

Fingermillet-Finger millet and Defatted Sesame Seeds Flours as Complementary Foods: Nutritional Evaluation and Protein Quality

Abstract

The role of germination and fermentation on finger millet and defatted sesame seeds complementary foods was studied comprising of non-germinated non-fermented finger millet and defatted sesame seeds (NGNFFS), non-germinated fermented finger millet and defatted sesame seeds (NGFFS), germinated non-fermented finger millet and defatted sesame seeds (GNFFS) and germinated fermented finger millet and defatted sesame seeds (GFFS).

All amino acids composition increased with germination and fermentation except for lysine and tryptophan. Amino acid composition increased with germination and fermentation except for lysine and tryptophan, which were limiting essential amino acids. Notable increments were Methionine 1.96 - 3.13, arginine 6.15 - 7.32 and leucine 9.67 - 10.66 respectively. The protein efficiency ratio (PER) and net protein retention (NPR) with their relative values R-PER and R-NPR were within a similar range as control.

Keywords: Complementary foods, Fermentation, Germination, Nutrients

INTRODUCTION

Infancy and early childhood require adequate nutrition which is fundamental to growth and development. This is a critical stage in the lives of children because it is when behavioural development, health and optimal growth take place. Complementary foods are foods other than breast milk or infant formula (liquids, semisolids, and solids) introduced to an infant to provide nutrients (Gahlawat *et al.*, 2020). These foods are formulated from a combination of food materials comprising of cereals, legumes, tubers, roots and seeds. Irrespective of their sources, complementary foods are expected to be high in energy density, rich in protein quality and possess low levels of anti-nutritional components (Malleshi *et al.*,

Commented [MN1]: Usually Abstract should describe the main Objective of the study also. That part is missing in your abstract

Formatted: Highlight

Commented [MN2]: Recheck these values and ranges

Formatted: Highlight

Commented [MN3]: Is it in similar range as control

Commented [MN4]: Since you have done an interesting work. Come up with strong findings here. You can mention interesting findings from your rat study, etc., to attract the reader. Otherwise reader gets fed up just after reading the abstract, So try to polish your abstract in a more scientific way.

Formatted: Highlight

Formatted: Font: Bold

2019). Animal proteins such as meat and milk are important for complementary feeding. However, these are expensive and unaffordable for the majority of the population in Nigeria.

Protein-energy malnutrition (PEM) is a major concern in Nigeria especially among children and expectant mothers in rural communities and internally displaced persons (IDP) camps. Milk, meat and fish are good sources of protein but are quite expensive and out of reach of vulnerable groups in Nigeria. Cereals like sorghum and maize constitute the major source of complementary foods in developing countries like Nigeria (Gernah *et al.*, 2020). These products may be inadequate in protein, essential amino acids and mineral quality and quantity ~~thus the need for complementary foods of,~~ thus the need for complementary foods which are available and affordable (Gernah *et al.*, 2020).

Finger millet ~~contain substantial amount of essential amino acids~~ contains a substantial amount of essential amino acids, which include arginine, leucine, threonine and valine (Ravindra, 2023). This makes finger millet highly important and a good source of complementary and functional foods.

Sesame is grown mainly for its seeds ~~that,~~ which contain approximately 50% oil and 25% protein (Burden, 2021). It is an important oil seed that is incorporated in several products due to its ~~fibrefiber~~ and protein quality (Muthamilarasan, *et al.*, 2020).

Germination and fermentation enhance the quality of complementary food products. Hence ~~proper processing and judicious blending of locally available foods could result to,~~ proper processing and judicious blending of locally available foods could result in improved intake of nutrients (Senappa, 2022). Therefore, the aim of the study is to ascertain the quality of protein, essential amino acids and minerals of finger millet and defatted sesame seeds.

MATERIALS AND ~~METHOD~~METHODS

Sources of raw materials and preliminary handling

Essentially 5 kg of red finger millet (*Eleusine coracana*) and white sesame seeds (*Sesamum indicum*) were bought from Wadata market, Makurdi, Nigeria. The raw materials were cleaned and sorted to remove defective and unwholesome particles. The basal diet ingredients were bought from a local shop in Makurdi, Nigeria. Rice husk was obtained from Mikap Nig. Ltd. Gboko road, Makurdi, Nigeria. A 25 kg acclimatization starter feed (chicken feed, Crown Flour Mill Ltd) was purchased from Wurukum market, Makurdi, Nigeria. Thirty Wister albino rats (male) were bought from department of animal science, Benue State University Teaching hospital, the Department of Animal Science, Benue State University Teaching Hospital, Makurdi, Benue State, Nigeria.

