AFS- ADVANCES IN FOOD SCIENCES

Contininuation of CMTL founded by F.Drawert

Production by PSP-Vimy Str. 1e,85354 Freising,Germany in

cooperation with PRT-Parlar Research & Technology

Vimy Str 1e,85354 Freising

Printed in Germany-ISSN 1431-7737

AFS

Advances in Food Sciences(AFS) is abstracted/indexed in:

Chemical Abstract Service, BIOSIS, CAB International, Cambridge Scientific Abstracts, Food Science and Technology Abstracts(FSTA) , Current Contents/Agriculture, CSA Civil Engineering Abstracts, CSA Mechanical & Transportation Engineering,ISI(Current Contents)

CONTENTS

REVIEW

ORIGINAL PAPERS

REVIEW

A REVIEW ON USAGE OF NUTRITION LABELLING: CONSUMER PREFERENCES AND CHOICES

Shubhangi Srivastav, Madhvi Awasthi*

Department of Nutrition and Dietetics, Faculty of Allied Health Sciences, Manav Rachna International Institute of Research and Studies, Faridabad 121001, India

ABSTRACT

This review study summarizes the information available in context to nutrition labelling usage, the factors affecting the use of nutrition labels, recent trends in food labels display, preference of the display format by different age groups, association of food label usage with certain noticeable parameters, etc. Several databases like Google Scholar, Medline were searched to compile the review study. Food and Nutrition labels are the prominent first-glance article which needs to be eye-catchy and comprehensible for the consumers. They play an extremely characteristic role in making purchase decisions for a healthier lifestyle. The escalation in non-communicable diseases around the globe have generated a serious concern for the betterment of the labels and promotion of its usage on a large scale. Many studies concluded that education and specific dietary requirements had a positive association with the food label use whereas gender and household income do not have any significant relation. A system of clear, concise, and symbol-intensive display format is promoted in all the studies. This study also emphasizes on the increment in font size and the provision of nutrition education.

KEYWORDS:
Food label, consumer, usage, prepackaged foods, preferences, symbol intensive

INTRODUCTION

A nutrition label is a visible summary displayed on food packets that has a notable effect on the health status, physical activity, and diet of an individual [1]. Nutritional labels are the tools for communication between the manufacturer, seller and consumers that convey the appropriate information. Nutrition labels are targeted for delivering out-and-out nutrition information and other mandatory details of the packaged food product [2]. They act as motivational and guiding tools for the consumers to make sensible choices regarding healthy foods [3]. Nutrition labels inform the consumers about the contents of the food along with the supplementary nutrition information [4].

According to a study, a nutrition label has three major roles to play: (1) providing the generic information about the product (2) providing allergen, storage, usage specifications (3) acting as a means for marketing and advertising source [5]. The nutritional labels act as a reliable guide which educates the consumers about the nutritional value of the packed foods [6]. According to the World Health Organization (WHO), food labelling includes "Any written, printed or graphic matter that is present on the label, accompanies the food, or is displayed near the food, including that for the purpose of promoting its sale or disposal"[7]. Food labels are the cost-effective, mandatory aids that promote the ideologies of right eating to foster the aim of disease-free nation. They are deliberated for the consumers to compare the products, to inform them about the nutrients and to make healthy choices [8].

MATERIALS AND METHODS

A narrative review approach was followed to read and interpret research papers and review articles. The papers studied had locales in and around the world viz., Global South, Chile, Korea, South Africa, South America, Europe, India, etc. In this review, the papers focusing majorly on consumer understanding of nutrition knowledge, preference regarding display formats, KAP study on food label use, determinants of consumers' label usage, role of nutrition labelling, consumer awareness and status of food labelling were reviewed for the study. Google Scholar, PubMed and Medline were surfed, and relevant papers were found and reviewed. The reviewed data was analyzed, paraphrased, and compiled based on their relevance.

RESULTS AND DISCUSSION

Who controls the Labelling regulations? Different countries have different controlling organizations which have mandated clear and precise label-

ling on the packaged products. Some foreign countries like New Zealand, Hong Kong, Canada, Brazil, United States, Australia have mandatory labelling regulations while countries like Jamaica, Kenya, Bangladesh, Pakistan, Cambodia have no such compulsory set of regulations for food labelling. The Gulf countries are regulated by Gulf Cooperation Council Standardization Organization (GSO) for labelling of pre-packaged foods [9]. In New Zealand and Australia, they have joint guidelines for the labelling purpose, which is counselled by Food Standards Australia New Zealand (FSANZ). In United Kingdom, the food labelling is enforced by Department for Environment, Food & Rural Affairs (DEFRA) under the aegis of Food Standards Agency.

In India, FSSAI governs the food and packaging related activities throughout and is also responsible for updating the packaging and labelling regulations from time-to-time. FSSAI in collaboration with the Codex Alimentarius Commission, established by FAO WHO develops standards and nutrition guidelines for the effective packaging of prepacked foods. Nutrition labels inform the consumers about the contents of the food along with the supplementary nutrition information [10]. FSSAI has a set of guidelines that is to be strictly followed by the manufacturers to sell their products without any hindrance from the Government. Every packaged food product should contain the following components: name of the food, list of ingredients, nutritional information, quantity and price, FSSAI registration number, storage and usage instructions, lot number, health claims, expiry date, details about the manufacturer, country of origin, allergen and fortificants specification, declaration about vegetarian or non-vegetarian foods, declaration about food additives or treatments, clarification about type of foods viz, irradiated or frozen or infant foods etc [10].

Growing trends in food labelling. People nowadays are living a sedentary lifestyle which has made them more lethargic and reluctant. They are exposed to many consequential diseases/disorders occurring due to various environmental, social, psychological, demographical factors. Diet related health issues have been at a great surge in the past few years, which has eventually led to the development of chronic diseases among the citizens [11]. As the diet-related diseases in the world are on escalation, the consumers are more inclined towards right eating and that is the reason why people tend to read food labels before making their choices. The trend of product reformulations is also quite common these days. The trend of healthy selection of foods is the result of westernization in the diets throughout [12].

Food labelling Formats. To satisfy the consumer's inquisitiveness, different formats of food labelling have been developed. The two most common of them are-: Front-of-pack labels and Back-of-pack labels. FOP labelling refers to when the front side of the packaged food product contains a printed nutritional label that tells the mandated information viz. nutritive values, specific additives and serving size [13]. It includes traffic light coding system which displays the ingredients in color codes according to their composition in the product. The ingredient present in higher proportion is shown in red color, ingredient present in medium amount is displayed in yellow and the component present in acceptable amount is shown in green color [14]. Back-of-pack label incorporates the detailed information guide at the back of the packet along with the mandatory details like usage, storage, allergen specifications, etc.

Consumer's awareness for nutrition labelling. Among the several papers reviewed, the basic inference drawn is that the metropolitan cities have higher literacy rate and the people over there have a higher ratio of food label knowledge as compared to rural cities and towns. The consumers if they occur to be knowledgeable, they do not practice it often. The urban consumers read the food labels for fats and sugars whereas the rural population rarely read for vitamin A and folic acid [12]. The findings of a study revealed that although 76 per cent consumers were aware of nutritional labelling but out of them only 58 per cent of the consumers were practicing the use of nutrition labels. The people showed a casual attitude towards brand and convenience in interpreting the food labels. The population surveyed in study reflected high awareness in context to nutrition labelling but still was not satisfied with the format of the labels. Among the six factors i.e., convenience, brand, taste, nutrition, quality, and price; majority of the population focused on quality and only 25 per cent preferred the nutritional content as their choice [15]. A study conducted in Srinagar India, reported that 78.4 per cent were aware about food product order; 55.8 per cent people were known of food adulteration, 1.96 per cent understood about Codex Alimentarius and 13.2 per cent had no information about these food standards. Around 94.11 per cent subjects looked for expiry date, which made it an extremely essential component of the packaged foods [16].

Consumer's preferences in nutrition labelling usage. Studies have indicated that consumers use nutritional labeling depending upon their own personal choice. The many factors have been reported till now in usage of nutritional labeling by consumers. The knowledge, understanding usage of nutritional label is mainly dependent either positively or negatively on the factors like gender, age, socio-economic status, qualification, dietary specifications, marital status, display format, frequency of purchasing and health consciousness.

Factors affecting usage of nutrition labels. The common reasons given by the consumers for not using labels are time-constraints, lack of interest in nutrition knowledge and font size of the print, which was supported by the findings that proved 9 per cent found it difficult to understand, 14 per cent suffered time-constraints, 22 per cent had no interest, 31 per cent had no opinion and the remaining either trusted themselves for their choices or did not understand the technical terms [15]. A researcher in his study found that about 38 per cent of the total consumers surveyed, reported that the information provided was not adequate as the font size was small, the terminologies were complicated however, the packages were so appealing that they got lured by the glare of the food packets but were not satisfied with the display of nutrition information panel [17]. A researcher from his review paper authenticated that the knowledge level and health status of an individual majorly affects the use of nutrition labels [18]. To support this outcome, one more study demonstrated that the people with low level of knowledge and financial status have difficulties in understanding the labels and that is why they do no us them [19].

When investigated for the reasons of not using nutrition information, it was revealed that the information might not be credible; lack of time and nutrition knowledge; complicated terminologies; trust built on the renowned brands were the common reasons [12]. A study concludes that the displayed information is mostly confusing and difficult to understand by the respondents which supports various other studies in context to nutritional labels [16]. According to a study, the consumers were able to understand some of the terms but were not able to interpret it completely [20]. Some authors in their respective studies concluded that the consumers found the printed information difficult and confusing which weakens their interest in reading the labels [21,22]. Similarly, two studies revealed that the lack of nutrition knowledge among the consumers was the primary factor which affects their labelling usage [23,24].

Other factors. Gender and Age. Gender is a deciding factor for the knowledge level and purchase options in almost 20 countries of the Global South [12]. A research done by demonstrated a strong association between increasing age and the awareness regarding the nutrition labels [25]. Several authors through their research works found that the women participants were more active in reading labels as compared to the men counterparts [17,26,27]. A study conducted in Puducherry reported no significant association of age, gender, education, special dietary requirements, occupation with the awareness of labels. It emphasizes on the interdependence of reading of labels with special dietary specifications of the consumers [28,29].

Educational background of the consumer. A study done by Darkwa basically explains and proves that the level of education not always plays an influencing role in making purchase decisions.[30] Somewhat similar findings were obtained from another study, that the level of education was not a deciding factor for nutritional knowledge among the consumers, but it had an association with the frequency of reading the nutritional labels [31] But various other studies contradicted the above proofs and showed a strong positive association of education with label usage. They demonstrated the positive association of educational background with the nutritional knowledge, attitude, and its practice. A clear positive association between the level of education and food labelling usage was obtained through the study which indicated that; higher the level of education, more is the knowledge and awareness among the respondents regarding the use of nutrition labels. Another research done also proved that people with high level of education are more likely to read labels [12, 5, 17, 32].

Special dietary requirements of consumers. The knowledge level of the gym-goers and the persons having certain special dietary restrictions is quite notable than the non-gym goers and the people having no such health conditions [5]. On the contrary, two authors were not able to build any association between the nutrition knowledge of gym-goers and non-gym goers [33]. Vemula in his study found that the adolescent girls and women usually avoid the foods rich in sugar because of their health consciousness [17]. Similar results were reported in Ireland that people who followed a special diet only make concerns about the use of nutritional labels [15]. A study found that the people who had some dietary restrictions regarding CVDs, showed notable understanding about the claims and labels [16]. The subjects suffering from obesity were into a habit of reading food labels properly than the normal weighted respondents [31].

Socio-economic status. The socio-economic status of the consumers has a strong influence on their knowledge and purchase decisions [12]. The situational and demographical factors do play a significant role in the usage of nutrition labels by the population [5]. There was no association found between the occupation of an individual with their purchase decisions and it was revealed that the subjects who had less income has lesser interest in reading food labels [18, 16].

Consumers recommendations for nutritional labelling. A study conducted in Ireland suggested to provide truthful and authentic food labelling information to encourage its usage among the

commoners [34]. The subjects studied by Madhvapaty and Dasgupta have advised for the transparent and simple format of nutritional labels for the sake of customers benefit [2]. The commonly stated suggestions by the subjects for the improvement of the nutritional labels on the food packages includes-increment in the font size of the information, more graphical & symbolic indications of healthy foods can be displayed, nutrition awareness must be created, and simpler terms must be used. People also wanted more of symbol-intensive labelling display for easy inference than the complicated, time consuming text-intensive display [17].

