Nutrient and Anti-Nutrient Composition of Cowpea (Vigna Biflorus) Flours Produced from Germination and Fermentation Process

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ABSTRACT

Background: *Cowpea is a legume that is extensively grown across Africa. It is an important cheap non-animal protein. Processing of cowpea into flour could reduce post-harvest pest damage, improve nutrient quality and increase diversity its uses. Although these practice are often underutilized in Nigeria.*

Objective: *The study evaluated the Nutrient and Anti-nutrient composition of cowpea (Vigna biflorus) flours produced from germination and fermentation process.*

Methods: *The cowpea harvested from a farm in Ovoko, Igbo-Eze South LGA of Enugu State, Nigeria. Cowpea grains were divided into three portions: unprocessed; germinated only; germinated and fermented for 48hrs. After the three portions of the cowpea were separately, washed, drained, sundried and milled into flours. Unprocessed cowpea flour (UCF); germinated cowpea flour (GCF); germinated and fermented cowpea flour (GFCF). The samples were subjected to nutrients and antinutrients analysis using standard methods. Data were analyzed using Statistical Product for Service Solution (SPSS) version 22. Statistical analysis was done using Analysis of Variance (ANOVA) and Duncan's multiple range tests was used to compare the means.*

Results: *Results showed that GFCF had higher protein (20.99%); Fat (2.88%), Fibre (3.29%) and least carbohydrate (68.43%), while UCF had higher ash content (2.98%). GFCF had higher zinc (0.12mg), iron (3.93mg), calcium (3.52mg) and phosphorous (68.90mg). GFCF had reduced in Phytate 4.33mg; Tannins (1.43); Hydrogen Cyanide (4.31); Oxalate (3.55); but increased in Saponin (0.13), respectively.*

Conclusions: *This study shows that germination and fermentation process of cowpea flour improve the nutrient content and reduces the carbohydrate and anti nutrient contents of the cowpea flour.*

Keywords: Cowpea flour, Germination, Fermentation, Nutrients

INTRODUCTION

Cowpea (*Vigna Unguiculata*) is an important legume that is extensively grown throughout Sub-Saharan Africa. Its grain contains about 23 to 32% protein [1] and 50 to 60% carbohydrate [2, 3], and serve as a cheap source plant protein for human consumers. Cowpea originated and was domesticated in southern Africa and was later moved to east and west Africa and Asia. Nigeria is the largest producer and consumer of cowpea, accounts for 61% of nearly 5.2 million tons produced in Africa and 58% of more than 5.4 million tons of dried cowpea produced worldwide

[4, 5]. Cowpea which is *agwa* in Igbo, *ewa* in Yoruba and *wanke* in Hausa language is among the important food crop that is commonly consumed as protein source food and apparently underexplored for commercial health food production [6]. Majority of the millions of African farmers that grow cowpea are women. More than half (52%) of Africa's production is used as food, 13% as animal feed, 10% for seeds, 9% for other used, while16% is wasted [7]. Cowpea is used both as a vegetable and grain, provides an inexpensive source of rich protein, carbohydrate, fiber, vitamins and minerals in the diet [8]. Despite the high nutritional value of cowpea, it is consumed as minor component of food diet and underutilized as food products due to its undesirable taste, cooking difficulty, long cooking time, and waste of recourses.

