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Effects of Storage Media and Duration on Nutritional Qualities of Cowpea (*Vigna unguiculata* L.Walp)

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Abstract: *The assessment of the level of effectiveness of storage media for storage of cowpea and level of nutrient deficiency of cowpea was investigated. The storage media used in the study are; fridge, freezer, airtight container, dry chilli pepper, phostosine and control experiment. Cowpea seeds were stored in each medium for 4 months and at every 4 weeks interval, proximate analysis test was carried out on cowpea in each storage media to determine the level of their protein, fat and moisture content. Analysis of variance (ANOVA) of two factor Completely Randomized Design (CRD) was used to evaluate the effect and significance of each level of duration of storage and storage media on percent moisture content, protein and fat and oil at alpha (α) = 0.05. The effect of duration of storage, storage media and interaction effect between duration and storage media on percent moisture content, protein and fat and oil of the cowpea stored were statistically analyzed using Duncan's Multiple Range Test (MDRT) at P < 0.05. The traditional method using pepper was observed to be most effective. It recorded the highest value of nutrient at the end of each period of storage. It has 9.2% moisture content, 2.3% fat and 25.10% protein at the 4th weeks of storage. At 16th weeks of storage it recorded 8.42% moisture content, 2.13% fat and 22.53% of protein. Equally, the phostosine method is also very effective, it recorded 8.86% moisture content, 2.81% fat and 23.03% protein at 4th weeks and 9.83% moisture content, 2.54% fat and 21.41% protein at 16th weeks. The storage media can be recommended based on the ability of the media to retain desired nutritive content for a required duration of storage, also cowpea that is to be stored must be kept under optimum moisture content of 12–13% and the storage should favour both environmental and room temperature.*

Keywords: *cowpea, storage media, storage duration, nutritional qualities.*

I. Introduction

Cowpea (Vigna unguiculata L. Walp.) is warm-season, annual, herbaceous legume categorized as erect, semi-erect, prostrate (trailing), or climbing depending on the variety. Cowpea originated in Africa and it's widely grown in Latin America, Southeast Asia and Southern part of United States. Nigeria is the center for world-wide collection and testing of cowpea germplasm (Broker, 1967). It is chiefly used as a grain crop for animal fodder, or as a vegetable. Worldwide cowpea production has increased dramatically in the last 25 years, United States production of dry cowpea has declined from 3/4 million acres to a few thousand over the same period. Cowpea seed is valued as a nutritional supplement to cereals and an extender of animal proteins (Ivbijaro, 1986).

Cowpea can be harvested at three different stages of maturity: green snaps, green-mature, and dry. Depending on temperature, fresh-market (green-mature) peas are ready for harvest 16 to 17 days after bloom (60 to 90 days after planting). Harvest date for green snap pods is normally specified by the processor. Harvested green cowpea exposed to heat tends to spoil unless kept cool. Post-harvest handling of cowpea must provide shade and adequate ventilation necessary to keep temperature at a low level. Cowpeas cooled below 7.2^{o}C may show chilling injury. Seed is cleaned, graded, fumigated and packed in small plastic bags for sale to consumers (Ehler, 1997).

Cowpea is a major staple food in Nigeria and serves as a source of protein in most developing countries. The storage of cowpea is essential to avoid loss, make the crop available throughout the year and seed preservation for planting (Adejumo and Raji, 2007). Cowpea possesses intrinsic properties that must be preserved in other to meet the nutritive value of the consumer. Common storage structures for cowpea include: Earthenware Granaries (Rhumbus), steel drums/tins, polythene bags, silos and pit method (Igbeka, 1983). Storage conditions may influence the production, yield and the grain quality. Mijinyawa (2002) classified postharvest losses of cowpea into quantitative, qualitative, nutritional, loss of seed viability and commercial loss which may be attributable to many factors like temperature, moisture content, relative humidity, oxygen and carbon dioxide.

Cowpea consists average 24.8%, protein, fat 1.9%, fibre 6.3% carbohydrates 63.6%, thiamine 0.00074%, Riboflavin 0.0042% and Niacin 0.00281%. The protein in cowpea seed is rich in amino acid, lysine and tryptophan and is most nutritious when compared to cereal grains (Bressain,1985). The effect of storage media on the storage of cowpea is essential in to prevent losses, retain essential nutrient and ensure maximum quality. The main objectives of this study is to assess the level of effectiveness of various storage media and level of nutrient deficiency of cowpea due to storage. .