A researcher in his study reiterated on the ideas of promoting public health education programs that may advertise the need of healthy eating. There is a strong need for simplifying the food labels for a better global health status throughout [35]. In a study executed the findings revealed that the consumers in Ghana exhibited varying levels of knowledge regarding the nutrition labels, which directly indicated that the consumers should be made more aware about the food labels usage [30]. A research study reiterated the importance of providing fundamental nutrition knowledge to the people and motivating them to bring those guidelines into daily practice [31]. Implementation of nutrition education programs is highlighted in this study which requires urgent administration among the population for the betterment of the dietary conditions in and around the world [36].

There exist numerous FOP formats which is an obstacle in the usage of nutrition labels as the multiple layouts leads to complications in the understanding [37]. According to a study done, 'per serving size' display format is more convenient to interpret by the participants of the survey [38]. A researcher surveyed the consumers of Chile and found that they too prefer the 'per serving size' nutrition information [39]. But on the contrary, a research study done in India, concluded that the food products should be with front-of-pack labels using images or logo and health profit for healthy selection of foods [40].

Very few studies are known which advances the use of front-of-pack nutrition labels or traffic-light labels around the world [41.] In a study conducted, the conclusion withdrawn is that the participants found the traffic light labelling format easier than other front-of-pack display [2]. Consumers also favour the format of traffic light labelling as it is easy for them to differentiate between the color codes [14]. The front-of-pack nutrition labelling is favored in several studies as it is easy to have a quick-glance at and more interactive [42, 43]. On the contrary, a researcher found that the participants were not satisfied with the front-of-pack display format at all and considered it as confusing as surveyed on consumers of UK [44].

CONCLUSION

Nutrition Labelling is a mandatory phenomenon that every packaged food product needs to undergo for their valuable selection by the consumers in majority of the countries across the world. The studies reviewed basically throws light upon the consumer understanding, factors affecting their use of labels, need to modify it and the advantages of its advancement. It can be very well understood by the respondent's point of view that the quality control agencies need to implement effective measures regarding the better utility of the food labels. The study also highlights the need of a provision of elementary nutrition knowledge at school and college level. Also, there should be a push on simplification of terminologies and usage of symbol-intensive display formats on the packaged foods. more detailed study on the association between the consumers' practice and their dietary conditions, simplification of symbols, etc. Nutritional labeling regulatory agencies should do amendments in regulations based on consumers review for better enforcement of law thereby proving easiness for consumers. More efforts are required to educate consumers so that they can be benefitted by nutritional information of food.

ACKNOWLEDGEMENT

This study was supported by Department of Nutrition and Dietetics, Manav Rachna International Institute of Research and Studies, Faridabad, Haryana.

Funding Source. We have received no financial support for the research, authorship, and/or publication of this article from nay agency.

REFERENCES

[1] Waxman, A. and Norum, K.R. (2004) Why a global strategy on diet, physical activity, and health? The growing burden of non-communicable diseases. Public Health Nutrition. 7(3), 381-383.

[2] Madhvapaty, H. and DasGupta, A. (2015) A study of food product labelling for products aimed at children. IOSR Journal of Business and Management. 17(3), 88-96.

[3] Roberto, C.A., Bragg, M.A., Livingston, K.A., Harris, J.L., Thompson, J.M., Seamans, M.J. and Brownell, K.D. (2012) Choosing front-of-package food labelling nutritional criteria: how smart were 'Smart Choices'? Public Health Nutrition. 15(2), 262-267.

[4] Codex Alimentarius Commission (1985) Codex Guidelines on Nutrition Labelling. CAC/GL 2-1985 (Rev. 1-1993). Retrieved June 4, p.2002.

[5] Donga, G., and Patel, N. (2018) A review of research studies on factors affecting consumers' use of nutritional labels. Nutrition and Food Science International Journal. 7(3), 555713.
[6] Jadapalli, M., and Somavarapu, S. (2018) A Survey on Perception of Food Labels Among the Population of Nellore District. American Journal of Food Science and Nutrition. 5(1), 1-16.
[7] Hawkes, C. (2004) Nutrition labels and health claims: the global regulatory environment. World Health Organization. 74.
[8] Campos, S., Doxey, J. and Hammond, D. (2011) Nutrition labels on pre-packaged foods: a systematic review. Public Health Nutrition. 14(8), 1496–1506.
[9] Hawkes, C. (2009) Government and voluntary policies on nutrition labelling: A global overview. Food and Nutrition Policy, France. 37-58.
[10] Ministry of Health and Family Welfare; "Food Safety and Standards (packaging and labelling) regulations" (2011). F.No. 2-15015/30/2010
[11] Astrup, A. (2001) The role of dietary fat in the prevention and treatment of obesity. Efficacy and safety of low-fat diets. International Journal of Obesity. 25(S1), S46–S50.
[12] Mandle, J., Tugendhaft, A., Michalow, J. and Hofman, K. (2015) Nutrition labelling: a review of research on consumer and industry response in the global South. Global Health Action. 8(1), 1-10.
[13] Hawley, K.L., Roberto, C.A., Bragg, M.A., Liu, P.J., Schwartz, M.B. ,and Brownell, K.D. (2013) The science on front-of-package food labels. Public Health Nutrition. 16(3), 430-439.
[14] Hieke, S. and Wilczynski, P. (2011) Colour Me In–an empirical study on consumer responses to the traffic light signposting system in nutrition labelling. Public Health Nutrition. 15(5), 773-782.
[15] Shine, A., O'Reilly, S., and O'Sullivan, K. (1997) Consumer use of nutrition labels. British Food Journal. 99(8), 290-296.
[16] Masoodi, N. and Mubarak, H. (2019) Nutritional knowledge and consumers use and understanding of food labels. International Journal of Physiology, Nutrition and Physical Education. 4(1), 1371-1376
[17] Vemula, S.R., Gavaravarapu, S.M., Mendu, V.V.R., Mathur, P., and Avula, L. (2014). Use of food label information by urban consumers in India–a study among supermarket shoppers. Public Health Nutrition. 17(9), 2104-2114.
[18] Drichoutis, A.C., Lazaridis, P., and Nayga, R.M. (2005) Nutrition knowledge and consumer use of nutritional food labels. European Review of Agricultural Economics. 32(1), 93-118.
[19] Malam, S. Clegg, S. Kirwan, S. McGinigal, S. Raats, M. Shepherd, R. Barnett, J. Senior, V. Hodgkins, C. and Dean, M. (2009) Comprehension and use of UK nutrition signpost labelling schemes. London: Food Standards Agency.
[20] Cowburn, G. and Stockley, L. (2016) Consumer Understanding and Use of Nutrition Labelling: A Systematic Review Article. Public Health Nutrition. 8(1), 21–28.
[21] Carrillo, E., Varela, P. and Fiszman, S. (2012) Influence of nutritional knowledge on the use and interpretation of Spanish nutritional food labels. Journal of Food Science .77(1), H1-H8.
[22] Bandara, B.E.S., De Silva, D.A.M., Maduwanthi, B.C.H., and Warunasinghe, W.A.A.I. (2016) Impact of food labeling information on consumer purchasing decision: with special reference to faculty of Agricultural Sciences. Procedia Food Science. 6, 309-313.
[23] Kelly, B., Hughes, C., Chapman, K., Louie, J.C.Y., Dixon, H., Crawford, H.J., King, L., Daube, M., and Slevin, T. (2009) Consumer testing of the acceptability and effectiveness of front-of-pack food labelling systems for the Australian grocery market. Health Promotion International Journal. 24(2), 120-129.
[24] Miller, L.M.S. and Cassady, D.L. (2015) The effects of nutrition knowledge on food label use. A review of the literature. Appetite. 92, 207-216.
[25] Fulgoni, V.L. and Miller, G.D. (2006) Dietary references intakes for food labelling. American Journal of Clinical Nutrition. 83(5), 1215-1216.
[26] Smed, S., Edenbrandt, A.K., Koch-Hansen, P., and Jansen, L. (2017) Who is the purchaser of nutrition-labelled products? British Food Journal. 119(9), 1934-1952.
[27] Su, D., Zhou, J., Jackson, H.L., Soliman, G.H., Huang, T.T., and Yaroch, A.L. (2015). Peer Reviewed: A Sex-Specific Analysis of Nutrition Label Use and Health, Douglas County, Nebraska. Preventing Chronic Disease. 12, E158.
[28] Peters-Texeira, A. and Badrie, N. (2005) Consumers' perception of food packaging in Trinidad, West Indies, and its related impact on food choices. International Journal of Consumer Studies. 29(6), 508-514.
[29] Kar, S., Jain, S., and Gomathi, R. (2018) Consumer awareness and status of food labeling in selected supermarkets of Puducherry: An exploratory study. International Journal of Advanced Medical and Health Research. 5, 36-40.
[30] Darkwa, S. (2014) Knowledge of nutrition facts on food labels and their impact on food choices on consumers in Koforidua, Ghana: a case study. South African Journal of Clinical Nutrition. 27(1), 13-17.

[31] Robert, S.D., Chandran, A. (2017) Survey on consumer knowledge and use of food labels. International Journal of Health Science and Research. 7(10), 203-209.
[32] Blistein, J.L., and Evans, W.D. (2006) Use of nutrition facts panel among adults who make household food purchasing decision. Journal of Nutritional Education and Behavior. 38(6), 360-364.
[33] Wade, S., and Kennedy, O.B. (2010) Does gym use impact upon nutritional knowledge? British Food Journal. 112(1), 44-54.
[34] Food Safety of Ireland (2009) Attitudes to Food Labelling. Northern Ireland Protocol Nutrition Labeling. F/No. 1169/2011.
[35] Nayga Jr, R.M. (1996) Determinants of consumer's use of nutritional information on food packages. Journal of Agricultural and Applied Economics. 28(2), 303-312.
[36] Buyuktuncer, Z., Ayaz, A., Dedebayraktar, D., Inan-Eroglu, E., Ellahi, B., and Besler, H.T. (2018). Promoting a healthy diet in young adults: The role of nutrition labelling. Nutrients. 10(10), 1335-1337
[37] Draper, A.K., Adamson, A.J., Clegg, S., Malam, S., Rigg, M. ,and Duncan, S. (2013). Front-of-pack nutrition labelling: Are multiple formats a problem for consumers? European Journal of Public Health. 23(3), 517–521.
[38] Singla, M. (2010). Usage and understanding of food and nutritional labels among Indian consumers. British Food Journal 12(1), 83-92.
[39] Gregori, D., Ballali, S., Vecchio, M.G., Contreras L.M.V., Correa, J.B., Perez, C.B., Luengo, J.B., Moyano, E., Arrieta, M., Gutierrez, A., and Ghidina, M. (2013). How to communicate nutritional information to people: the attitudes of Chile population toward food. The Open Obesity Journal. 5(1), 36-42.
[40] Pande, R., Gavaravarapu, S.M., and Kulkarni, B. (2020). Front-of-pack nutrition labelling in India. The Lancet Public Health 5(4), E195.
[41] Kim, W.K. and Kim, J. (2009). A study on the consumer's perception of front-of-pack nutrition labeling. Nutrition Research and Practice. 3(4), 300-306.
[42] Kleef Van E. Trijp Van, H. Paeps, F. Celemín, L.F. (2008). Consumer preferences for front-of-pack calories labelling. Public Health Nutrition. 11(2), 203-213.
[43] Herpen Van, E. and Trijp Van, J.C.M. (2011). Front-of-pack nutrition labels. Their effect on attention and choices when consumers have varying goals and time constraints. Appetite. 57(1), 148-160.
[44] Grunert, K.G. Wills, J.M. and Fernández-Celemín, L. (2010). Nutrition knowledge and use and understanding of nutrition information on food labels among consumers in the UK. Appetite. 55(2), 177-189.