Food fermentation enriches food substrates with protein, essential amino acids, and vitamins; and the diet through development of a diversity of flavors, aromas, and textures in food substrates; eliminate anti nutrients; reduces the post harvest loss, cooking difficulties and provide a desirable taste on the food product [9]. Fermentation and germination are traditional food processing technique used to produce ogi [9] from sorghum, maize or millet; garri or fufu from cassava; ogiri from mellon seed, castor oil seed, which are widely consumed across West Africa [10] and in Nigeria. Fermentation and germination of food not only enhance nutrient availability and reduce anti-nutritional factors [11] but also reduced post harvest losses and shelf life of the food. Cowpea grain is suscptible to high post harvest pest damage, which could lead to increas in the use of pesticides to prolonge the shelf life and reduce post harvest pest damage. Ground cowpea is a favorite and traditional processing technique recently used in some households to produce cowpea flour. Cowpea flour is less susceptible to post-harvest pest damage and can be used in many different dishes thus enhancing food security between harvests [12]. Economic importance of cowpea could be through export and marketing of its products, job creation and income generation. Although it is often underutilized in many homes and commercial eating-houses in Nigeria. This cowpea flour has created convenience in the diverse use of this food product as food in Nigeria, it has also created job for people that produce the flour[5.6]. The challenge in the use of cowpea flour despite its rich source of nutrients such as starch, protein, fibre, B-group vitamins and some minerals are the anti nutritional factors and the bean flavor it has. Germination (Sprouting) is a traditional non-thermal process of improving the quality such as increasing nutrient, digestibility, reducing the level or activities of anti nutritional compounds in cereals and pulses. Improving the nutrient quality of the cowpea through germination and combine germination and fermentation of cowpea and production of flours from its products have not been fully explored. Hence, the study evaluated the nutrients and antinutriets composition of cowpea *(Vigna bifiorus*) *orarudi* flour produced from germination and fermentation process.

MATERIALS AND METHODS

Procurement of materials

The cowpeas (*Vigna biflorus*) also known as *oraludi* used for the study were harvested from a non commercial farm purposesively selected from farms in Ovoko, Igbo-Eze South Local Government Area in Enugu State, Nigeria. The *orarudi* was harvestedwith hand in month of July about 90 days after planting and when the pods turned brown. The pods were sundried, split the pods to collect the grains, cleaned and dried the grains.

Processing of the cowpea

The cowpeas (*orarudi*) were divided into three portions: unprocessed; germinated 72 hrs only; germinated for 72hrs and fermented for 48hrs, then de-hulled, washed, drained, sundried and milled into flour using grinding machine in the Department of Nutrition and Dietetics, University of Nigeria Nsukka. The three flour products were packaged in an airtight containers as: unprocessed cowpea flour (UCF); germinated cowpea flour (GCF); germinated and fermented cowpea flour (GFCF). The three flour samples were subjected to nutrients and antinutrientsl analysis using standard methods.