II. Materials And Methods

The main materials used for this study include: white plastic container, polythene bags, paper tape, nylon tape, dried pepper, phostosine, cowpea, sealing machine, weighing balance and stapler.

2.1 Sample Preparation and Storage Media

Brown-eyed cowpea variety was used for this study, viable and healthy seeds were purchased from market Bodija market in Ibadan, Oyo State, Nigeria. The seeds were cleaned and sorted based on quality fractions. The study was carried out at Biology/Livestock laboratory of the Federal College of Agriculture, Institute of Agricultural Research and Training, Moor Plantation Ibadan. Different storage media were used for the study including fridge, freezer, big plastic containers with tight lids and small plastic containers with threaded covers. The big plastic containers are white and made up of synthetic resinous materials. The big plastic containers have tight cover while the small plastic containers have threaded covers.

2.2 Treatment of Samples

Cowpea seeds were placed in sealed polythene bags of 150g each and stored in fridge, freezer and plastic containers according to the following procedures:

i. Four sample bags, each containing 150g of grains, stapled at the mouth were labeled T_1A (1-4 weeks), T_1B (5-8 weeks), T_1C (9-12 weeks), T_1D (13-16 weeks) were stored in the fridge.
ii. Four other samples, each containing 150g of grains, stapled at the mouth and labeled TF_1A, TF_1B, TF_1C, and TF_1D were stored in the freezer.
iii. Four sample bags, sealed at mouth with a sealing machine, were placed in plastic containers which were sealed and labeled T_2A, T_2B, T_2C, T_2D were stored in a room at a room temperature. The plastics were nylon taped at the cover to make it airtight.
iv. Four sample bags, each with 2.0g of phostosine were placed in four plastic containers and labeled PH_3TA, PH_3TB, PH_3TC, and PH_3TD and then stored at room temperature.
v. 2.0g of dry pepper each was placed in four sample bags which was put in four plastic containers labeled PT_3A, PT_3B, PT_3C, PT_3D and then stored at room temperature.
vi. Four samples bags, without pepper and phostosine placed in four plastic containers and labeled P_4A, P_4B, P_4C, P_4D used as control were stored in the same room as above.

Samples were stored accordingly in the six (6) storage media for four months; samples were taken from each storage media three times per month for proximate analysis thus making a total of 72 experimental units.

2.3 Proximate Analysis of Stored Cowpea

The proximate analysis of the cowpea was carried out in order to detect the effects of the storage media and duration on the nutritional qualities of cowpea. At the end of each month, each treatment was taken out for analysis. The samples were grinded before the chemical analysis was carried out, the following parameters were determined:

i. **Crude Protein:** determined by the routine semi-micro kjeldah procedure/technique consisting of three techniques of analysis namely Digestion, Distillation and Titration. For the digestion, 0.5g of each finely ground dried sample was weighed carefully into the Kjeldahl digestion tube. To this was added 1 Kjeldahl catalyst tablet and 10ml of conc. H_2SO_4. These were set in the appropriate hole of the Digestion. Block heaters in a flame cupboard. The digestion was left on for four hours, after which a clear colourless solution was left in the tube. The digest was cooled and carefully transferred into 100ml volumetric flask, thoroughly rinsing the digestion tube with distilled water and the flask was made up to mark with distilled water. The distillation was done with Markham Distillation apparatus which allows volatile substances (ammonia) to be steam distilled with complete of the distillate. The 5ml portion of the digest above was then added to 5ml of 40%(W/V) NaOH through opening of 5ml pipettle. The mixture was steam-distilled for 2 minutes into a 50ml conical flask containing 10ml of 2% Boric Acid plus indicator Solution changes colour from red to green. Distillation was done with Markham distillation apparatus which allow volatile substances such as ammonia to be steam distilled with complete collection of the distillate. The apparatus was steam out for about 10 minutes. The steam generator is then removed from the heat source to allow the developing vacuum to remove condensed water. The titration process involves obtaining a green colour

solution which was then titrated against 0.01N HCL contained in a 50ml Burette. At the end point, the green-colour turns to wine which indicates that all the Nitrogen trapped as Ammonium Borate $(NH4)_2BO_3$ have been removed as ammonium Chloride (NH4Cl). The percentage nitrogen in this analysis was calculated using the formula

$$\% \text{ N} = \frac{\text{titre value} \times \text{molarity of HCL used} \times \text{atomic mass of N}}{\text{volume of flask containing the digest}} \times 100 \quad (1)$$