Received: **24.05.2021**
Accepted: **30.06.2021**

CORRESPONDING AUTHOR

Madhvi Awasthi
Department of Nutrition and Dietetics,
Faculty of Allied Health Sciences,
Manav Rachna International Institute of Research and Studies,
Faridabad 121001 – India

e-mail: madhviawasthi84@gmail.com

BIONOMICS OF *Squilla mantis* (LINNAEUS, 1758) FROM MAKOKO AREA OF THE LAGOS LAGOON, NIGERIA

Mosunmola Florence Akinwunmi[1,*], Oluayo Anthony Bello-Olusoji[2], Hannah Ene Egwu[1]

[1]Department of Marine Sciences, Faculty of Science, University of Lagos, Nigeria
[2]Department of Fisheries and Aquaculture Technology, School of Agriculture and Agricultural Technology, Federal University of Technology Akure, Nigeria

ABSTRACT

Spot-tail mantis shrimp, is a marine crustacean belonging to the Family Squillidae and a member of the genus - Squilla. Mantis shrimps are so called because the second pair of limbs are greatly enlarged and shaped like the large grasping forelimbs of the praying mantis (an insect). The physico-chemical parameters of the water samples from the study site were measured in-situ and recorded. Samples of *Squilla mantis* were collected monthly between April and September, 2019. The size composition and growth pattern, length weight relationship, food composition and the reproductive biology of *Squilla mantis* were examined for a period of 6 months. The physico-chemical parameters of water samples collected from the Lagos Lagoon indicated that the condition of the lagoon was conducive to the aquatic organisms inhabiting it. *S. mantis* Total Length (TL) ranged between 7.3 and 15.0 cm, Carapace Length (CL) - 1.4 - 4.0 cm and Total Weight (TW) - 3.0 - 30.0 g. The length-weight relationship of the species exhibited a negative allometric ($b<3$) growth. The coefficient of determination (R^2) value for *S. mantis* was 0.2771, it showed a weak and negative growth pattern. The condition factor (K) for the combined sexes of *S. mantis* ranged from 0.9 - 1.5. The stomach contents were made up fish parts (17.0%), worms (19.0%), molluscs (oysters/squids) (6.0%), Crustaceans (small crabs and shrimps), (3.0), some unidentified food masses (1.0) and detrital organisms (5.0%). The food composition showed that *S. mantis* is a predatory species. The sex ratio (male: female) for *S. mantis* was 1:0.38 and there was significant difference ($P < 0.05$) in the sex ratio. The fecundity estimated ranged between 225 and 1960 eggs with a mean of 318.36 eggs. There was a strong and positive correlation ($r > 0.5$) between fecundity and total length, fecundity and body weight ($r > 0.5$) for *S. mantis* from the lagoon. The study provided a baseline information and an addition to knowledge on Mantis Shrimp for fisheries management, taxonomist and for the population monitoring.

KEYWORDS:
Food composition, Lagos Lagoon, length-weight, physico-chemical, sex ratio, *Squilla mantis*

INTRODUCTION

Spot-tail mantis shrimp is a marine crustacean that can grow up to the length of 30 cm (12 inches) and a times can grow up to 15 inches. It's not actually a shrimp, but gets its name because it resembles a praying mantis and a shrimp. Spot-tail mantis shrimp is distributed off West Africa from Gibraltar to Angola and can also be found in the Mediterranean. It occurs from sublittoral areas to more than 200 metres depth, but generally in 120 metres or less [1]. It is generally dull brown in coloration, has two brown eye spots, circled in white and they possess chelipeds which are jaw bearing limbs [2-3].

S. Mantis dig burrows in muddy and sandy bottoms and it remains in its burrow during the day and comes out at twilight or night to hunt or mate. They are usually abundant where there is significant runoff from rivers and where the substrate is suitable for burrowing [4].

There are two main types of mantis shrimp: 'spearers' and 'smashers' while the *Squilla mantis* is of the spearer type which use a tactic called ambush predation in feeding. 'Spearers' are usually claw lined with numerous sharp teeth and they hunt by impaling prey on these teeth. They hunt primarily during the night using mostly long duration attacks [5]. They feed on soft- bodied aquatic organisms like fish, marine worms and few other aquatic organisms. The mantis shrimp is a benthic species, strongly related to bottom sediments, as demonstrated by its burrowing behaviour and by the composition of its diet [4]. They are very quick and deadly predators, which prey on crustaceans such as clams, fish or other small invertebrates. They can either pierce or smash their prey with their fierce appendages. They can pierce the soft shell of a fish or smash and break open the shell of a clam to retrieve the soft tissue.

They play an important role in marine ecosystems because they help in regulating the numbers of other species and high promoter of the overall species richness. Also, where the seabed is soft, their burrowing behavior contributes to the turnover and oxygenation of sediments. The usual danger associated with consuming sea foods caught in contaminated waters still applies to mantis shrimp [6].

Length weight relationship is a useful tool in fisheries research and assessment because it allows for the conversion of growth in length assessment, estimation of the biomass from the length observation, estimation of the condition of the species and to determine the growth pattern [7].

Due to the paucity of information on the identification and diversity of Mantis shrimp in Nigeria, the bionomics of this species becomes necessary because the detailed scientific study is still at its infancy and besides, the biology of an organisms attracts the attention of biologists. Hence, the project aimed at studying the bionomics of *Squilla mantis* in order to provide a baseline data on the species and towards the management and conservation of the fisheries resources in Lagos Lagoon.

MATERIALS AND METHODS

Brief description of study area. The study area is located at the South Western coast of Nigeria which lies within the Lagos Lagoon and receives fresh water from Lekki Lagoon via Epe Lagoon in the North-East and discharges from Majidun, Agboyi and Ogudu creeks as well as Ogun River in the North West [8-9]. Makoko is located at coordinates of 6°29′44″N latitude and 3°23′39″E longitude while the Lagos Lagoon (Figure 1) have coordinates of 6°26′59.99″N latitude and 3°22′59.99″E longitude and a surface area of 6, 354 km^2.

SAMPLES COLLECTION

Collection of water samples. Water samples were collected from the study area with a plastic bottle and measured in-situ to determine the physico-chemical parameters of the water. The temperature (^{0}C), salinity (‰), Dissolved oxygen (mg/L), pH and conductivity (µS/cm) were measured using a Mercury-in-glass thermometer, hand refractometer (Model No: RHS-10), Hanna DO meter (Model: HI 9146), Hanna pH meter (Model: HI 2210) and a Hanna conductivity meter (Model: EC 215) respectively.

Collection of specimen (*Squilla mantis*). Monthly samples of *S. mantis* were collected from the local fish traders at the Makoko fish market for a period of six (6) months between April and September, 2019. Collection of the specimen was done randomly from the traps landing which were set in the lagoons. A total of 234 specimens of *S. mantis* (Plate 1) used for this study were obtained from the lagoon and they were immediately preserved in an ice-chest and later transported into a deep freezer at temperature of -20 ^{o}C in the Marine Research Laboratory of the Department of Marine Sciences for further studies.

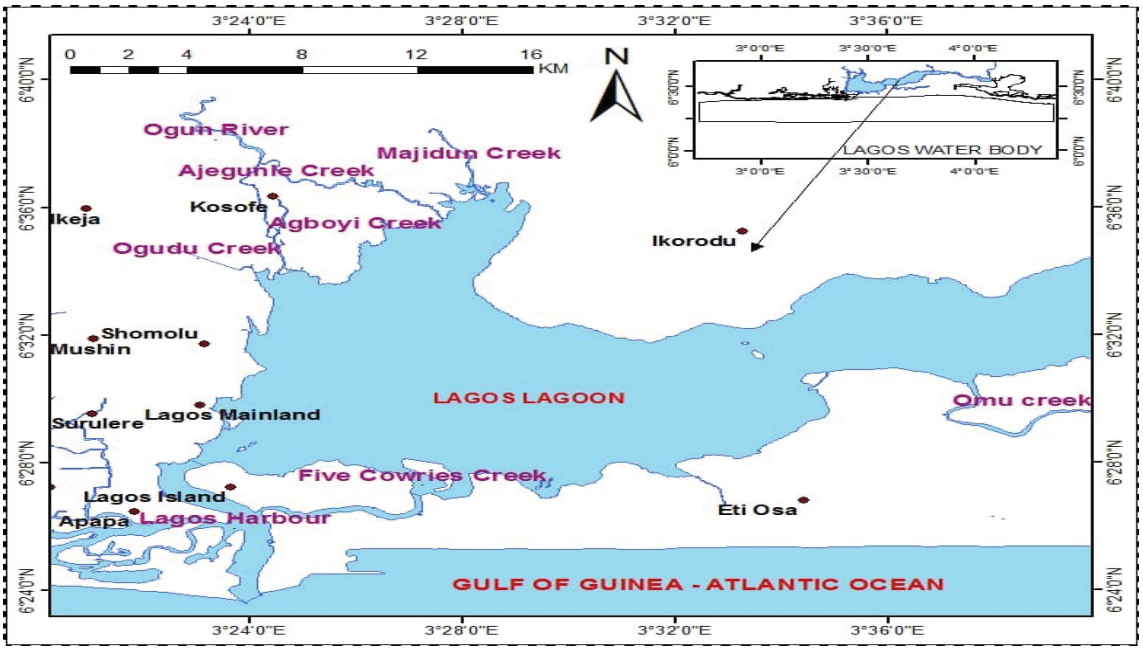


FIGURE 1
Map showing the sampling area and other surrounding water bodies [10].

PLATE 1
The dorsal view of Mantis Shrimp (*Squilla mantis*)

LABORATORY PROCEDURES

The Mantis shrimps (*Squilla mantis*) were removed from the freezer and allowed to thaw. Excess water was removed from the specimens using a pile of filter papers. The total length of the shrimps (from the tip of the rostrum to the end of the telson) and the carapace length (from eye socket to posterior end of the carapace) was measured using a tape rule. The total body weight and the carapace weight was measured on an electronic weighing balance (Model: DT 1001A) to the nearest 0.01 gram. Other length measurement of the specimen that were measured and recorded include: Standard length (SL), Abdominal length (AL), Ocular length (OL), Telson length (Tel-L), Right cheliped length (RCL), Left cheliped length (LCL). All these parameters were measured using a tape rule.

Growth pattern. Length – Frequency distribution. The length-frequency distribution and weight-frequency distribution were determined monthly from the data of individual specimens on lengths and weight.

Length –Weight relationship. The length-weight relationship was estimated using the equation: [11-12]

Where W= weight of the shrimps in grams.
L = total length of the shrimps in cm.
a = regression constant
b = regression coefficient

The values of constant a and b were estimated from log transformation values of length and weight as:

$$Log\ W = Log\ a + b\ Log\ L \qquad [13]$$

Condition factor (K) in *Squilla mantis*

The condition factor (K) which indicates the state or general well-being of the prawn was determined using the formula:

Where W = weight of the prawn in gram
L = total length of the prawn in cm

Food and feeding habits in *Squilla mantis*. The stomachs of the collected shrimps were examined and scored with regards to whether they are empty ($^0/_4$), ¼ full, ½ full, ¾ full and/or full ($^4/_4$). Each stomach contents were dissected out with the contents washed into a Petri dish. These contents were examined under a binocular microscope. The analysis of the stomach contents was carried out by both the numerical and frequency of occurrence methods as described by [15-16]. The food items were identified with the aid of the keys using several texts [17-19].

Reproductive biology. Sex Ratio in *Squilla mantis.* *S. mantis* was separated into males and females by physical observation. On the underside of the male mantis, below the last pair of walking legs, is two inwards facing sticks ("V" shape right behind the last set of walking legs or right in front of the first swimmate). The males also have paired penes that arise from the last pair of walking legs on the eighth thoracic sternite. This is absent in the females but in its place, are two white bumps. Females have three internal cement glands that are visible through the exoskeleton on the thoracic sternite surface and they also carry eggs on their underside similar to other arthropods [20].

Fecundity in *Squilla mantis.* A total of 26 fecund ***Squilla mantis*** from Lagos Lagoon were examined. The eggs were removed from the female prawns and excess fluid was dried with the aid of filter papers. They were then weighed on an electronic weighing balance (Model: DT 1001A) to the nearest 0.01 gram and the weight recorded. The total number of ripe eggs was estimated using the gravimetric method [21]. The relationship between the fecundity, length and weight of the prawns was expressed as:

Where Y = fecundity estimate
X = total length (cm) / total weight (g)
a = regression constant
b = regression coefficient

STATISTICAL ANALYSIS

The statistical analysis was carried out using the Predictive Analytics SoftWare (PASW) Statistics version 18 and PAleontological STatistics (PAST) version 2. Test for goodness of fits was determined statistically using chi-square (χ^2) test on sex ratios of the species.

RESULTS

The summary of the physico-chemical parameters of Lagos Lagoon is presented in Table 1. The ranges and mean values of the water temperature, dissolved oxygen, pH, salinity and the conductivity are 23 – 29 ºC (27.33 ºC), 4.5 – 7.9 mg/L (5.87 mg/L), 6.8 – 8.7 (7.43), 15.2 – 29.2 ‰ (20.4 ‰) and 652 – 13850 µS/cm (8816.83 µS/cm) respectively.