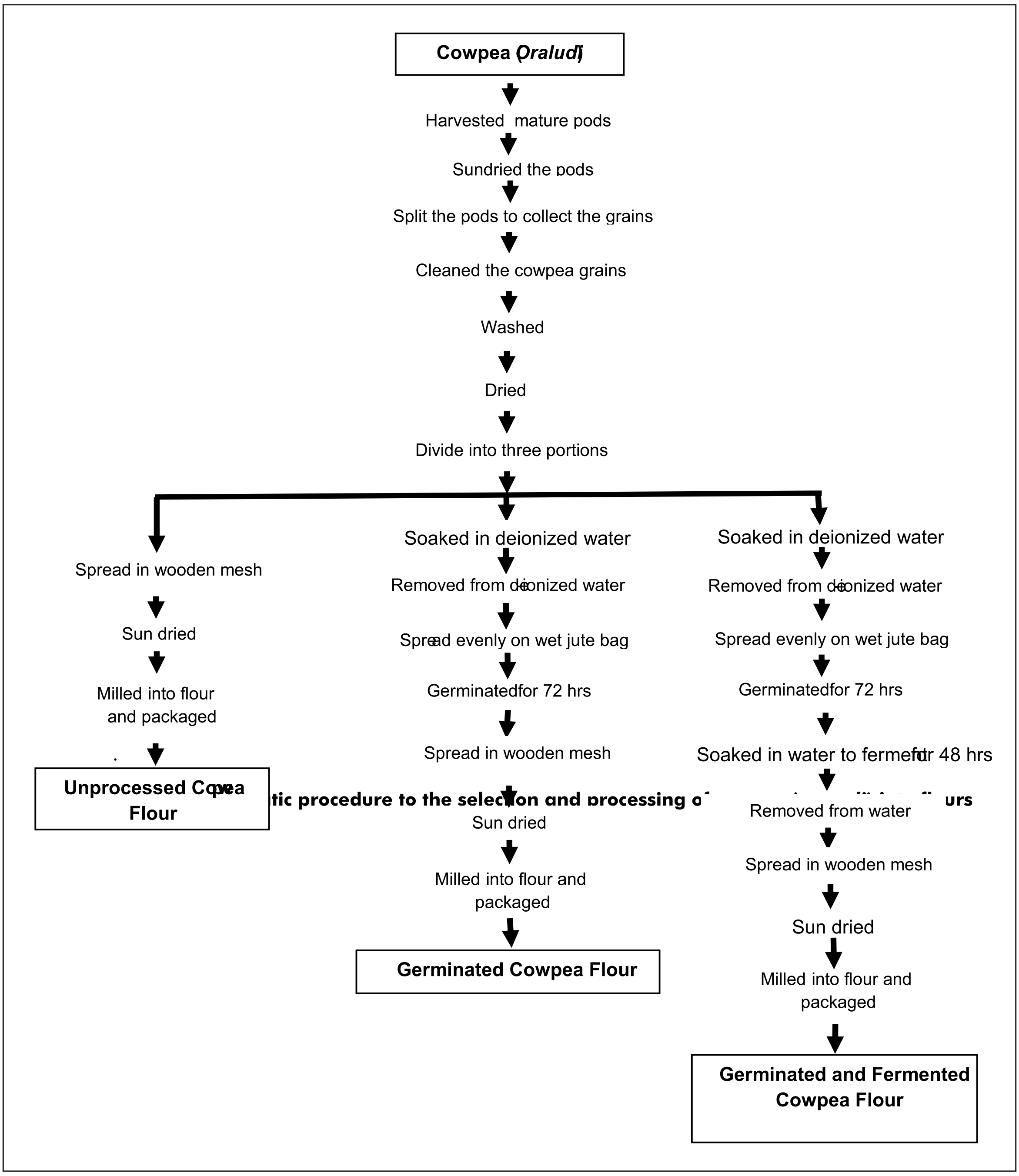

Figure1: Systematic procedure to the selection and processing of cowpea (orarudi) into flours

Nutrients and antinutrients analysis

Proximate analysis:

The protein, crude fibre, crude fats and ash contents of the cowpea flour samples were determined using standard chemical methods described by Association of Official Analytical Chemistry [13]. Protein content of the samples was determined using Kjedahl method as described by AOAC –[14. 15].. Fat content determination was done by Soxhlet extraction technique using petroleum ether (40-50°C) as described by Folch et al. –[16]. The Ash content was determined using incineration of 2 g of each sample in a muffle furnace at 500°C for 2 hours as described by AOAC [13]. The crude fibre was determined by method of AOAC International, [13] and McCleary & Prosky, [17]. The carbohydrate content of the flour samples was determined by difference, after addition of all the percentages of moisture, crude protein, ash, fat, and crude fibre and was subtracted from 100%. This gave the amount of nitrogen free extract otherwise known as carbohydrate content. Carbohydrate (%) = 100% - [Moisture (%) +Crude Protein (%) + Ash (%) + Fat (%) + Crude fibre (%)].

Minerals content of cowpea flour samples

Mineral compositions of the cowpea flour samples were determined according to methods recommended by AOAC International [13, 15, 18] using absorption spectrophotometer. Iron, zinc, calcium and magnesium contents were analyzed by Atomic Absorption Spectrophotometer [19, 20, 21]. All the analyses were in triplicate determinations.

Determination of anti-nutrient properties of cowpea flours

The anti-nutrient contents of cowpea flour samples subjected to difference processing methods such as fermentation and germination methods were determined with the following methods: Phytate determination was carried out by the method of Makar & Becker [22]. Tannin was determined by the method of Kirk & Sawyer[23]. Oxalate was determined by spectrometric method by Dye, [24]. Saponin was determined by the modified method of Makkar & Becker, [22]. Hydrogen cyanide (HCN) was determined by the method of AOAC International [13].

Statistical analyses

The data generated were analyzed using Statistical Product for Service Solution (SPSS) version 22 software. Analysis of variance (ANOVA) was used to determine the mean differences of the chemical values of the flour samples while Duncan multiple range test was used to separated and compared the means at significant level of P= 0.05.

RESULTS

Table 1 shows the proximate composition of unprocessed, germinated, germinated and fermented cowpea flour on dry matter basis. The germinated and fermented cowpea flour had the highest protein, fat and fiber content value of 20.99%, 2.88% and 3.29%, respectively, as well as the lowest Ash and carbohydrate content value of 2.22% and 68.43%, respectively. The unprocessed cowpea flour had the highest ash and carbohydrate content value of 2.98% and 73.84%, respectively.