The crude protein content was determined by multiplying percentage Nitrogen by a constant factor of 6.25 (Lynch and Fleming, 1999).

ii. **Moisture determination:** this was determined using 2g sample weighed crucible. The crucible plus sample taken was then transferred into oven to dry to a constant weight for 24 hours. At the end, the crucible plus sample was removed from the oven and transferred to dessicator and cooled (10 minutes). The weight of empty crucible is W_0, Weight of crucible plus sample is W_1, Weight of crucible plus oven dried sample W_3

$$\% \text{ moisture} = \frac{W_1 - W_3}{W_1 - W_0} \text{ x } 100 \quad (2)$$

iii. **Crude fat Determination:** 1g of each dried sample was weighed into fat free extraction thimble and pug lightly with cotton wool. The timble was placed in the extractor and fitted up with reflux condenser. The soxhlet flask was then filled to ¾ of its volume with petroleum ether (b.pt. 40-60^{0}C) and the soxhlet flask extractor plus condenser set was placed on the heater. The heater was put on for six hours with constant running water from the tap for condensation of ether vapour. The ether is left to siphon over several time stay over about at least 10-12 time until it is short of siphoning. It is after this is noticed that any ether content of the extractor is carefully drained into the ether stock bottle. The thumble containing sample is then removed and dry on a clock glass on the bench top. The extractor flask and condenser is replaced and the distillation continues until the flask is practically dry. The flask which now contains the fat or oil is detached, its exterior cleaned and dried to a constant weight in the oven. If the initial weight of dry soxchlet flask is Wo and the final weight of oven dried flask toil + fat is W_1, percentage fat is obtained by the formula:

$W_1 - Wo \text{ x } 100$.

2.4 Statistical Method for Analysis

Analysis of variance (ANOVA) of two factor Completely Randomized Design (CRD) was used to evaluate the effect and significance of each level of duration of storage and storage media on percent moisture content, crude protein and fat and oil of cowpea used for the experiment at alpha (α) = 0.05. The effect of duration of storage, storage media and interaction effect between duration and storage media on percent moisture content, protein and fat and oil of the stored seeds were statistically analyzed using Duncan's Multiple Range Test (MDRT) at $P < 0.05$.

III. Results And Discussion

The result of the effect of each level of duration of storage and storage media on percent protein, fat and moisture content in the cowpea needed for the experiment are presented in Tables 1 – 6:

Table 1: Analysis of Variance (% Protein)

Source	Degree of freedom	Sum of square	Mean square	F calculated	F (α =0.05) Tabulated
Storage	5	34.84457361	6.96891472	232.68	2.0001
Period	3	56.22494861	18.74164954	625.76	2.0001
Storage period*	15	1.47367639	0.09824509	3.28	0.9999
Error	48	1.43760001	0.02995000		
Total	71	93.98079861			

Table 2: Analysis of Variance (% Fat and Oil)

Source	Degree of freedom	Sum of square	Mean square	F calculated	F (α =0.05) Tabulated
Storage	5	14.51896111	2.90379222	8278.27	<.0001
Period	3	65.7003708	0.22362593	2252.58	<.0001
Storage period*	15	32.8782208	0.00016481	225.48	0.9999
Error	48	0.05526667	0.00115139		
Total	71	15.24757778			

Table 3: Analysis of Variance (% Moisture Content)

Source	Degree of freedom	Sum of square	Mean square	F calculated	F (α =0.05) Tabulated
Storage	5	402.4159069	80.4831814	8278.27	<.0001
Period	3	65.7003708		2252.58	<.0001
Storage period*	15	32.8782208	2.1918814	225.48	<.0001
Error	48	0.4666667	0.0097222		
Total	71	501.4611653			

Table 4: Mean Effect of Duration of Storage on Protein, Fat, Moisture Content

Storage duration	N (OBS)	% Protein	% Fat	% Moisture content
1-4 weeks	18	23.6361111	1.9883333	10.7588889
S. D.		0.7985070	0.4682540	1.4050158
S. E.		0.1882099	0.1103685	0.3311654
5-8 weeks	18	22.8000000	1.79027778	11.2672222
S. D.		0.7700191	0.4616824	2.4456030
S. E.		0.1814952	0.1888196	0.5764342
9-12 weeks	18	21.9922222	1.8177778	12.2572222
LSD		0.7129356	0.4607610	2.7850775
S. E.		0.1680405	0.1086024	0.6564491
13-16 weeks	18	21.2722222	1.7288889	13.2494444
S. D.		0.6943432	0.4612373	3.1498076
S. E.		0.1636583	0.1087147	0.7424168
Total mean	72	22.4251389	1.8594444	11.8831944
L. S. D. (0.05)		1.1505100	0.4634162	2.6575992
S. E.		0.1355889	0.0546141	0.3132011