A total of 234 specimens of *S. mantis* were collected from the Lagos Lagoon, but no samples of the mantis shrimps were available for collection in the month of June and September, 2019. The sizes of *S. mantis* from the Lagoon ranged from 7.3 – 15.0 cm (total length), 1.4 – 4.0 cm (carapace length) and 3.0 – 30.0 g (total weight) with mean values of 10.97 cm, 2.53 cm and 15.20 g respectively (Table 2). The relationship between total length and weight of *S. mantis* is illustrated in Figures 2-4 for male, female and the combined sexes respectively. The relationship between log total length and log weight measurements of the species is presented in the regression equations shown below:

Male: Log Y = -1.5785 + 2.6299 Log X
n = 169, R^2 = 0.9686
Female: Log Y = -1.2416 + 2.3201 Log X
n = 65, R^2 = 0.8645
Combined sexes: Log Y = -0.1524 + 1.2638 Log X
n = 234, R^2 = 0.2771

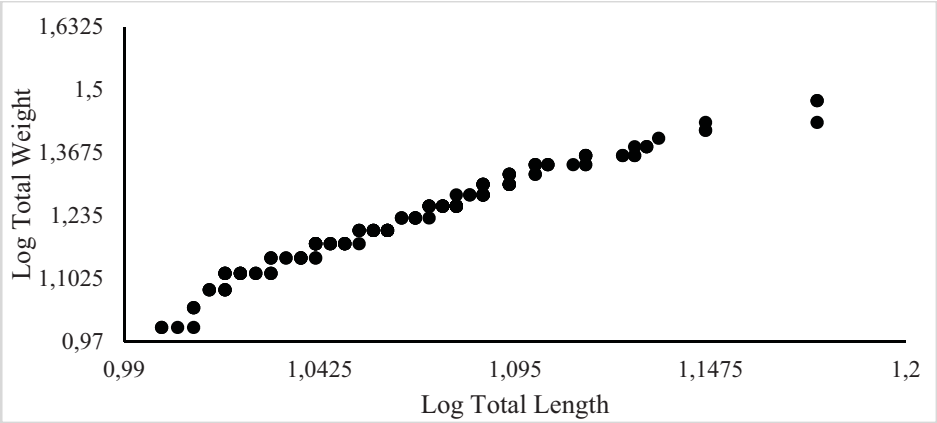
The variations in condition factor (K) by size and sex of *S. mantis* from the lagoons is presented in Table 3. The K values ranged from 0.9 – 1.1 (male), 1.1 – 1.5 (female) and 0.9 – 1.5 (combined sexes). The highest K-value (1.5) was recorded for the female within the size group of 7.5 – 8.4 while the lowest mean K value (0.9) was recorded within the males within the size group of 14.5 – 15.4.

TABLE 1
Ranges and Means of physico-chemical parameters of Lagos Lagoon (April - September, 2019)

PARAMETERS	Range	Mean
Water temperature (ºC)	23 - 29	27.33
Dissolved oxygen (mg/L)	4.5 – 7.9	5.87
pH	6.8 – 8.7	7.43
Salinity (‰)	15.2 – 29.2	20.4
Conductivity (µS/cm)	652 – 13850	8816.83

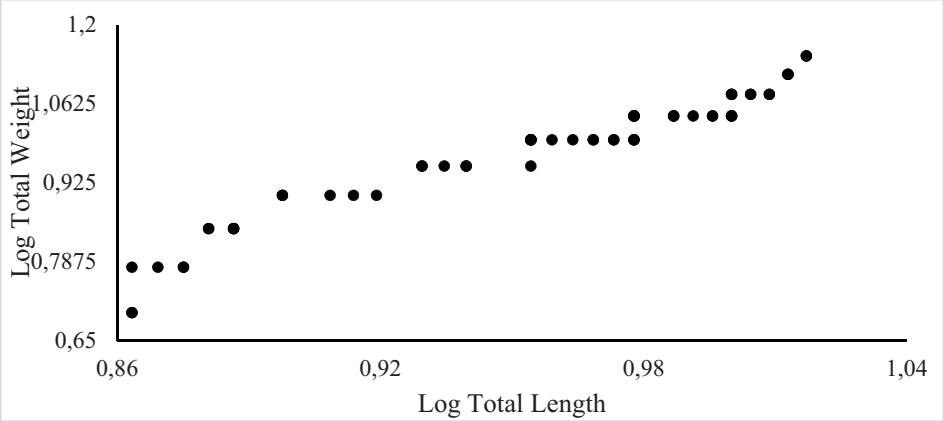
TABLE 2
Morphometric and meristic (Morphomerics) features of *Squilla mantis* from the Lagos Lagoon (April – September, 2019)

Measurements	Minimum values	Maximum values	Mean values
Total Length (TL) cm	7.3	15.0	10.97
Standard Length (SL) cm	5.4	13.3	9.24
Abdominal Length (AL) cm	1.5	11.8	7.08
Telson Length (Tel-L) cm	0.8	4.3	1.78
Right Cheliped Length (RCL) cm	2.5	5.0	4.03
Left Cheliped Length (LCL) cm	2.6	5.2	4.01
Carapace Length (CL) cm	1.4	4.0	2.53
Ocular Length (OL) cm	7.0	15.5	11.86
Total Weight (TW) g	3.0	30.0	15.20
Carapace Weight (CW) g	0.5	9.0	3.84
Flesh Weight (FW) g	2.0	21.0	9.60
FECUNDITY			
Total Length (TL) cm	8.5	13.3	10.93
Total Weight (TW) g	6.0	26.0	15.58
Egg Count	225	1960	318.36



Log Y = -1.5785 + 2.6299 Log X
n = 169, R^2 = 0.9686

FIGURE 2
Log total length – Log weight relationship of *Squilla mantis* (male) from Lagos Lagoon (April to September, 2019).



Log Y = -1.2416 + 2.3201 Log X
n = 65, R^2 = 0.8645

FIGURE 3
Log total length – Log weight relationship of *Squilla mantis* (female) from Lagos Lagoon (April to September, 2019).

A total of 234 specimens of *S. mantis* were examined for food and feeding habits. 23 (9.8 %) of the shrimps from the lagoon had empty stomachs as shown in Table 4. The food items found in the stomachs of species are presented in Table 5. The food items were made up fish, worms, molluscs (Oysters/squids), Crustaceans (crabs, shrimps), some unidentified food masses and detrital organisms.

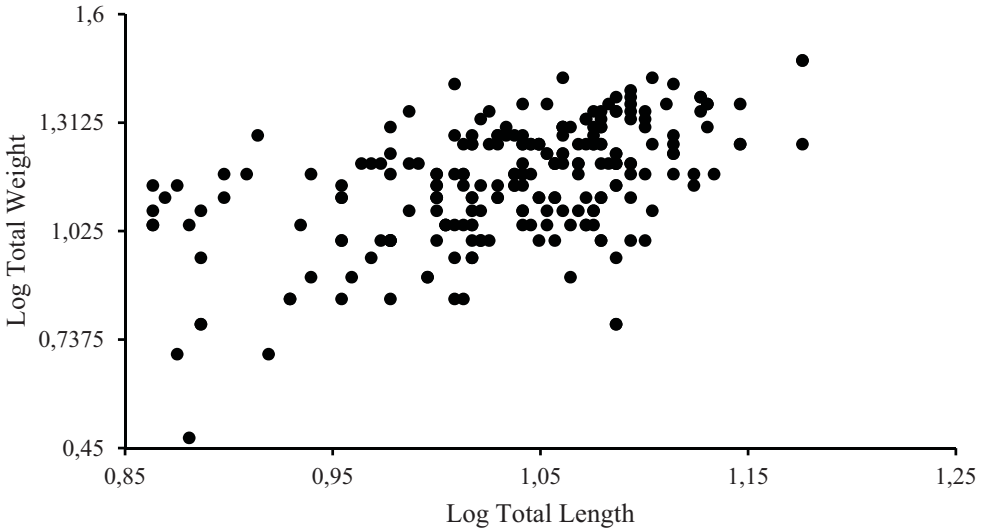
From the total of 234 specimens of mantis shrimps collected from the Lagos Lagoon, 169 were males (72.22%) and 65 were females (27.78%) giving a sex ratio of 1:0.38 (male: female) as shown in Table 6. A total of twenty-six (26) *S. mantis* collected from the lagoon were examined for fecundity. The fecund species ranged from 8.5 – 13.3 cm (total length) and weighed between 6.0 and 26.0 g. The fecundity varied from 225 - 1960 eggs with an average fecundity of 318 eggs as presented in Table 2. The Log fecundity – Log total length and Log fecundity – Log weight relationships are shown in Figures 5 and 6 respectively with the linear regression equations presented below:

Log Y = -2.272 + 4.8386 Log X
n = 26, R^2 = 0.8608
Log Y = 0.9509 + 1.531 Log X
n = 26, R^2 = 0.7861

DISCUSSION

The physico-chemical parameters of water samples collected in the lagoon from this study indicated that the values of the temperature, dissolved oxygen, pH, salinity and conductivity recorded has not changed drastically from the result obtained from previous authors. Hence, the conditions of the lagoon was favorable to the aquatic organisms inhabiting it. This supports the work of [22] on Lagos Lagoon. The salinity of the lagoon also reduces with

the onset of rainfall and dilution of the saltiness with the rain water. According to [10], the rainfall distribution has a great impact on the chemistry of lagoon water which is expressed in the salinity values. This is because the rain water is a fresh water which when added to the lagoon water, greatly reduces the salinity and the water conditions.



Log Y = -0.1524 + 1.2638 Log X
n = 234, R^2 = 0.2771

FIGURE 4
Log total length – Log weight relationship of *Squilla mantis* (combined sexes) from Lagos Lagoon (April to September, 2019).

TABLE 3
Mean monthly condition factor (K) by sex and size of *Squilla mantis* from Lagos Lagoon (April – September, 2019)

Total length (cm)	MALE				FEMALE				COMBINED SEX			
	N	TL(cm)	WT(g)	K	N	TL(cm)	WT(g)	K	N	TL(cm)	WT(g)	K
6.5-7.4					5	7.32	5.0	1.3	5	7.32	5.0	1.3
7.5-8.4					13	7.8	7.23	1.5	13	7.8	7.23	1.5
8.5-9.4					17	8.98	9.65	1.3	17	8.98	9.65	1.3
9.5-10.4	19	10.35	12.53	1.1	30	9.86	10.93	1.1	49	10.05	11.55	1.1
10.5-11.4	53	10.98	14.58	1.1					53	10.98	14.58	1.1
11.5-12.4	67	11.96	18.27	1.1					67	11.96	18.27	1.1
12.5-13.4	20	12.94	22.40	1.0					20	12.94	22.40	1.0
13.5 -14.4	7	13.73	25.14	1.0					7	13.73	25.14	1.0
14.5 - 15.4	3	15.00	29.00	0.9					3	15.00	29.00	0.9
	169			1.0	65			1.3	234			1.1

Key: N = Number, TL = Total length in (cm), WT = Total weight in (g), K = Condition factor

TABLE 4
Monthly variation in empty stomach in *Squilla mantis* from Lagos Lagoon (April – September, 2019)

YEAR/MONTH	NUMBER OF MANTIS SHRIMPS EXAMINED	NUMBER OF MANTIS SHRIMPS WITH EMPTY STOMACH	NUMBER OF MANTIS SHRIMPS WITH FOOD ITEMS	% EMPTY STOMACH
April, 2019	60	6	54	10.0
May, 2019	54	3	51	5.6
June, 2019	-	-	-	-
July, 2019	60	10	50	16.7
August, 2019	60	4	56	6.7
September, 2019	-	-	-	-
Total	234	23	211	9.8

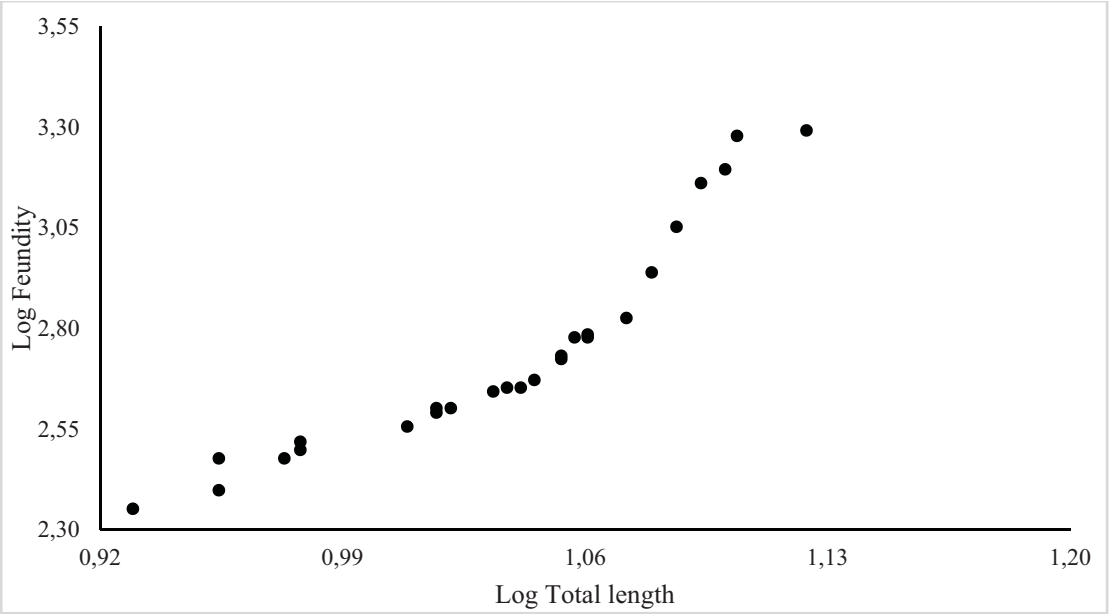
TABLE 5
Food items in *Squilla mantis* from Lagos Lagoon (April – September, 2019)