Table 1: Proximate composition of unprocessed, germinated and fermented cowpea flours (%) in dry matter base

Flour Samples	Protein	Fat	Ash	Fiber	Carbohydrate	Dry Matter
UCF	17.90[a]	2.21[a]	2.98[b]	2.98[a]	73.84[b]	92.22
GCF	20.97[b]	2.22[a]	2.93[b]	2.93[b]	69.51[a]	91.86
GFCF	20.99[b]	2.88[a]	2.22[a]	3.29[c]	68.43[a]	91.76
LSD	1.0832	1.0922	1.0945	1.0844	1.0886	1.0817

Mean values with same alphabet superscript across the samples indicated no significant difference (p>0.05); different alphabet superscript across the samples indicated significant difference (p < 0.05; UCF = Unprocessed cow pea flour; GCF = Germinated cow pea flour; GFCF = Germinated fermented cow pea flour

Table 2 Selected Mineral composition of unprocessed, germinated, germinated and fermented cowpea flours on dry matter basis (mg/100g).

Minerals	UCF	GCF	GFCF
	Mean ± SD	Mean ± SD	Mean ± SD
Zinc	0.07 ± 0.01	0.08 ± 0.01	0.12± 0.02
Iron	2.69± 0.07	3.78 ± 0.05	3.93± 0.03
Calcium	3.04± 0.09	3.20± 0.10	3.52± 0.07
Phosphorus	59.88± 0.03	65.47± 0.03	68.90± 0.02

Mean ± SD indicated mean plus or minus standard deviation. The samples were in triplicate determination

Table 3: Selected anti-nutrient content of unprocessed, germinated, germinated and fermented cowpea flours on dry matter basis (mg/100g)

Anti-nutrient	UCF	GCF	GFCF
Phytate	5.06^{b}	4.47^{a}	4.33^{a}
Tannins	1.56^{b}	1.51^{b}	1.43^{a}
HCN	6.47^{c}	5.32^{b}	4.31^{a}
Oxalate	5.43^{c}	4.52^{b}	3.55^{a}
Saponin	0.11^{b}	0.10^{a}	0.13^{c}

Each antinutrients mean values with same alphabet superscript across the samples indicated no significant difference ($p>0.05$); different alphabet superscript across the samples indicated significant difference ($p < 0.05$).