Production of flour samples

Preparation of germinated and non-germinated finger millet flours

The process for production of finger millet flours is shown in Fig. 1, the production of finger millet flour is shown in Fig. 1, as described by Ariahu et al., 1999(b). Finger millet grains were washed and steeped in water at room temperature using the ratio of 1:3 (w/v grain: water) in a bucket. The steep water was replaced every 4 hours for a period of 12 hours and then drained. Wet grains were spread out to germinate while water was continuously sprinkled on it, spread out to germinate while water was continuously sprinkled on them. The non-germinated and germinated grains were removed at 0 and 72 hours and dried. The dried seeds were rubbed between the palms to separate testa and rootlets from cotyledons and then the seeds were winnowed. The resulted dried seeds were milled using a blender, resulting dried seeds were milled using a blender and sieved with recycling of the seeds to improve output. The non-germinated finger millet (NGF) and germinated finger millet (GF) flours were sealed and stored properly for analysis.

Preparation of fermented finger millet flour

Commented [MN5]: Just a suggestion: Here it would be more scientific if you obtained authenticated red finger millet and white sesame seeds from the Department of Agriculture, Nigeria

For other basal diet ingredients and rice husk also it would have been better if you obtained them from a standardized research body, at your locality

Formatted: Highlight

Formatted: Highlight

Formatted: Highlight

Formatted: Highlight

Formatted: Highlight

Formatted: Highlight

Formatted: Highlight

The fermented finger millet was obtained by natural lactic acid fermentation (Pradhan, 2021) ~~as also shown in Fig 1.~~ 100g each of the germinated (GF) and non-germinated (NGF) finger millet was mixed with distilled water to form slurry, ~~as also shown in Fig 1.~~ 100g each of the germinated (GF) and non-germinated (NGF) finger millet was mixed with distilled water to form a slurry and then allowed to ferment naturally for 24 hours. At the end of the period, 50% of the fermented mixture was used as a starter culture for a new fermentation cycle. pH and titratable acidity (an index of lactic acid bacteria activity) ~~was~~ were monitored during this period. The process was allowed to continue to enable a stable and constant pH. The resultant concentrates were dried in an oven, ~~and~~ milled in a blender to obtain germinated and fermented finger millet (GFF) and non-germinated fermented finger millet (NGFF).

Formatted: Highlight

Formatted: Highlight

Preparation of defatted sesame flour

The process for sesame seeds flour formulation by Ravindra, 2023 ~~is shown in Fig.2.~~ The seeds were sorted and cleaned to remove debris and stones. The seeds were soaked in warm water to soften the seed coat and dehulled by rubbing between palms followed by washing. The resultant seeds were dried, milled and defatted by a screw press method as described by Muthamilarasan, 2019. Defatted flour was analyzed for fat until a fat content of 15% in the cake. The cake was dried in an oven and milled.

Commented [MN8]: It is not shown in Fig 2. Include the relevant figure with correct figure numbering

Formatted: Highlight

Food Products Development

The effect of germination and fermentation on four different food formulations comprising non-germinated finger millet + defatted sesame (NGNFFS), germinated finger millet+ defatted sesame (GNFFS), non-germinated fermented finger millet + defatted sesame (NGFFS) and germinated fermented finger millet + defatted sesame (GFFS) were made by incorporating finger millet flours with defatted sesame flour to obtain 16_g protein and 9_g fat/100g as recommended by the Protein Advisory Group (PAG). Material balancing was used to obtain the

amount of the four blends of finger millet/sesame seeds flour from their respective proximate compositions (Devi *et al.*, 2020).