FOOD ITEMS	Numerical Method		Occurrence Method	
	Number	%	Number	%
Fish	83	36	35	17
Worms	66	29	41	19
Molluscs (Oysters/squids)	38	16	13	6
Crustaceans (crabs, shrimps)	16	7	6	3
Unidentified food mass	8	3	3	1
Detritus	20	9	10	5

TABLE 6
Monthly variation in sex ratio in *Squilla mantis* from Lagos Lagoon (April –September, 2019)

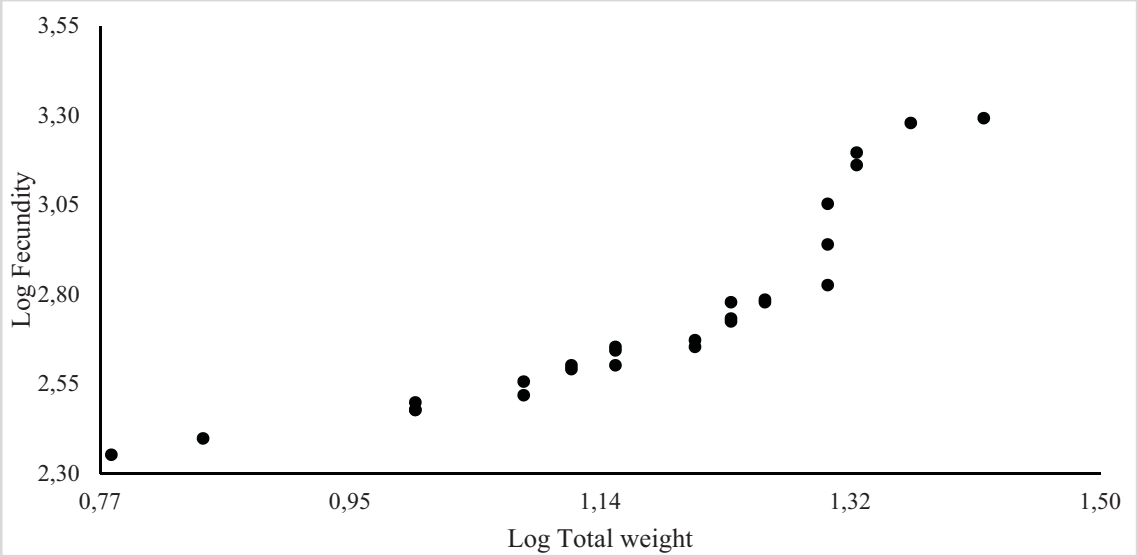
Year/Month	Number Examined	Male	Female	Sex Ratio Male : Female	Chi-square (χ^2)
Apr-19	60	50	10	1:0.20	26.67*
May-19	54	42	12	1:0.29	16.67*
Jun-19	-	-	-	-	-
Jul-19	60	42	18	1:0.43	9.60*
Aug-19	60	35	25	1:0.71	1.67
Sept-19	-	-	-	-	-
Total	234	169	65	1:0.38	46.22*

* = Significant



Log Y = -2.272 + 4.8386 Log X
n = 26, R^2 = 0.8608

FIGURE 5
Log total length / Log fecundity relationship of *Squilla mantis* from Lagos Lagoon (April – September, 2019)



Log Y = 0.9509 + 1.531 Log X
n = 26, R^2 = 0.7861

FIGURE 6
Log total weight / Log fecundity relationship of *Squilla mantis* from Lagos Lagoon (April – September, 2019)

The size frequency distribution of the total length (10.0 – 15.0, 7.3 – 10.4 and 7.3 – 15.0 cm) and total weight (10.0 – 30.0, 3.0 – 14.0 and 3.0 – 30.0 g) for the males, females and combined sexes respectively, showed that the males grew larger and weighed more than the females but the females were noticed to have had a wider telson, which was in agreement with the work of [23]. The length-weight relationship of the species exhibited a negative allometric growth (b<3) for the males, females and the combined sexes. It was depicted from these findings that the body form of this species did not grow at the same proportion (growth in length is not proportional to weight). The coefficient of determination (R^2) values for the combined sexes of the species was 0.2771, which showed a weak and negative relationship. This implied that as the shrimp increases

in length, it does not cumulate to an increase in weight. Thus, indicating that the shrimp body forms did not grow at the same proportion (growth in length is not proportional to weight). This supports the works of [24-25] on Mantis Shrimp (*Harpiosquilla raphidea*) and (*Harpiosquilla harpax*) from the Banten Bay waters, Indonesia and coastal waters of Pantai Remis, Malaysia respectively. An allometric growth pattern (but a positive growth) was also observed by [26] in Mantis Shrimp (*Harpiosquilla spp*) from Andaman Sea of Satun Province, Thailand.

Low K values showed that the animal is light for its lengths and an indication of low feeding intensity and/or spawning activity while high K value is an assumption of high feeding intensity and gradual increase in accumulated fat that also suggests preparation for a new reproductive period [27]. An aquatic animal is said to be in good condition factor when the K is equal to 1 or greater [28]. The result showed that the mean values of the condition factor were 1.0 (male), 1.3 (female) and 1.1 (combined sexes). These values are greater than 1 and it could be deduced that the mantis shrimps are in good conditions in their ecosystems. The outcome from this was in agreement with the findings of [24, 29] on Mantis shrimps.

The food composition in the stomach of these species revealed that the major food content is in animal form and mostly shellfishes (Crustaceans and Molluscs). Hence, it could be said that Mantis shrimps are predatory animals and cannibalistic in nature. There is correlation between these findings and the works of [5, 30].

Sex ratio in the present study showed that there were more males to females, giving a sex-ratio of 1:0.38 (Male: Female). However, there was also significant difference ($P < 0.05$) in the sex ratio. The findings of [25, 26] on Mantis shrimps also recorded a significant difference ($P < 0.05$) in the male to female ratios. This is in conformity with the findings from this work. However, this is contrary to the findings of [23-24, 29 and 31] as females were found to be more than the males. These could be due to differences in the location, time/year of sampling, as well as environmental changes.

The fecundity of *S. mantis* from these lagoon ranged from 225 – 1,960 eggs with a mean of 318.36 eggs. This average fecundity does not support the result from the work of [32]. They recorded an average relative fecundity of 1618±632 eggs/g of the Mantis shrimp from three Tunisian Gulfs. These variation might be attributed to the different sampling sites. Fecundity could also be affected by environmental pollution [33], [34] reported that the Lagos Lagoon could be said to be under intense pressure from pollution (untreated sewage, sawdust, detergent, industrial effluents and so on). The result showed that there existed a strong correlation ($R^2 = 0.9278$ and 0.7861) between fecundity and total length, fecundity and total body weight of *S. mantis* from the lagoon respectively. This implied that the relationship was significant and that fecundity was a measure of size. Thus, the observation implied that as the length and weight of the species increases, the number of eggs in the females also increased. The increase of fecundity with body size seems to be a rule that is applicable to many crustaceans [35-36]. A decline in the number of eggs with an increase in the size of *Penaeus plebejus* was reported by [37] and found out that this could possibly be due to ovarian senescence in large (old) females.

CONCLUSION

The study provided the prevailing physicochemical parameters of the Lagos Lagoon which determines the size composition and growth pattern, length weight relationship, food composition and the reproductive biology of *Squilla mantis*. The bionomics of *Squilla mantis* also needs to be studied to provide more information on its biological features, composition, conservation and the management, as the species are not available in this lagoon all through the year. The study provided a baseline information and an addition to knowledge on Mantis Shrimp for fisheries management, taxonomist and for the population monitoring.

ACKNOWLEDGEMENTS

The authors are grateful to the Department of Marine Sciences, Faculty of Science and the University of Lagos for providing the facilities and the enabling environment to carry out this research.

REFERENCES

[1] Food and Agriculture Organization of the United Nations (FAO), (1981) Agriculture: Toward 2000. FAO, Rome. 196 pp.
[2] D'Udekem d'Acoz, C. (2003) "Squilla mantis Crustikon – Crustacean photographic website. 8, pp 46.
[3] Caldwell, R. (2011) External anatomy and explanatory notes". Roy's List of Stomatopods for the Aquarium. 5, 5-8.
[4] Sartor, P., Abello, P. and Maynou, F. (2004) A review of the Fisheries Biology of the Mantis Shrimp, Squilla mantis (L., 1758) (stomatopoda Squidillae) in the Mediterranean. Crustaceana. 77(9), 1081-1099.
[5] DeVries, M.S., Murphy, E.A. and Patek, S.N. (2012) Strike mechanics of an ambush predator: the spearing mantis shrimp. Journal of Experimental Biology. 215, 4374-4384.

[6] James, G. (2003). "Large shrimp thriving in Ala Wai Canal muck. The Honolulu Advertiser, Hawaii's Newspaper. 33(5), 15-18.
[7] Moutopoulos, D.K. and Stergiou, K.I. (2002) Length-Weight and Length-Length relationships of fish species from the Aegean Sea (Greece). Journal of Applied Ichthyology. 18(3), 200-203.
[8] Soyinka, O.O. (2008) The feeding ecology of *Mugil cephalus* (Linnaeus) from a high brackish tropical lagoon in South-west, Nigeria. African Journal of Biotechnology. 7(22), 4192-4198.
[9] Lawal-Are, A.O. and Nwankwo, H. (2011) Biology of the Hairy Mangrove Crab, *Sersema huzardii* (Decapoda: Graspidae) from a Tropical Estuarine Lagoon. Nature and Science. 9, 158-164.
[10] Onyema, I.C. and Nwankwo, D.I. (2009) Chlorophyll: A dynamics and environmental factors in a tropical estuarine lagoon. Academia Arena. 1(1), 18-30.
[11] Edwards, A.L. (1976) An introduction to linear regression and correlation. W.H. Freeman and Company, USA, 213 pp.
[12] Pauly, D. (1983) Some simple methods for the assessment of tropical stocks. FAO Fish. Tech. Pap., 234, 52.
[13] Parsons, R. (1988) Statistical Analysis: A Decision making Approach. (2nd Edition), Harper and Row Publishers, New York, 791 pp.
[14] Bannister, J.V. (1976) The length-weight relationship, condition factor and gut contents of the dolphin fish, *Coryphaena hippurus* (L) in the Mediterranean. Journal of Fish Biology. 9, 335-338.
[15] Lagler, K.F. (1978) Freshwater fisheries biology. C. Brown Company Ltd. 421 pp.
[16] Hyslop, E.J. (1980) Stomach contents analysis: A review of methods and their applications. Journal of Fish Biology. 17, 411-429.
[17] Edmunds, J. (1978) Sea shells and other molluscs found on West African coast estuaries. Ghana University Press, Accra, Ghana. 146pp.
[18] Schneider, W. (1990) Field guide to the commercial marine resources of the Gulf of Guinea. Food and Agriculture Organization of the United Nations, Rome-Italy. 268 pp.
[19] Anderson, D.T. (1999) Invertebrate Zoology. Oxford University Press, New York. 467pp.
[20] Wortham-Neal, J.L. (2002) Reproductive Morphology and Biology of Male and Female Mantis Shrimp (Stomatopoda: Squillidae). Journal of Crustacean Biology. 22(4), 728–741.
[21] Bagenal, T.B. (1978) Aspects of fish fecundity. In: Method of Assessment of Ecology of freshwater fish production, (Gerking S. D., Ed), Blackwell Scientific Publications, Blackwell, Oxford. 75-101.
[22] Akinwunmi, M.F. and Lawal-Are, A.O. (2018) Occurrence, Size Composition and Growth Pattern of Two Caridean Species from Three Interconnecting Lagoons in South-West, Nigeria. Unilag Journal of Medicine, Science and Technology (UJMST). 6(1), 69-82.
[23] Thodoros, E.K., Emmanouil, K., Marianna, L. and Ioannis, E.B. (2018) Length-weight relationships of *Squilla mantis* (Linnaeus, 1758) (Crustacea, Stomatopoda, Squillidae) from Thermaikos Gulf, North-West Aegean Sea, Greece. International Journal of Fisheries and Aquatic Studies. 6(6), 241-246.
[24] Mulyono, M., Patria, M.P., Abinawanto, A. and Affandi, R. (2013) Length-weight relationship and condition factor in giant harpiosquillid Mantis shrimp, *Harpiosquilla raphidea* (Crustacea: Stomatopoda) in Banten Bay waters, Indonesia. International Journal of Aquatic Biology. 1(4), 185-187.
[25] Arshad, A., Sofea, T., Zamri, Z., Amin, S.M.N. and Ara, R. (2015) Population dynamics of mantis shrimp, *Harpiosquilla harpax* in the coastal waters of Pantai Remis, Perak, Peninsular Malaysia. Iranian Journal of Fisheries Sciences. 14(1), 15-26.
[26] Samphan, P. and Ratanamusik, A. (2018) The Length -Weight relationship factor and sex-ratio of Mantis Shrimp (*Harpiosquilla* spp) in Andaman Sea of Satun Province, Thailand. International Journal of Agricultural Technology. 14(1), 61-71.
[27] Braga, F.M. and Gennari-Filho, O. (1990) Contribution to the knowledge of reproduction Moenkhausia intermedia (Characidae, Tetragonopterinae) in the Barra Bonita, Rio Piracicaba, SP. Naturalist. 15, 171–188.
[28] Wade, J.W. (1992) The relationship between temperature, food intake and growth of brown trout, *Salmo trutta* (L) fed natural and artificial pelleted diet in earthen pond. Journal of Aquatic Science. 7, 59-71.
[29] Sağlam, N.E., Sağlam, Y.D. and Sağlam, C. (2018) A study on some population parameters of mantis shrimp (*Squilla mantis* L., 1758) in Izmir Bay (Aegean Sea). Journal of the Marine Biological Association of the United Kingdom. 98(4), 721–726.
[30] Mili, S., Bouriga, N., Ennouri, R., Jarboui, O. and Missaoui, H. (2013) Food and biochemical composition of the spot-tail mantis shrimp *Squilla mantis* caught in three Tunisian Gulfs: Tunis, Hammamet and Gabes. Cahiers de Biologie Marine. 54, 271-280.
[31] SeongEun, K., HanJu, K., HoJin, B., Hyeong-Gi, K. and Chul-Woong, O. (2017) Growth and Reproduction of the Japanese Mantis Shrimp, *Oratosquilla oratoria* (De Haan 1844) in the Coastal Area of Tongyeong, Korea. Ocean Sci. J. 52(2), 257-265.