DISCUSSION

The study observed that the germinated and fermented cowpea flour had the highest protein, fat and fiber content while they were found to have low ash and carbohydrate content. Increased protein content in germinated and fermented cowpea could be attributed to the enzymes activities that are activated during the germination process which hydrolyze the bonds between protein and anti nutrients to release more free amino acids to synthesize new protein. The findings in this study is similar to previous study by Ghavidel & Prakash —[25] that reported an increase in protein in Green gram, cowpea, lentil, chickpea which wereSoaked in water for 12 hours at 22–25°C, and germination for 24 hours. High protein content could further be attributed to increase in number of free amino acids during germination, which are more available due to amino acid destruction or for formation of new linkages that human are unable to hydrolyze during digestion [26]. Sprouting causes mobilization of protein with the help of protease leading to the formation of peptides, oligopeptides and free amino acid [27]. The increase in protein content could be attributed toa net synthesis of enzymic protein (e.g. proteases) by germinating seeds [28]. Similarly, fermentation process is responsible for breaking down of the protein to increase digestibility and to a lesser extent amino acid and protein substrates are necessary for bacteria growth and activities and enhances the nutritive value of food by increasing protein content [29]. Increased fat content of the germinated and fermented cowpea flour agree with the findings of Inyang and Idoko [30] and Samia El-Safy et al. [31] in their study, reported a reduction in fat content in malted millet during production and a decrease in fat content in all germinated samples of peas, lentils, chickpea, fada bean, oats and wheat, respectively. Similar findings were reported by El-Adewy et al.[32], Ghavided and Prakash [25] and Hahm et al.[33] that fat content decreases with an incresase in the time of germintion. The decrease in fat content could be due to increase in lipolytic enzymes during germination or due to hydrolysis of fat by β-oxidation to fatty acids and glycerol for energy purpose in embroyodevelopment and in the synthesis of new fat. The increase in the fiber content and the decrease in the carbohydrate are attributed to the hydrolysis of complex carbohydrate and the utilization of the simple carbohydrates by the bacteria for growth. Decrease in ash and carbohydrate content of the germinated and fermented cowpea flow could be attributed to the released minerals from the hydrolysis of complex bond of minerals and anti nutrients compounds and the release of glucose and other simple sugar from the hydrolysis of complex carbohydrates by enzyme and bacteria activities. And the use of these nutrients released for the growth and activities of the bacteria and enzyme in the process. The low carbohydrate content in the germinated and fermented cowpea flour could be relevant in Diabetes management. This finding is similar to the report that sugars are the most common substrate of fermentation, and typical examples of fermentation products are ethanol, lactic acid, carbon dioxide, and hydrogen gas (H2) [9]. Ash content was higher in germinated and fermented cowpea flour. This findings disagree with the results by Samina El-Safy et al.[31] that reported a significant decreased in ash content in all germinated samples. The increase in Ash content represents increase in minerals in cowpea flours. Minerals plays a crucial role in the maintenance of health. Cowpeas are rich in minerals but the bioavailability of these minerals is usually low due to the presence of antinutritional factors such as phytate, trypsin inhibitor and polyphenoles [31]. The findings is similar to the study by Samina El-Safy et al. [31] that reported that germination process technique improved the mineral composition of selected samples than that of raw legumes and cereal samples. The higher Zinc, Iron, Calcium and Phosphorus content in germinated and fermented cowpea flour could be attributed to biosynthesis and activities of enzymes during germination process [34, 35]. Fermentation increased minerals such as magnesium, iron, calcium, and zinc content of fermented foods and decrease the amount of phytates [36]. However, the increase in mineral content could be attributed to loss of dry matter during fermentation as microbes degrade carbohydrates and protein [37].

The germination and fermentation process increase the minerals content. Complex bonds between anti nutrients and nutrients are hydrolyzed by active enzymes in the cowpea and that of micro-organisms. This processing technique is suitable for the preparation of micronutrient quality food for the prevention and treatment of micronutrient deficiencies. The difference in mineral availability from the cowpea flour subjected to different processing methods is similar to the report by Nkhata et al, [38] that mineral availability in cereals and legumes are related to differences in phytate content, phytase activation, extent of binding of minerals within the matrix, or interaction of these factors. It was observed that germinated and fermented cowpea flour had the least phytate, tannins, hydrogen cyanide (HCN) and oxalate content but higher saponnin content compared to other samples. The finding is similar to previous study by Yasmin et al,–[39] that reported a decrease in cyanide, tannins, polyphenols, and phytic acid in red kidney beans (soaked in water for 6 hr at room temperature, germination for 4 days at 22°C). This implies that germination and fermentation processes reduce the anti-nutritional compounds in food samples

CONCLUSION

The study reported the proximate, minerals and antinutrients content of unprocessed cowpea, germinated and combined germinated and fermented cowpea flours. The germinated and fermented cowpea flour shows improved protein, fat, fiber and mineral quality and reduced anti-nutrient content compared to germinated and unprocessed cowpea flour. Combined germination and fermentation process improve nutrient quality and reduces the anti nutrient contents of the cowpea flour.

REFERENCES

1. José, F., Cruz, R., Júnior De Almeida, H., Maria, D., and Dos Santos, M., (2014). Growth, nutritional status and nitrogen metabolism in Vignaunguiculata (L.) Walp is affected by aluminum. Aust J Crop Sci 8:1132–1139.

2. Khalid, I. I., and Elharadallou, S. B., (2013). Functional properties of cowpea (Vignaungiculata L. Walp), and Lupin (Lupinus termis) flour and proteinisolates. J Nutr Food Sci 3:1–6 (2013).