Feeding Tests

Animal feeding experiment was performed using Wistar male rats with a modification of the method described by Pellet *et al.*, 2023. A complete randomized design (CRD) was used, and 30 Wistar rats were weighed into six wooden cages with five animals per cage at the beginning of each feeding trial. The cages were placed on a platform for the collection of faeces. The animals were acclimatized by ~~feeding with~~ poultry starter feed for one week. After acclimatization, test diets ~~formulated, and control was mixed with basal diet to obtain protein level (9g) while were formulated, and control was mixed with a basal diet to obtain a protein level (9 g) while the~~ basal diet was fed directly. The rats were fed the diets and water *ad libitum* for 28 days. The average food intake of each group was determined daily while average weight gain was determined at 2 days interval. ~~Amount~~The amount of Protein consumed was calculated by material balance from food intake. Faeces collected from day 10-28 ~~was were~~ dried, weighed and milled into fine powder for analysis. Protein efficiency ratio (PER) and net protein ratio ~~was estimated from average feed intake and average weight gain at 14 and 28 day were estimated from average feed intake and average weight gain at 14 and 28 days~~ of feeding.

Evaluation of protein quality indices

Amino Acids Determinations

Sample Preparations: Amino acid composition of products was evaluated using an amino acid analyser/analyzer based on ~~the high performance~~high-performance liquid chromatography

technique described by Rhon *et al.*, 2018. Samples ~~hydrolysate was of hydrolysate were~~ prepared as described by Pellet and Young 2023. A mixture of standard amino acids was carried through the hydrolysis and incubation process.

HPLC Analysis: An aliquot of 150 μ l of each hydrolysate was injected into the amino acid ~~analyser~~ analyzer (Skykam S7130) using a cation separation glass column at 130 $^{\circ}$ C.

The reaction mixture was detected at 570nm and 440nm on a ~~dual-channel~~ dual-channel photometer. The amino ~~acids composition was calculated from areas of standards obtained from the integrator and expressed as~~ acid composition was calculated from areas of standards obtained from the integrator and expressed as a percentage of total protein. The amino acid scores were obtained relative to FAO/WHO reference patterns.

***In-vivo* protein Evaluation**

Protein quality indices were determined using standard methods. From the values of Mean Daily Feed Intake (MFDI) and Mean Daily Weight Gain (MDWG) obtained, Protein Efficiency Ratio (PER), ~~Net and net~~ Protein Retention (NPR) ~~was were~~ estimated by the method of Jena *et al.*,

2020 as follows:
$$NPR = \frac{W_t - W_b}{W_p} \quad \text{---(8)}$$

$$PER = \frac{W_t}{W_p} \quad (9)$$

W_t = average weight gain of test animals on a given diet, W_b = average weight loss of animals on basal diet, W_p = protein consumed by test animals on the given diet

The relative PER (R-PER) and relative and relative NPR (R-NPR) were obtained by relating the PER and NPR values respectively to those of Animal Nutrition Council (ANCR) casein which are 2.5 for PER (Malleshi *et al.*, 2019) and 4.02 for NPR (Malleshi *et al.*, 2019) as follows

$$R - PER = \frac{PER \text{ of diet}}{PER \text{ of ANCR - CASEIN}} \quad \text{---(10)}$$

Formatted: Font: Italic

Formatted: Tab stops: 3.25", Left

Formatted: Tab stops: 4.5", Left

$$R - NPR = \frac{NPR \text{ of diet}}{NPR \text{ of ANCR-CASEIN}} \quad (11)$$

Feeding experiments

The four test diets were formulated using material balancing (Devi *et al.*, 2020) by blending 43.75 g of the basal (nitrogen-free) diet ~~to 56.25g of NGNFFS, GNFFS, NGFFS and GFFS respectively~~ with 56.25g of NGNFFS, GNFFS, NGFFS and GFFS, respectively, to give 9 g protein/100g of each test diet. The basal diet consisted of corn starch: 80 g/100 g, corn oil: 10 g/100 g, common table salt: 4 g/100 g, ~~sugar: 1g/100g, vitamin: 1g/100g and non-nutritive fiber~~ Sugar: 1 g/100 g, vitamin: 1 g/100 g and non-nutritive fibre (rice husk): 4 g/100 g.

RESULTS AND DISCUSSION

Protein quality indices

Essential amino acid profile of complementary foods.

Essential amino acids composition is shown in Table 1 ~~and the FAO/WHO reference pattern was used as a means of comparing the result, and the FAO/WHO reference pattern was used as a means of comparing the results.~~ The result of the essential amino acids composition of the formulated products was within the FAO/WHO/UNU standards. It was observed that leucine had the highest (10.66) mean value compared to other amino acids. ~~GNFFS and NGNFFS had highest amounts of arginine, isoleucine, lysine, histidine, threonine, phenylalanine and valine having values ranging from 2.96- 4.05~~ the highest amounts of arginine, isoleucine, lysine, histidine, threonine, phenylalanine and valine, having values ranging from 2.96 - 4.05, while tryptophan and methionine values ranged from 0.88 - 3.13. The total amino acids (TAA) of food samples were above average recommended values. This indicates that the complementary foods are nutritionally adequate to meet the essential amino acid demand of children. In comparison, the total amino acid profiles of GNFFS and GFFS in the current study were higher.

