	17.79
1:3/5.0 $\mu\text{l/g}$	92.73	77.74	2.65	2.88	73.04	18.27

EAAI, Essential Amino Acid Index

PER, Protein Efficiency Ratio calculated according to Alsmeyer *et al.* (1974) equation

BV, Biological Value

NPV, Net Protein Utilization.

4. Discussion

The ranges of all amino acid concentration in this study are in the range of values reported by other workers who studied on the chemical composition and nutritional values of a cowpea protein concentrate [15,23,24]. This implies that neem-moringa oils used for the storage did not cause a major deviation in the in the chemical composition of the grains after the 270 days of storage.

The protein quality or the nutrient value of food depends on its amino acid content and on the physiological utilization of specific amino acid after digestion, absorption and utilization observed significant improvement in the amino acid concentration of samples treated with ratio 1:3/5.0 $\mu\text{l/g}$, mixture of neem and

moringa seed oils compared with other samples confirms an earlier report [25] that moringa is very rich in amino acids. Cowpea grains treated with ratio 1:3/2.5 $\mu\text{l/g}$ favorably compared with the control even at this, the concentrations of many of the amino acids slightly improved, further confirming the findings of an earlier study [11]. Generally, higher lysine and leucine, lower tryptophan and sulphur amino acids contents are common to most legume proteins [12].

However at the end of 180 and 270 days, the samples treated with ratio 1:2 neem – moringa seed oils showed significant increase in amino acid concentrations. This sudden increment could be attributed to infestation (presence of *Callosbruchus maculatus* eggs and larvae in the samples) because the moringa oil concentration was low and so the cowpea samples were attacked by *Callosbruchus maculatus*. This finding is in line with earlier studies where it was

reported that low concentration of moringa oil translates to lower behenic acid (the active ingredient) in the moringa oil and the potent antibiotic and fungicide terygosperrin content in moringa oil [12,26,27]. This perhaps could be the substance responsible for the preservation effect of cowpea grains against *Callosobruchus maculatus*.

Generally, at 90 days of storage, the interaction between the period of storage and ratio 1:3/5.0 $\mu\text{l/g}$ treated grain samples recorded the highest amino acid content while the interaction between the period of storage and ratio 1:2/2.5 $\mu\text{l/g}$ recorded the least amino acid contents. At 180 and 270 days of storage, interaction between the storage period and ratio 1:2/2.5 $\mu\text{l/g}$ recorded the highest amino acid contents. This affirmed that ratio 1:3/5.0 $\mu\text{l/g}$ mixture of neem and moringa seed oils appeared to be the best for cowpea grains preservation as it improved the amino acids content of the stored cowpea grains at 90 days (before infestation sets in). However, the high amino acid contents observed in ratio 1:2/2.5 $\mu\text{l/g}$ treated cowpea grains at 180 – 270 days could be because of the eggs and larvae of cowpea bruchid infestation, so it is an apparent increment not real. The results of protein content in the proximate composition from this study shows increased protein content even in samples that *Callosobruchus maculatus* was effectively controlled as a result of the treatment with the neem and moringa seed oils. Based on this study it appears as if the treatment oils had no adverse effect on the amino acids concentration of cowpea.

The nature and quantity of amino acids contained in a dietary protein determines the efficiency with which an organism could use the protein. The quality of proteins, based on chemical score, as assessed in comparison with the FAO/WHO [18] recommended pattern of essential amino acids indicated that the limiting amino acids for all the treatments was tryphen. This is common to most legumes including cowpea [12,13].

Protein efficiency ratio of cowpea treated with mixtures of neem and moringa seed oils were in the range of those reported for beach pea (2.75 – 2.81) and higher than those reported for lupin protein extracts (2.22 – 2.32) [28,29]. Essential amino acid index (EAAI) and Biological value (67.93-77.74) and 62.34-73.04) respectively are in the range reported for cowpea flour (74.27 and 69.26) [23] these results showed that the use of these mixtures of oils in the preservation of cowpea does not affect its protein quality.

5. Conclusion

The study concluded that treatment of cowpea with ratio 1:3/5.0 $\mu\text{l/g}$ mixture of neem and moringa seed oils significantly ($P < 0.05$) improved the amino acids concentration of cowpea grains. Therefore, it is recommended that ratio 1:3 mixtures of neem and moringa seed oils should be used for cowpea preservation for maximum nutritional benefits.

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