3. Kirse, A., and Karklina, D. (2015), Integrated evaluation of cowpea (Vignaunguiculata (L.) Walp.) and maple pea (Pisum sativum var. arvenseL.) spreads. Agron Res 13:956–968 (2015). (13) (PDF) Cowpea: An overview on its nutritional facts and health benefits: Nutritional and Health Properties of Cowpea. Available from: https://www.researchgate.net/publication/324534058_Cowpea_An_overview_on_its_nutritional_facts_and_health_benefits_Nutritional_and_Health_Properties_of_Cowpea [accessed Apr 01 2022].

4. Ronner, E., and Giller, K.E., (2012). Background information on agronomy, farming systems and ongoing projects on grain legumes in Uganda, www.N2Africa.org pp. 17-18.

5. Ddungu, S. P., Ekere, W., Bisikwa, J., Kawooya, R., Okello Kalule, D., and Biruma, M (2015). Marketing and market integration of cowpea (Vigna unguiculata L. Walp) in Uganda. Journal of Development and Agricultural Economics Vol.7(1), pp.1-11, DOI: 10.5897/JDAE14.0577

6. Food and Agriculture Organization of the United Nations (FAO)/FAOSTAT, (2019). Statistical database, statistical Division Rome. Rome: Food and Agriculture Organization of the United Nations.

7. Agro Nigeria (2015). Cowpea Production in Nigeria. https://agronigeria.ng/cowpea-production-in-nigeria/accessedon01April2022.

8. Mamiro P.S., Mbwaga A. M., Mamiro, D. P., Mwanri, A. W., and Kinabo, J.L., (2011). Nutritional Quality and Utilization of Local and Improved Cowpea Varieties in some Regions in Tanzania. African Journal of Food Agriculture Nutrition and Development, vol.11(1). pp 4490-450.

9. Cooper, G. (2018). *Food Microbiology*. Library Press

10. Omemu, A. M. (2011). Fermentation dynamics during production of ogi, a Nigerian fermented cereal porridge. *Report and Opinion*, *3*, 8–17.

11. Hotz, C., and Gibson, R. S. (2007). Traditional food- processing and prepa-ration practices to enhance the bioavailability of micronutrients in plants-based diets. *Journal of Nutrition*, *137*, 1097–1100. https://doi.org/https://doi.org/10.1093/jn/137.4.1097

12. Mutungi, C., Ndunguru, G., Gaspar, A. and Abass, A. (2020). Improved postharvest practices for reduction of losses and improvement of produce quality: A trainer's manual for smallholder maize farmers in Tanzania. Ibadan, Nigeria: IITA.

13. AOAC International. (2016). *Official methods of analysis* (20th edn.). AOAC International.

14. Wiles, P., Gray, I., and Kissling, R. (1998). Routine analysis of proteins by Kjeldahl and Dumas methods: review and interlaboratory study using dairy products. *J AOAC Int*, *81*, 620–632.

15. Pearson, D. A. (1976). *Chemical Analysis of Food* (7th ed). Churchil Living Stone.

16. Folch, J., Lees, M., and Stanley, G. (1957). A simple method for the isolation and purification of total lipids from animal tissues. *J Biol Chem*, *226*, 297–509.

17. McCleary, B., & Prosky, L. (2001). *Advanced dietary fibre technology*. Blackwell Science.

18. Onwuka, G. . (2005). *Food Analysis and instrumentation; Theory and practice*. Naphtali Prints.

19. Beaty, R., and Kerber, J. (1993). *Concepts, instrumentation and techniques in atomic absorption spectrophotometry* (2nd ed.). The Perkin-Elmer Corporation.

20. Welz, B. (1985). *Atomic Absorption Spectrometry*. VCH.

21. Yeung, V., Miller, D. D., and Rutzke, M. A. (2017). Atomic Absorption Emission Spectroscopy, Spectroscopy, Atomic Coupled Plasma-Mass and Inductively Spectrometry. In S. Nielsen (Ed.), *Food Analysis, Food Science Text Series* ((ed), p. 129). Springer International Publishing. https://doi.org/DOI 10.1007/978-3-319-45776-5_9

22. Makkar, H. P. S., and Becker, K. (1996). Nutritional Value and anti-nutritional components of and ethanol extracted moringa oleifera leaves. *Animal Feed Science Technology*, *63*, 211–238.