Commented [MN9]: Recheck these values and ranges

compared to melon (53.4_g/100_g) and pumpkin (38.3_g/100_g)~~respectively, respectively,~~ as reported by Davidson *et al.*, 2022. The values for amino acids ~~of-in~~ the formulated diets were superior to ~~that those~~ of popcorn-based diets (Ijarotimi *et al.*, 2019). Cereals are deficient in lysine and tryptophan (Mensah *et al.*, 2023)~~but on addition of legumes, but in addition to legumes,~~ which are rich in tryptophan and lysine but deficient in sulphur-containing amino acids, a desirable pattern of essential amino acids comparable to or higher than the reference protein is obtained (Nnam, 2021). The use of finger ~~millet and sesame-based foods are therefore suggested as alternative protein and energy source~~millet and sesame-based foods are therefore suggested as alternative protein and energy sources for infant and adult food products (Mensah *et al.*, 2023).

In-vivo protein quality indices: A comparison of the NPR and PER mean values of the formulated food with the corresponding ANRC (casein) resulted in the R-NPR and R-PER data which ranged from 0.21 - 0.95 and 0.85 - 0.94 as well as 0.06 - 0.13 and 0.33 - 0.475, as shown in Table 2 respectively. The PER values of GFFS and GNFFS were high (2.36 & 2.25) compared to ~~control diet which had the least~~the control diet, which had the lowest value (2.12). The PER relates weight gain in the test animals and the corresponding protein intake while NPR indicates the relationship between weight changes in the animals fed the test diets relative to those fed the basal diet (Briggs *et al.*, 2020).

As shown in Fig.1 and 2, there was a steady increase in the feed intake by the test animals~~with control diet being consumed higher (108.16g) than the basal diet (43g-39g), with the control diet being consumed higher (108.16 g) than the basal diet (43 g - 39 g),~~ notably with a decrease in consumption to the other test diets. The high daily feed intake by animals fed with a control diet compared with the experimental food samples might be due to the fortification of the formula with quality protein like casein or other quality protein food materials. The GNFFS recorded a consumption rate of 56.37_g - 85.70_g. This was the most consumed diet among the test diets,

which could be due to the formation of better ~~flavors~~ flavors and taste by germination (Ijarotimi *et al.*, 2021)

Table 4: Essential amino acid profile of finger millet and defatted sesame based complementary food

Commented [MN10]: Products are not mentioned in the relevant columns

Lysine	2.96	3.16	2.88	3.25	5.8
Histidine	3.21	3.76	3.06	3.18	1.9
Arginine	6.66	7.32	6.15	6.82	2.0
Threonine	3.55	3.66	3.45	3.33	3.4
Valine	3.75	3.81	3.56	3.48	3.5
Methionine	1.96	2.44	2.37	3.13	2.2
Isoleucine	4.16	4.05	4.28	4.18	2.8
Leucine	10.66	9.78	9.92	9.67	6.6
Tryptophan	0.88	0.92	1.13	0.98	1.1
Phenylalanine	3.69	4.11	4.28	4.34	2.8

Results are means \pm standard deviation of triplicate determinations. **Key:** NGNFFS= non-germinated non-fermented finger millet and defatted sesame flour, GFFS= germinated fermented finger millet and defatted sesame flour, NGFFS= non-germinated fermented finger millet and defatted sesame flour, GNFFS= germinated non-fermented finger millet and defatted sesame flour