[32] Mili, S., Ennouri, R., Jarboui, O. and Missaoui, H. (2014) Reproductive biology of the spot-tail mantis shrimp, (*Squilla mantis*) in three Tunisian gulfs: Tunis, Hammamet and Gabes. Bulletin de la Société zoologique de France. 139(1-4), 215-232.
[33] Johnson, L.L., Misitiano, D., Sol, S.Y., Nelson, G.M., French, B., Ylitalo, G.M. and Hom, T. (1998) Contamination effects on ovarian development and spawning success in rock sole from Puget Sound, Washington. Trans. Am. Fish. Soc. 127, 375-392.
[34] Nwankwo, D.I. and Akinsoji, A. (1989) The Benthic Algal Community of a Sawdust Deposition Site in Lagos Lagoon. International Journal of Ecology and Environmental Sciences. 15, 197-204.
[35] Udo, P.J and Ekpe, E.D. (1991) Fecundity in the African river prawn, *Macrobrachium vollenhovenii* (Herklots, 1957) from natural habitats. Journal of Tropical Aquaculture. 6 (2), 173-177.
[36] Llodra, E.R., Tyler, P.A. and Copley, J.T. (2000) Reproductive biology of three caridean shrimp, *Rimicaris exoculata*, *Chorocaris chacei* and *Mirocaris fortunate* (Caridea: Decapoda), from Hydrothermal vents. Journal of Marine Biological Association U.K. 80, 473–484.
[37] Courtney A.J., Die, D.J. and Macgilvray, J.G. (1996) Lunar periodicity in catch rates and reproductive conditions of adult eastern King prawns, *Penaeus plebejus* in coastal waters of Southeastern Queensland, Australia. Marine and Freshwater Research. 47, 67–76.

Received: **04.06.2020**
Accepted: **03.04.2021**

CORRESPONDING AUTHOR

Mosunmola Florence Akinwunmi
Department of Marine Sciences,
Faculty of Science,
University of Lagos,
Nigeria

e-mail: mfakinwunmi@gmail.com

NUTRITIONAL AND TECHNOLOGICAL EVALUATION OF BEEF BURGER SUBSTITUTED WITH QUINOA FLOUR

Nahla A Felyes[2], Waleed Z Badawy[1,*], Mohamed A El-Bana[2], Hanan E Kassab[1]

[1]Food Technology Department, Faculty of Agriculture, Kaferelsheikh University, Egypt
[2]Food Technology Department, Food Tech. Res. Institute, Agricultural Research Center, Giza, Egypt

ABSTRACT

Quinoa is one of the oldest crops of the world. Chemical composition, amino acid analysis, mineral contents and fatty acids profile of quinoa were estimated. In addition, quinoa powder was partially substituted of beef meat by different levels (2.5, 5 and 7.5 %), then compared to the control. Also, the sensory evaluation, quality parameters and physiochemical properties of different levels of beef burger were determined. The data cleared that the quinoa powder contained great contents of protein (13.49 %) and ash (7.81%). On the other hand, amino acids contents of quinoa powder contained 4.20%, 1.95%, 4.80%, 3.20% and 1.14% for valine, methionine, lysine, threonine and tryptophan respectively. Furthermore, minerals contents of quinoa flour contain higher values in all determined elements except for manganese (Mn). All of sensory evaluation of beef burger (taste, odor, texture color and overall acceptability) was acceptable. Moreover, decrease the cost of manufacture of beef burger without significant changes in its sensory properties. In contrast, protein content of beef burger substituted with quinoa powder increased by increasing the replacement levels from 2.5 to 7.5 %. Also, Addition of quinoa powder to beef burger led to improve of physiochemical properties such as rise of water holding capacity (WHC). Finally, we can mention that, the replacement of beef burger by quinoa can develop its functional and nutritional properties.

KEYWORDS:
Quinoa, beef burger, quality and physiochemical properties

INTRODUCTION

Quinoa civilized in the Andes of South America as an essential food element on behalf of numerous thousand years [1]. In modern years, quinoa has concerned increasing attention not only in South America but also worldwide, due to its abundant inflexibility to different growing conditions, its numerous customs, besides the well-balanced dietary values [2, 3].

Nowadays, many countries have expanded the production of this quinoa seed concentrating on great commercial and technological importance not only for human diet but also caused by the releases of by-products that offer good nutritional changes for animals feeding as well as applications in pharmaceutical manufacturing [4, 5, 6].

Quinoa seeds have high quality proteins and higher levels of energy, calcium, phosphorus, magnesium, iron, fiber and B-vitamins than barley, oats, have an upper nutritional rate [9].The protein content of quinoa a seed differs from 8% to 22%, which is higher on middling than that in public cereals for instance rice, wheat and barley. However, it presents less than 50% of the protein content found in most legumes. In quinoa, most of the protein is found in the embryo. In pseudo cereals, such as quinoa, globulins and albumins are the main protein fraction (44–77% of total protein), which is bigger than that of prolamins (0.5–7.0%). Quinoa is reflected to be a gluten-free grain for it contains very slight or no prolamin. Quinoa offers an economical, nutritional, easy-to-prepare, flavorful food source which is of particular application for people with gluten intolerance, for example those with celiac disease [10].

Quinoa has high protein content and its essential amino acid balance is excellent, because of a wider amino acid spectrum than that of cereal and legumes, with higher lysine and methionine contents [11, 12].

Quinoa seed is used to make diverse food products containing cookies, bread, crepes, biscuits, pancakes, muffins and tortillas. More newly, attention, has been given to quinoa for people suffering celiac disease, as an alternative to rye, wheat and barley which all hold gluten [13].

Replacement of meat with other non-meat ingredients has been practiced between processed meat industries. This additional is done too many causes such as, economic or health purposes and quality. For instance, the replacement of constituents from animal source with that of plants has been applied in food industrial [14].

The objectives of this work are to evaluate the nutritional value of quinoa seeds cultivated in Egypt.

As well as, chemical composition, amino acid analysis, mineral contents and fatty acids profile of quinoa powder were estimated. In addition, quinoa powder was partially substituted of beef meat by different levels (2.5, 5 and 7.5 %). Also, the sensory properties, quality and physiochemical characteristics of beef burger prepared using different levels of quinoa powder were evaluated.

MATERIALS AND METHODS

Materials: Quinoa seeds (Chenopodium quinoa Willd.) were got from Desert Research Center, Cairo, Egypt during the season 2017 under the recommended conditions for date of culture, fertilization, harvesting time and irrigation.

Methods: Preparation of quinoa powder: Quinoa seeds were washed numerous times with cold water to eliminate saponins until there was no more foam in the washing water, and then dried at 50 °C. The quinoa seeds were milled to fine powder in an electric grinder stainless steel using a laboratorial disc mill into flour that could pass through a 60 -80 mesh screen [15].

Chemical composition of quinoa powder and burgers: The moisture, crude protein, crude fat, ash and crude fiber contents of the quinoa flour and burger samples were determined according to [16]. The available carbohydrates were calculated by difference as follows:

Carbohydrates (%) = 100 - (crude fiber + crude protein + crude ash + crude fat).

Amino acids analysis: Amino acids content of quinoa powder was determined using Beackman amino acid analyzer according to the method of (17). Tryptophan content was analysis colorimetrically after exposing to alkaline hydrolysis as defined by (18).

Determination of minerals content of quinoa. Minerals were determined according to [19]. After wet ashing, the zinc (Zn), magnesium (Mg), copper (Cu) and manganese (Mn) contents were determined using the atomic absorption spectrophotometer (Zeiss FMD3). Calcium (Ca), sodium (Na), ferric (Fe), phosphor (P) and potassium (K) were determined using a flame photometer.

Analysis of fatty acids of quinoa: Fatty acids were extracted as descended by AOAC at National Food Institution and transmitted to methyl esters. The methyl-esters were ready by a weight of 10 mg sample in a 2 mL test tube (with a screw cap). Close the sample in 1 mL of hexane then add 10 Ml of 2N hydroxide in methanol (11.2g in 100 mL). After that close the tube and vortex for 30 sec. Samples were examined using gas chromatography Ultra GCDSQ (Thermo Fisher Scientific, Austin, USA). And the results were expressed as a percent of the relative area as described by [20].

Preparation of burgers: Four burger formulations were prepared in Food Technology Department, Faculty of Agriculture, Kafrelsheikh University according to [21] with some modifications by replacing the beef meat with three different levels (2.5, 5 and 7.5 %) of quinoa powder. All ingredients were mixed together in a blender, to ensure uniform distribution of the added ingredients, and the resulting paste was added to minced beef and mixed with quinoa powder in a separate blender. This mixture was formed by a commercial burger maker into disc pieces of 70 g and a thickness around two cm and diameter around seven cm to obtain burger.

The Sensory evaluation of burgers: The Sensory properties of substituted burgers by different levels of quinoa powder were evaluated as described by [22].

Physiochemical properties of burgers: pH value: The pH value of burgers was estimated according to (23) with a slight modification. 10 g of burgers were weighed into a conical flask (50 mL) and 20 mL of deionized water was added. Samples were homogenized for 30 seconds and the pH value of homogenate was determined using a pH meter (Mettler toledo FE20/EL20, Shanghai, China) calibrated using three buffers (pH 4, 7 and 9).

Water-holding capacity (WHC): WHC of burgers was measured according to the method adapted by (24).The WHC was calculated depending on the following equation (1)

Oil uptake: The oil content of raw and cooked burgers was determined using the soxhlet extraction method [16]. The oil uptake (%) was calculated according to the following equation:

Oil uptake (%) = $o_f - o_r / o_r \times 100$ Where: o_f is the oil content of cooked burger and o_r is the oil content of raw burger expressed as dry matter.