23. Kirk, R., and Sawyer, R. (1991). *Pearson composition and analysis of foods*. Longman Scientific and Technical.

24. Dye, G. (1956). *The Chemical Analysis of Food* (7th ed.). Churchill, Living Stone

25. .Ghavidel, R. A., and Prakash, J. (2007). The impact of germination and de- hulling on nutrients, anti- nutrients, in- vitro iron and calcium bioavail- ability and in- vitro starch and protein digestibility of some legume seeds. *LWT – Food Science and Technology*, *40*(7), 1292–1299.

https://doi.org/https://doi.org/10.1016/j.lwt.2006.08.002.

26. Ruiz, C., and Bressani, R. (1990). Effect of Germination on the Chemical Composition and Nutritive Value of Amaranth Grain. *Cereal Chem.*, *67*(6), 519–522.

27. Jood, S., Chauhan, B.M., and Kapoor, A. C., (1988). Contents and digestibility of carbohydrate of chickpea and blackgram as affected by domestic processing and cooking. Food Chem. 34: 417-420

28. Nzeribe, H.C., and Nwasike, C.C. (1995). The brewing potential of acha (*Digiteria exlils*) malt compared with pearl millet (*Pennisetum glaucum*) malt and sorghum (*Sorghum bicolar*) malts. J. Inst. Brewing 101: 345-350.

29. Onofiok N.O., and Nnanyelugo, D. O., (2005) Weaning foods in West Africa: nutritional problems and possible solutions. In early programming of adult disease in resource poor countries, ed. AM Prentice, SE Moore. Arch Dis Child, pp: 429- 432.

30. Inyang, C.U., and Idoko, C.A., (2006).Assessment of the quality of "Ogi" made from malted millet. Af.J. Biotechnol., 5: 2334-2337

31. Samina El-Safy, F., Rabab, H.A.S., and Ensaf Mukhtar, Y. Y., (2013) The Impact of Soaking and Germination on Chemical Composition, Carbohydrate Fractions, Digestibility, Antinutritional Factors and Minerals Content of Some Legumes and Cereals Grain Seeds. Alexandria Science Exchange Journal., Vol. 34(4); 499-512.

32. El-Adewy, T.A., Rahma, E.H., El-bedawey, A.A., and El-Beltagy, A.E., (2003). Nutritional Potential and Functional propertiesof germinated mungbean, pea and lentil seeds. Plant Foods for Hum. Nutr., 58:1-13.

33. Hahm, T., Park, S., and Lo, Y. M., (2008). Effects of germination on chemical composition and functional properties of sesame (*Sesamum indicum* L.) seeds. Bioresource Technology 100: 1643-1647.

34. Gabriel, R.A.O and Akharaiyi, F.C., (2007). Effect of spontaneous fermentation on the chemical composition of thermally treated jack beans (*Canavalia ensiformis* L.) Int. J. Biol. Chem. 1:91-97

35. .Bau, H. M., Villaume, C., Nicolas, J., and Mejean, L., (1997). Effect of germination on chemical composition, biochemical constituents and antinutritional factors of soybean (*Glycine max*) seeds. Journal of the Science of Food and Agriculture, 73:1-9.

36. Pranoto, Y., Anggrahini, S., and Efendi, Z. (2013). Effect of natural and *Lactobacillus plantarum* fermentation on invitro protein and starch digestibilities of sorghum flours. *Food Bioscience*, *2*, 46–52. https://doi.org/10.1016/j.fbio.2013.04.001

37. Day, C. N., and Morawicki, R. O. (2018). Effects of fermentation by yeast and amylolytic lactic acid bacteria on grain sorghum protein content and digestibility. *Hindawi Journal of Food Quality*, *2018*, 1–8. https://doi.org/10.1155/2018/3964392

38. 18. Nkhata, S. G., Ayua, E., Kamau, E. H., and Shingiro, J. B. (2018). Fermentation and germination improve nutritional value of cereals and legumes through activation of endogenous enzymes. *Food Science & Nutritionn*, *6*(September), 2446–2458. https://doi.org/10.1002/fsn3.846

39. 19. Yasmin, A., Zeb, A., Khalil, A. W., Paracha, G. M., and Khattak, A. B. (2008). Effect of processing on anti- nutritional factors of red kidney bean (Phaseolus vulgaris) grains. *Food and Bioprocess Technology*, *1*, 415– 419.