Table 2: In-vivo protein quality of test diets

Parameter	Products					
	NGNFFS	NGFFS	GNFFS	GFFS	NUTREND	LSD
NPR	3.45 ^c \pm 0.01	3.46 ^c \pm 0.07	3.51 ^d \pm 0.02	3.81 ^b \pm 0.02	3.23 ^a \pm 0.02	0.046
PER	2.17 ^c \pm 0.07	2.21 ^c \pm 0.01	2.25 ^c \pm 0.07	2.36 ^b \pm 0.07	2.12 ^a \pm 0.10	0.124
R-NPR	0.85 ^b \pm 0.02	0.86 ^b \pm 0.02	0.21 ^b \pm 0.07	0.95 ^a \pm 0.07	0.80 ^a \pm 0.01	0.453
R-PER	0.87 ^c \pm 0.00	0.88 ^c \pm 0.07	0.90 ^c \pm 0.07	0.94 ^b \pm 0.07	0.85 ^a \pm 0.01	0.023

Formatted: Highlight

Commented [MN11]: What is suggested by NUTREND and LSD

Formatted: Highlight

Results are means of \pm standard deviation of duplicate expressed on dry weight. Values along each row with superscripts are not significantly ($p < 0.05$) different.

Key: GNFFS= germinated non-fermented finger millet and defatted sesame flour, GFFS= germinated fermented finger millet, NGFFS= non-germinated fermented finger millet and defatted sesame flour

UNDER PEER REVIEW

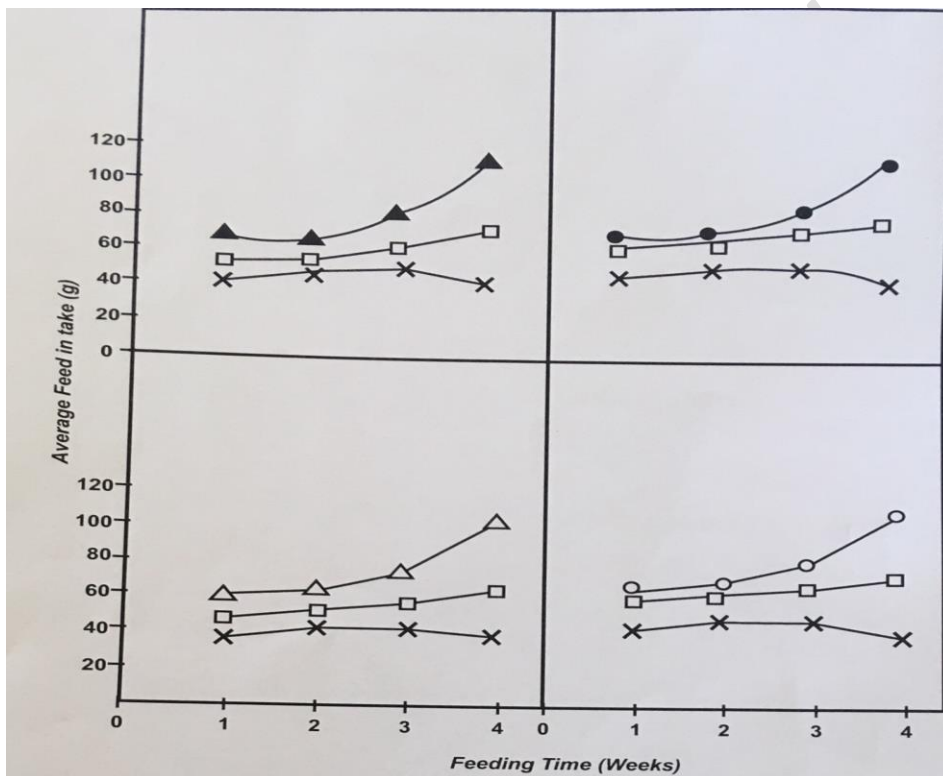


Fig. 4.2: Effect of germination & fermentation on feed intake of test animals

Keys:

- ▲ = Non-germinated fermented finger millet + sesame
- ◻ = Nutrend
- × = Basal diet
- = Germinated & fermented finger millet + sesame
- △ = Non-germinated non-fermented finger millet + sesame
- = Germinated non-fermented finger millet + sesame