Table 5: Effect of Storage Media on % Protein, % Fat and Oil, % Moisture Content

Storage media	N (OBS)	% Protein	% Fat	% Moisture content
Fridge (T_1)	12	21.7183333	1.4975000	13.8391667
S. D		0.9265560	0.1032319	1.4635353
S. E		02674737	0.298005	0.4224863
Freezer (TF_1)	12	22.8175000	1.5850000	14.7000000
S. D		1.0526687	0.734408	1.5980727
S. E		0.3038793	0.0298608	0.4613239
Airtight (T_2)	12	22.1583333	1.5083333	13.0141667
S. D		0.9328435	0.0985193	1.3930768
S. E		0.2692887	0.284401	0.4021466
Pepper (PT_3)	12	23.8016667	2.2633333	8.5408333
S. D		1.0105429	0.1097380	0.4140149
S. E		0.2917188	0.0316786	0.1195158
Phostosine (PHT_3)	12	22.0500000	26741667	8.8641667
S. D		0.7252836	0.1066394	0.7230046
S. E		0.2093714	0.0307842	0.2087134
Control (T_4)	12	21.9250000	1.6283000	12.3408000
S. D		0.9959783	0.1084463	1.2936520
S. E		0.2875140	0.0313058	0.3734452
Total mean	72	22.42513888	1.859444433	11.88319445
LSD (0.05)		0.9406455	0.105002616	1.147559383
S. E		0.271541016	0.030311666	0.331271866

Table 6: Mean for the Interaction Effect between Duration of Storage and Storage Media on % Protein, % Fat and Oil and % Moisture Content

Week	Storage media	% Protein	% Fat & Oil	%Moisture content
4^{th}	Fridge	45.43444	3.48583	24.59806
	Freezer	46.45361	3.57333	25.45889
	Air tight	45.79444	3.49666	23.77306
	Pepper	47.43778	4.25166	19.29972
	Phostosine	45.68611	4.6625	19.62306
	Control	45.56111	3.61666	23.09972
8^{th}	Fridge	44.59883	3.40028	25.10639

	Freezer	45.617	53.48778	25.96722
	Air tight	44.95833	3.41111	24.28139
	Pepper	46.60167	4.16611	19.80805
	Phostosine	44.85	4.57695	20.13139
	Control	44.725	3.53111	23.60805
12^{th}	Fridge	43.79055	3.31528	26.09639
	Freezer	44.80972	3.40278	26.95722
	Air tight	44.15055	3.32611	25.27139
	Pepper	45.79389	4.08111	20.79805
	Phostosine	44.04222	4.49195	21.12139
	Control	43.07055	3.44611	24.59805
16^{th}	Fridge	43.07055	3.48583	27.08861
	Freezer	44.08972	3.31389	27.94944
	Air tight	43.43055	3.23722	26.26361
	Pepper	45.07389	3.99222	21.79027
	Phostosine	43.32222	4.40306	22.11361
	Control	43.19722	3.35722	25.59027
LSD	(0.05)	0.2581	0.0506	0.147
S. E.		0.0599	2.302	0.019444

3.1 Discussions

a) Percentage of Protein Content

Table 1 shows that there is significance difference at (α=0.05) in the effect of each level of duration of storage and that of storage media percentage of protein in cowpea used for the experiment. The result of Duncan's test as shown in Table 4 indicates that the cowpea stored for just 4 weeks obtained the highest % protein (23.636%), it was observed also that the longer the duration of storage the lower the percentage of protein. The cowpea stored for 16 weeks retained the lowest percentage of protein (21.27%). This is in tandem with Hellevong (2000) who reported that protein deteriorates with time as a result of microbial activities. Table 5 indicates that cowpea stored freezer and traditional (pepper) storage media produced the highest percentage of protein. The presence of pepper and temperature of freezer in the storage media must have created a non-conducive environment for insect infestation. This infestation can leads to deterioration in the protein contents and the nutritive value of the stored cowpea.