Texture: The texture measurement of burgers was measured using a texture analyzer as outlined by [25].

$$\%WHC = \frac{\text{Weight of sample before centrifugation (g)} - \text{Weight of sample after centrifugation (g)}}{\text{Initial moisture content of the sample (g)}} \times 100 \quad (1)$$

Statistical analysis: Most of the received data were examined statistically by means of the analysis

of variance and the means were additional tasted using the least significant variance test (LSD) as outlined by [26].

RESULTS AND DISCUSSION

Proximate chemical composition of quinoa powder: Data presented in Table (1) indicated that, crude protein, ether extract, ash and crude fiber contents of quinoa powder were 13.49, 4.62, 7.81 and 2.43, respectively. These results are in agreement with [27, 28] they reported that quinoa flour contained high amount of crude protein, ether extract, ash and all dietary fiber.

Amino acids content of quinoa: The essential and non essential amino acids content of quinoa powder are given in Table (2). The results revealed that amino acids composition of quinoa powder contained 4.20%, 1.95%, 4.80%, 3.20% and 1.14% for valine, methionine, lysine, threonine and tryptophan respectively, among the essential amino acids. Results in this respect were recorded by [29]. On the other hand, the results of non-essential amino acids indicated that, quinoa powder contained high amount of non-essential amino acids. The obtained results are in agreement with those obtained by [30, 31], who reported that, quinoa proteins have higher histidine content than barley, soy or wheat proteins.

Minerals content: Minerals or elements play an important role in human nutrition, some are essential for much component as hem for blood. Magnesium and manganese for the activation of some enzymes and stimulation insulin activity, calcium and phosphorus are essential for bones and some of them are very important for vitamins. Potassium is very important for cardiovascular diseases and zinc made the same function in helping control blood sugar levels [32].

TABLE 1
Chemical composition (% on dry weight) of quinoa powder.

Sample	Chemical composition					
	Moisture (%)	Crude Protein (%)	Ether extract (%)	Ash (%)	Crud Fiber (%)	Carbohydrates (%)
Quinoa powder	9.66±0.88	13.49±0.32	4.62±0.17	7.81±0.22	2.43±0.13	71.65±1.3

** Each value was an average of three determinations.

TABLE 2
Amino acids contents of quinoa powder (g/100g protein).

Quinoa flour			
Essential amino acids	%	Non-essential amino acids	%
Valine	4.20	Arginine	8.1
Methionine	1.95	Aspartic	7.42
Isoleucine	3.20	Serine	5.0
Leucine	5.0	Glutamic	11.9
Tyrosine	2.6	Proline	4.1
Phenylalanine	3.20	Glycine	4.9
Lysine	4.80	Alanine	3.90
Cystine	1.73	**Total non-essential amino acids**	**45.32**
Hisitidine	3.5		
Therionine	3.2		
Tryptophan	1.14		
Total essential amino acids	**34.66**		

TABLE 3

Minerals content of quinoa flour (mg/100g).

Samples	Minerals(mg/100g)								
	P	K	Na	Ca	Mg	Fe	Mn	Cu	Zn
Quinoa flour	414	881	85.7	130.4	198.6	13.2	0.26	1.86	0.95

TABLE 4
Fatty acids composition of quinoa flour (%)

Components	Fatty acids (%)
Caprylic acid(C 14:0)	0.11%
Palmitic acid(C 16:0)	15.81%
Palmitoleic acid(C 16:1)	3.11%
Stearic acid(C 18:0)	3.25%
Oleic acid(C 18:1)	41.61%
Linoleic acid(C 18:2)	36.11%
Total saturated fatty acids %	19.17%
Total unsaturated fatty acids %	80.83%
Total fatty acids %	100%

Results of minerals composition of quinoa flour are summarized in Table (3); the data indicate quinoa flour contains high values in all determined elements except for manganese (Mn) Furthermore, quinoa flour is very rich in Potassium (K) 881 mg/100g. The iron content of quinoa flour is higher eight times than that of wheat flour (82% extraction). The iron is important for the schoolchildren, which mostly needs more iron to avoid the anemia especially in developing countries. The results indicated source that quinoa flour is a good for the minerals. The results are in agreement with that reported by [33, 34].

Fatty acids analysis of quinoa flour. Fatty acids are divided into 2 main groups' essential fatty acids and non-essential fatty acids. On the other hand, the body cannot produce all types of fatty acids it needed. Non-essential fatty acids are synthesized by human body. So, they are not essentially needed, some must come from the diet; these fatty acids are called "essential fatty acids". The results presented in Table (4) cleared that fat is characterized by a high content of nutritionally valuable unsaturated fatty acids with oleic acid accounting for 41.61%, linoleic acid 36.11% and palmitoleic acid 3.11%. On the other hand, the content of saturated fatty acid is low. The quinoa fat contained 15.81% palmitic acid, 3.25% Stearic acid and 0.11% caprylic acid. From the obtained result, it can be noticed that quinoa fat was rich in unsaturated fatty acids with the higher saturated/unsaturated ratio observed from quinoa (80.83-19.17). These results in agreement with [35] who confirmed the high degree of unsaturated (87%) to saturated (13%) in quinoa fat.

The sensory evaluation of burgers: Data presented in Table (5) show the sensory properties of burger samples prepared with different levels of quinoa. Results indicate that there were no significant variances at $p \leq 0.05$ for texture, color, flavor and overall acceptability between burgers substituted by quinoa and the control burger. Therefore, supplemented burger with quinoa till 5 % could be recommended to be made as burger with good quality suitable sensory quality characteristics.

Quality characteristics of burgers: The moisture, fat, protein, ash and carbohydrates contents of control burger and burger samples replaced by different levels (2.5, 5 and 7.5 %) of quinoa powder were cleared in Table (6). The results show that the control of burger contained 57.9 % moisture, 71.0 % protein, 19.38 % fat, 9.45 % ash and 4.22 % carbohydrates. From the tabulated data, it could be noticed that the moisture content of burgers reduced such as the percentage of quinoa significantly improved.

TABLE 5

Sensory evaluation of beef burgers substituted by different levels of quinoa powder.

Formulations	Sensory properties					
	Color	Taste	Odor	Texture	Appearance	Overall Acceptability
Control	8.2 ±0.11[a]	7.8 ±0.72[a]	8.1 ±0.61[a]	8.4 ±0.46[a]	8.1 ±0.35[a]	8.2 ±0.47[a]
2.5 %	8.2 ±0.18[a]	7.8 ±0.34[a]	8.0 ±0.44[a]	8.3 ±0.73[a]	8.0 ±0.28[a]	8.2 ±0.32[a]
5 %	8.1 ±0.23[a]	7.6 ±0.46[a]	7.9 ±0.48[a]	8.1 ±0.49[a]	7.8 ±0.49[a]	8.0 ±0.33[a]
7.5 %	7.9±0.27[a]	7.4±0.45[a]	7.7±0.37[a]	7.9±0.40[a]	7.7±0.17[a]	7.7±0.22[a]

Where: Means within column with diverse letters are significantly different at $p \leq 0.05$.

TABLE 6
Quality parameters (dry weight) of burger substituted with different levels of quinoa.

Formulations	% Moisture	% Protein	% Ash	% Fat	% Carbohydrates
Control	57.9±1.0[a]	71.0±0.79[a]	9.45±0.12[a]	19.38±0.51[a]	4.22±0.14[b]
2.5 %	57.1±1.0[a]	69.5±1.1[a]	9.84±0.20[a]	19.73±0.32[a]	9.42±0.11[a]
5 %	56.7±1.1[b]	67.2±1.0[b]	10.11±0.21[a]	19.84±0.77[a]	11.85±0.13[a]
7.5 %	55.9±1.2[b]	65.5±1.2[c]	10.33±0.17[a]	19.95±0.78[a]	13.4±0.19[a]

The fat and protein contents of raw burgers had a trend like that of moisture. On the other hand, the cooking process caused significantly increases in fat content which may be related to the oil used in frying process [36]. A like observation has been reported by [37] for beef patties prepared with common bean flour and in buffalo meat patties prepared using different legume flours.

Physicochemical properties of burgers: Measuring of pH value is essential because of its effect on several properties of meat products, for example color, shelf-life, texture and water holding capacity. As shown in Table (7), the replacing of meat with 2.5, 5 and 7.5 % quinoa caused a slight decrease in the pH values of burgers compared with pH value of control burger. [38, 39] found that, the pH values of burgers fortified with different levels of lemon albedo and orange peel were lower than the control sample, these results may be due to its organic acids content such as citric and ascorbic acids.

From the same table, it could be also observed that water holding capacity (WHC) of burgers increased by increasing quinoa level from 2.5 to 7.5 %. WHC values of burgers fortified with 2.5 to 7.5 % quinoa ranged from 80.4 to 91.3 %, respectively. Compared to 72.7 % for control burger. Water Holding Capacity (WHC) of meat is reflected as one of the essential factors of quality features to determine the chance of using this meat in inducting of meat product. It is responsible for the eating quality, cooking loss, tenderness, juiciness and thawing drip of meat [39]. This property is affected by two chief causes, the level of pH value and the muscle protein. Besides, tenderness directly effects on WHC of meat protein [40].

These results showed that there was an improvement in WHC, a juicier product, with increasing concentrations of quinoa for all treatment study. Quinoa had high ability to hold water where, the increasing of quinoa level increased the WHC values which reveal increasing the ability of meat protein to holding water. The increase of WHC can be described by the increases in the water absorption capacity of protein and the gelatinization of starch at high temperatures, which absorbs water into its granules in addition to the swelling of the fiber [41]. In addition to these results, the texture of prepared burgers decreased with increasing of quinoa levels (Table 7). The decrease in the texture of fortified burgers may be related with the replacement of meat muscles with quinoa.

TABLE 7

Physiochemical properties of beef burgers substituted with different levels of quinoa.

Formulations	pH	WHC %	Oil uptake %	Texture (lb)
Control	6.01	72.7	7.10	0.85
2.5 %	6.10	80.4	13.22	0.79
5 %	6.13	88.1	22.41	0.74
7.5 %	6.14	91.3	31.19	0.60

CONCLUSIONS

It can be concluded that, the crude protein, ash, crude fat and crude fiber contents of quinoa powder were high. In addition, Most of minerals, essential amino acids and fatty acids of quinoa powder were high. On the other hand, there were no significantly variance ($P \leq 0.05$) between beef burger substituted with 2.5 to 5 % and control. Also, the nutritional and functional properties of beef burger improved. Finally, quinoa can be used as protein and nutrients sources.

REFERENCES

[1] Abugoch, L.E. (2009) Quinoa (*Chenopodium quinoa Willd*.): composition, chemistry, nutritional and functional properties. Advances in Food and Nutr. Res. 58, 1–31.

[2] Abderrahim, F., Huanatico, E., Segura, R., Arribas, S., Carmen-Gonzalez, M., Condezo-Hoyos, L. (2015) Physical features, phenolic compounds, betalains and total antioxidant capacity of coloured quinoa seeds (*Chenopodium quinoa Willd*.) from Peruvian Altiplano. Food Chem. 183, 83–90.

[3] Wang, S., Zhu, F. (2016) Formulation and quality attributes of quinoa food products. Food and Bioprocess Technology. 9, 49–68.

[4] Repo-Carrasco, R., Espinoza, C., Jacobsen, S.E. (2003) Nutritional value and use of the andean crops quinoa (*Chenopodium quinoa*) and kaniwa (*Chenopodium pallidicaule*). Food Rev. Int. 19(2), 179-189.

[5] Bhargava, A., Shukla, S., Ohari, D. (2006) Chenopodium quinoa- An Indian perspective. Industrial Crops Products. 23, 73-87.

[6] Gely, M.C., Santalla, E.M. (2007) Moisture diffusivity in quinoa (Chenopodium quinoa Willd) seeds: Effect of air temperature and initial moisture content of seeds. J. Food Eng. 78, 1029-1033.

[7] Dini, I., Tenore, G.C., Dini, A. (2005) Nutritional and antinutritional composition of kancolla seeds: an interesting and underexploited andine food plant. Food Chem. 92, 125-132.

[8] Comai, S., Bertazzo, A., Bailoni, L., Zancato, M., Costa, C., Allegri, G. (2007) The content of proteic and non proteic (free and protein-bound) tryptophan in quinoa and cereal flours. Food Chem. 100, 1350-1355.

[9] Matiacevich, S.B., Castellion, M.L., Maldonado, S.B., Buera, M.P. (2006) Water- dependent thermal transitions in quinoa embryos. Thermochimica Acta. 448, 117-122.

[10] Valencia-Chamorro, S.A. (2003) Quinoa In: Caballero B. editor Encyclopedia of Food Science and Nutrition. Academic Press, Amsterdam. 8, 4895–4902.