Fig 1. **Caption?**

Formatted: Font: Bold

UNDER PEER REVIEW

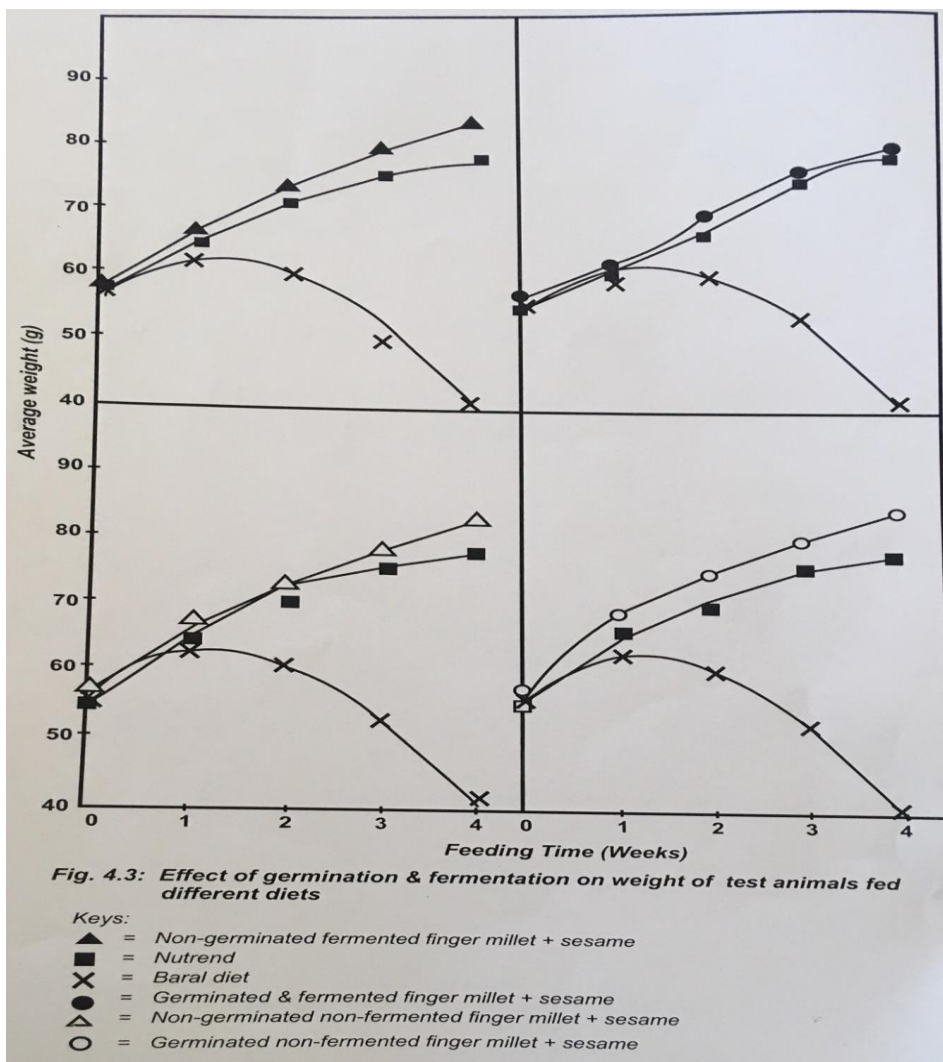


Fig 2. **Caption?**

Formatted: Highlight

Conclusion

The essential amino acid of the test diets was above the recommended values of FAO/WHO which may be due to germination and fermentation. There was an increase in ~~weight of the test animals; this may be due to the weight of the test animals; this may be due to the~~ bioavailability of nutrients made accessible by germination and fermentation.

Formatted: Justified

Commented [MN12]: You have done an interesting research. So come up with a more solid conclusion

Ethical Approval (Not Applicable)

Commented [MN13]: For rat studies?

Consent to participate (Not Applicable)

Statement of Informed Consent: Informed consent was obtained prior to performing the procedure, including permission for publication of photographs/ images included herein.

Availability of data and material: The data supporting the findings of this study are available from the corresponding author, upon reasonable request.

Code availability (Not Applicable)

REFERENCES

- Siwela, M., Taylor, J. N. R., de Milliano, W. A. J., & Doudu, K. G. (2021). Influence of phenolic in finger millet on grains and malt fungal load, and malt quality. *Food Chemistry*, 121, 443–449.
- Omary Z., Lupiana D., Mtenzi F., Wu B. (2019). Challenges to E-Healthcare Adoption in Developing countries: A case study of Tanzania. *First Intl conference on Networked Digital Technologies*. Ostrava