b) Percentage (%) Fat and Oil

It was discovered that the effect of duration of storage and storage media on fat of cowpea stored were significantly different at level of (P<0.05 or 0.001) i.e. f-calculated is greater than f-tabulated at 5% significant difference (Table 2). The result of Duncan's test shown in Table 4 shows that the cowpea sample stored for just 4 weeks retained the highest fat (1.988), indicating that most of the fat in the cowpea were still retained for 8weeks and the least fat content was observed on the cowpea stored for 16 weeks. It was also observed that the longer the duration of storage, the lower the percentage fat retained. It was presumed that the fats of cowpea were depreciated by microbial feeding actions taking place inside the tissue of cowpea as the duration increases. The experiment proved that the fat of the cowpea stored reduced with time. Table 5 shows that pepper storage retained the highest percentage of fat followed by phostosine and control storage media. Freezer, fridge and airtight retained the least fat content. This indicates that pepper, phostosine and control created non-conducive environment for enzymatic and microbial activities inside the cowpea to deteriorate the fat constituents.

c) Percentage of Moisture Content

From the analysis of variance on Table 3, f-calculated is greater than f-tabulated and this implies that there is significant difference in the effect of each level of duration of storage and storage media on percentage moisture content in cowpea used for the experiment. The result of Duncan's test shown in Table 4 reveals that the cowpea stored for 4 weeks produced the lowest moisture content (10.75%), followed by this are the cowpea stored for 8weeks, next was 12 weeks and the highest moisture content was recorded on the cowpea stored for 16 weeks. It is deduced that the longer the duration of storage the higher the percentage of moisture content retained. This effect was due to the enzymatic reaction in the tissue of the cowpea and the respiratory activities of the cowpea which were favoured by the storage media. From Table 5, freezer storage and fridge storage

produced the highest percentage of moisture content, followed by airtight, control and pepper retained the least moisture content.

d) Effects of Storage Duration on Nutritional Qualities of Cowpea

Duration of storage also has significant effects at level of ($P<0.05$) on protein fat and moisture content of the stored cowpea. As the duration of storage increases the protein content decreases. The 4^{th} week of storage recorded the highest amount of protein (23.64%) while the 16^{th} weeks of storage recorded the lowest amount of protein content 21.27% (Table 4). The fat also decreases in the same trend as in protein with 4^{th} week recorded highest value of (1.988%) and the least value of 1.728% (Table 4) 16^{th} week of storage. The increase in moisture content with increase duration of storage is responsible for the decrease in protein and fat content of the stored cowpea, since high moisture content of the grains aids spoilage (Sopade et al., 1995). At the 4^{th} week of storage, the moisture content increased to 10.75% (Table 4). Increasing moisture was found to give room for microbial infestation and enhances the activities of weevil, these organisms feed on the cowpeas and hence, decreases to nutritive value (Adeyoye and Ashama, 1996).

e) Effects of Storage Media on Nutritional Qualities of Cowpea

The storage media used have various effects on the nutritive value of the stored cowpeas. The storage media has significant effect at level of ($P<0.05$) on protein and fat content ($P<0.05$) of the stored cowpea and here is significant effect on moisture content of the stored cowpeas. The additional storage pepper recorded the highest protein contain 23.80%. Table 5 and phostosine storage recorded the highest fat content 2.674%. (Table 5) while the control storage recorded the least number of protein 21.925% and cold storage in fridge recorded least amount of fat 1.498% (Table 5).

f) Interaction Effect

Table 6 shows that cowpea stored for 4^{th} week with pepper and phostosine storage media retained the lowest percentage of moisture content while cowpea store for 16 week in freezer storage media retained the highest percentage of moisture content at ($P<0.05$). Cowpea stored for 4 weeks with pepper storage media retained the highest percentage of protein content followed by phostosine while airtight storage media at 16 weeks has the lowest protein content. Cowpea stored for 4 weeks with pepper and phostosine storage media retained the highest percentage of fat those of 16 weeks, stored in airtight storage media retained the lowest percentage of fat content.

IV. Conclusion

The traditional method using pepper was observed to be most effective. It recorded the highest value of nutrient at the end of each period of storage. It has 9.2% moisture content, 2.3% fat and 25.10% protein at 4^{th} weeks of storage. At 16^{th} weeks of storage it recorded 8.42% moisture content, 2.13% fat and 22.53% of protein. Equally, the phostosine method is also very effective, it recorded 8.86% moisture content, 2.81% fat and 23.03% protein at 4^{th} weeks and 9.83% moisture content, 2.54% fat and 21.41% protein at 16^{th} weeks. It was also observed that all the storage media are good but the traditional method is more effective than the others for the period employed in this course of study.

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