- Abdelrahman S.M., Hagir B.E., Wisal H.I (2019). Anti-nutritional factor content and hydrochloric acid extractability of minerals in pearl millet cultivars as affected by germination. *Intl. J.Food sciences & Nutrition* 58(1) : 6-17 Doi : 10. 1080/09637480601093236.
- Ravindra V. (2023). Feed resources for poultry production in Asia and the pacific. II plant protein sources. Vol. 48, issue 3 pp. 205-231 doi: 10.1079/WPS19920017
- Devi, P.B., Vijaya bharathi, R., Sathyabama, S., Malleshi, N.G., and Priyadarisini, V.B.(2019). Health benefits of finger millet (*Eleusin coracana L*)polyphenols and dietary fibre: a review.*J .Food Sci. Technol.*51, 1021- 1040.
- Gahlaw, J.R.A.(2020). The nature of the malting process in Barley and malt- Biology,Biostry,Technology , edited by A. H. cook. London: Academic press inc
- Rhon T.C. (2023). Malt Whiskies: Raw Materials and processing. In *Whisky: Technology, Production and Marketing*, edited by I. Rusell. London: Academic press
- Lewis, M. J., and T.W. Young (2023). *Malting Technology: malt, specializes malts, and non-malt adjuncts*.In *Brewing*. New York;Kluwer Academic.
- Malleshi, G. (2019). *Malting*.In *Cereals Processing Technology*, edited by G. Owens. Cambridge, MA: Woodhead Publishing Limited
- Food and Agriculturl Organisation/World Health Organisation/UNICEF (2019). *International conference of nutrition. Final report*. Rome: FAO/WHO/UNICEF
- Davidson and Brown (2003). *Guiding principles for complementary feeding of the breastfed child*. Pan American Health Organization: World Health Organization. Food and nutrition program

- Gernah D.H., Levitt E., Thairu L. (2003). Improving feeding practices: current patterns, common constraints and the design of interventions. *Food and Nutrition Bulletin* 24, 45-82.
- Burden H.S., Issa- Zacharia A., Kinabo J., (2011). Formulation and sensory evaluation of complementary foods from local, cheap and readily available cereals and legumes in Iringa, Tanzania. *Afri. J. Food Sci.*, 5, 26-31.
- Egbujie A.E.& Okoye J.I., (2019). Chemical and sensory evaluation of complementary foods produced from sorghum, African yam bean and crayfish flours.
- Ariahu CC, Ukpabi U and KO Mbajunwa (199a) Production of African Breadfruit (*Treculia africana*) and Soyabean (*Glycine max*) Seed based Food Formulations. 1: Effects of Germination and Fermentation on Nutritional and Organoleptic quality. *Pl. Foods Hum.Nutr.* 54:123 – 266.
- Nnam K.D (2021). Social ecology and living system theory. *Systems Research and Behavioral science/ vol.15,Doi:org/10.1002/(SCI)*
- Briggs W.F., Mccance M.F. (2020). *Laboratory methods in food and dairy microbiology (Revised Ed.)*. 452 S., 24 Abb. London-New York- San Francisco. Academic press.
- Jena G., Sun Q., Gu P (2022). Experimental Study of the cooling Characteristics of polymer filaments in FDM and impact on the Mesostructures and properties of Prototypes. 3(6) 567-588
- Muthamilarasan, M., Dhaka, A., Yadav, R., & Prasad, M. (2019). Exploration of millet models for developing nutrient rich graminaceous crops. *Plant Science*, 242, 89–97

Davidson A., Maha E.H., Abdulrahman M.H., Abdurrahman A.F. (2020). Current Opinion Health care professional Development: Working as a team to improve patient care. Sudanese Journal of Paediatrics 14(2): 9-16.

Pradhan, A., Nag, S.K., and Patil, S.K. (2021). Dietary management of finger millet controls diabetes. *Curr.Sci.* 98,763-765

Ijarotimi O.S & Ashipa F.,(2019).Evaluation of nutritional composition, sensory and physical properties of home processed weaning food based on low cost locally available food materials. *Nutr & Food Sci.* 36 (1):6-17

Senappa M (2022). Sorghum and millets in east Africa with reference to their use in weaning foods In: Proceedings of workshop, Nairobi, Kenya, 12-16 October, 1987, Improving young child feeding in eastern and southern Africa: household level food technology. Alnwick D, Moses S, Schmidt O G eds. Ottawa, International Development Research Centre. pp. 39-54

Pellet P.L., Young, V.R. (2023). Nutritional evaluation of protein foods. The United Nations University Hunger Programme. *Food Nutr. Bull sup 4.* Tokyo: The United Nations University