[11] Elsohaimy, S.A., Refaay, T.M., Zaytoun, M.A.M. (2015) Physicochemical and functional properties of quinoa protein isolate. Ann. Agri. Sci. 60(2), 297-305.

[12] Ruiz, G.A., Xiao, W., Boeke, M., Minor, M., Stieger, M. (2016) Effect of extraction pH on heat-induced aggregation, gelation and microstructure of protein isolate from quinoa (*Chenopodium quinoa Willd*). Food Chem. 209, 203-210.

[13] Jacobsen, S.E. (2011) The situation for quinoa and its production in Southern Bolivila:from Economic Success to Environmental Disaster. J. Agronomy Crop Sci. 10, 1439.

[14] Egbert, W., Payne, C. (2009) Plant proteins in ingredients in meat products: Properties, functionality and applications. Tarte, R., ed. Springer New York. 111-129.

[15] Atef, A., El-Faham, S.Y., Wafaa, H. (2012) Use of Quinoa Meal to Produce Bakery Products to Celiac and Autism Stuffs. Inter. J. Sci. and Res. 3(9), 1344-1354.

[16] A.O.A.C. Association of Official Analytical Chemistry. (2005) Officia Methods of Analysis of the Association of Official Analytical Chemists.18th Ed. Washington, DC, USA.

[17] Sadasivam, S., Manickam, A. (1992) Determination of total sugars, reducing sugars and amino acids, Agric. Sci., Wiley Eastern Limited, New Delhi, pp. 6 and 40, India.

[18] Miller, E.L. (1967) Determination of the tryptophan content of feeding stuffs with particular reference to cereals. J. Sci. Food Agric. 18, 381-387.

[19] Chapman, H.D., Pratt, P.F. (1978) Methods of

Analysis for Soil Plants and Waters, PP. 50. University of California, Division of Agriculture Science Priced Publication. 4034.
[20] Dabbou, S., Issaoui, M., Servili, M., Taticchi, A., Sifi, S., Montedoro, G.F., Hammami, M. (2009) Characteriziation of virgin olive oils from European olive cultivars introduced in Tunisia. Eur. J. Lipid Sci. Technol. 111, 392-401.
[21] Yousefi, N., Zeynali, F., Alizadeh, M. (2018) Optimization of low-fat meat hamburger formulation containing quince seed gum using response surface methodology. J. of Food Science and Technology. 55, 598-604.
[22] Meilgaard, M.C., Civileand, G.V., Carr, B.T. (2007) Sensory Evaluation Techniques, 4th Ed. CRC Press: New York.
[23] Tan, Y.W., Zhang, Y., Bolotin, K., Zhao, Y., Adam, S., Hwang, E.H., Das Sarma, S., Stormer, H.L., Kim, P. (2007) Measurement of Scattering Rate and Minimum Conductivity in Graphene. Physical Review Letters. 99, 246803.
[24] Troy, D.J., Desmond, E.M., Buckley, D.J. (1999) Eating quality of low-fat beef burgers containing fat-replacing functional blends. J. of the Science of Food and Agriculture. 79, 507-516.
[25] Ngadi, M., Li, Y., Oluka, S. (2007) Quality changes in chicken nuggets fried in oils with different degrees of hydrogenatation. LWT-Food Science and Technology. 40, 1784-1791.
[26] Steel, R.G., Torrie, J.H., Dickey, D.A. (1980) Principles and Procedures of Statistics 2nd Ed. McGraw-Hill, New York, USA.1200.
[27] Jancurova, M., Minarovicova, L., Dandar, A. (2009) Quinoa-a review. Czech J. Food Sci. 27, 71-79.
[28] Wafaa, K.G., Zeinab, A.A., Gomaa, A.R. (2015) Production of high dietary fiber bread fortified with quinoa flour. Adv. Food Sci. 37(2), 44-49.
[29] Gęsiński, K., Nowak, K. (2011) Comparative analysis of the biological value of protein of (*Chenopodium quinoa willd*). And Chenopodium albumL. Part I. amino acid composition of the seed protein .Acta Sci. Pol. Agric. 10(3), 47-56.
[30] Hareedy, L.A.M., Mahafauz, A.M., Kamel, A.S. (2009) Soybean-quinoa drinks from newly introduced quinoa varieties in Egypt. Egyptian J. Nutr. XXIIII(1), 125-160.
[31] James, L.E.A. (2009) Quinoa (*Chenopodium quinoa willd.*): composition, chemistry, nutritional, and functional properties. Advances Food Nutr. Research. 58, 1-31.
[32] National Academies of Sciences, Institute of Medicine (2001) Fruits and vegetables yield less vitamin A than previously thought; upper limits set for daily intake of vitamin A and Nine Other Nutrients. Press Release Jan. 9.
[33] Hareedy, L.A.M., Mahafauz, A.M., Kamel, A.S. (2009) Soybean-quinoa drinks from newly introduced quinoa varieties in Egypt. Egyptian J. Nutr. (1), 125-160.
[34] Bhargava, A., Shukla, S., Ohari, D. (2006) Chenopodium quinoa- An Indian perspective. Industrial Crops Products. 23, 73-87.
[35] Alvarez-Jubete, L., Arendt, E.K., Gallagher, E. (2010) Nutritive value of pseudocereals and their increasing use as functional gluten-free ingredients. Trends Food Sci. Technol. 21(2), 106–113.
[36] Sheridan, P.S., Shilton, N.C. (2002) Analysis of yield while cooking beefburger patties using far infrared radiation. J. of Food Engineering. 51, 3-11.
[37] Dzudie, T., Scher, J., Hardy, J. (2002) Common bean flour as an extender in beef sausages. J. of Food Engineering. 52, 143-147.
[38] Aleson-Carbonell, L., Fernandez-Lopez, J., Perez-Alvarez, J.A., Kuri, V. (2005) Characteristics of beef burger as influenced by various types of lemon albedo. Innovative Food Science & Emerging Technologies. 6, 247-255.
[39] Mahmoud, M.H., Abou-Arab, A.A., Abu-Salem, F.M. (2017) Quality Characteristics of Beef Burger as Influenced by Different Levels of Orange Peel Powder. American J. of Food Technology. 12, 262-270.
[40] El-Seesy, T.A. (2000) Quality and safety of meal burger patties using HACCP system 3. Proceedings of the 3rd Conference of Food Industry at the Service of Turisum, April. 12-14.
[41] Ali, R.F., El-Anany, A., Gaafar, A. (2011) Effect of potato flakes as fat replacer on the quality attributes of low-fat beef patties. Advance Journal of Food Science and Technology. 3, 173-180.

Received: **22.06.2020**
Accepted: **15.05.2021**

CORRESPONDING AUTHOR

Waleed Z Badawy
Food Technology Department,
Faculty of Agriculture,
Kaferelsheikh University,
Egypt.

e-mail: walid.metwali@agr.kfs.edu.eg

EXPLOITATION AND VALUATION OF SARDINE CO-PRODUCTS IN FOOD BIOTECHNOLOGY FISH NUTRITION

Faiza Alliouche[1,2,*], Redhouane Benfares[1], Soumia Benrabah[1], Lynda Frai[1], Maha Kherarba[1], Kamal Bodjema[1], Abdeslam Arab[2]

[1]Formulation of Artificial Feeds For Species of Aquaculture Interest, Cnrdpa, 11 Boulevard Colonel Amirouche 42415 Bou Ismaïl Tipaza Algeria
[2]Dynamics and Biodiversity Laboratory, FSB, USTHB, LP 32 El-Alia, Bab-Ezzouar, Algiers, Algeria

ABSTRACT

This present study is a contribution to the valorization and exploitation of the coproducts of the *Sardina pilchardus*.

The main objective is to make fishmeal with good nutritional quality

The production of fish meal has gone through several stages: cooking, pressing, decantation and centrifugation, drying and grinding. A sample of this meal was taken for a qualitative study (biochemical and microbiological).

The results of the biochemical analysis reveal 45.88% of protein, 15.45% of the fat, 0.57% of the total sugars, 3.9% of the carbohydrates, 26.98% of crude ash and 350.4 K cal/100g of energetic value, with a yield of 26.1% and absence of contamination microbiological. In a second time, we made an experiment to compare the biochemical quality of the meal resulting from the steamed and non-steamed wastes. The results of the non-steamed wastes are: 45.34 % of protein, 29.73 % of the fat, 0.24% of the total sugars, 10.01% of the carbohydrates and 488.97 K cal/100g of energetic value

These results are encouraging for the creation of a fish waste processing and transformation plant.

KEYWORDS:
Sardina pilchardus, fish meal, co-products, valuation, aquaculture

INTRODUCTION

Fish and fisheries are integral parts of most societies and contribute greatly to socio-economic health and well-being in many countries and regions [1]. In the Mediterranean, 78% of fish stocks are subject to overfishing [2]. The fishery production in Algeria represents only 12% of the overall production of the Mediterranean, or about 120,000 t per year [3]. In terms of the fishing fleet, that of sardine boats represents 30% of the total Mediterranean fishing fleet. Indeed, no less than 1,200 boats are fishing for sardines [4]. Overall, small pelagics constitute about 80% of the country's total catch and 50% of the Mediterranean catch. Sardines are the most consumed and family-friendly species for all Algerians in the 4 corners of the country. This species is especially of great economic interest in the context of the commercialization and processing industry in fishmeal and fish oil, due to its rapid growth, early maturity, short life cycle and its planktophagous diet (which feed on zooplankton and phytoplankton). The valuation of fishery co-products occupies an important place in biotechnology. In Algeria, sardines are intended directly for human consumption following sharp reductions in catches. As a consequence, the increase in the price of the raw material which represents 42% of the total cost of production of sardines in vegetable oil in 2015 [5]. In Algeria, the "Capten el mersa" sardine canning factory imports frozen sardines from Tunisia. This factory spends a fee to throw sardine waste into the sea, this gesture has environmental impacts. To this end, to protect the marine environment, minimize expenses for this plant, and bring an economic impact, we have proposed recycling this waste by transforming it into fish meal and oil. In aquaculture, this sardine waste processing procedure is a good solution for the supply of fishmeal and fish oil, which is considered to be the best source of animal protein and to minimize the prices of fish feed which constitute between 60 and 80% of expenditure for aquaculture projects.

MATERIALS AND METHODS

Description of Capten el marsa. CAPTEN EL MERSA, is an Algerian food company, located on the road to Mostaganem, 07 km west of Ténès, wilaya of Chleff, while the head office is located in Algiers. This factory specializes in the processing of canned sardines and was created in 1990. SARL CAPTEN has a staff of around 104 employees. The main activities of this cannery are the processing, freezing, packaging and marketing of fishery products, mainly sardines and tuna.

FIGURE 1
Location of Capten el Mersa

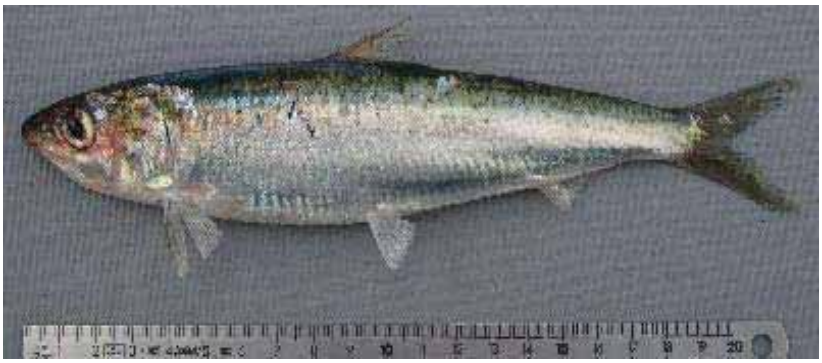


FIGURE 2
Photograph of the Sardine (*Sardina pilchardus*), source: Fish base

Biological material. Description de Sardina pilchardus, walbaum 1792. This species has a body subcylindrical, belly rather rounded (but body more compressed in juveniles).

Hind margin of gill opening smoothly rounded (without fleshy outgrowths); 3 to 5 distinct bony Strive radiating downward on lower part of operculum; lower gillrakers 44 to 106, not becoming shorter at angle of first gill arch, ping the lower [6]. It's caracterised by a series of dark spots along upper flanks, sometimes with a second or even third series below.

Habitat and biology: it is a coastal pelagic fish, up to 180 m deep, exactly 25-55 m during the day, and 15-35 m at night. Lives in shoals very large and performs great migrations. Reproduction from September to June in the Mediterranean, from June to August in the Black Sea. Pelagic eggs. Sexual maturity at one year (10-20 cm). Fertility 5,300 to 38,500 eggs (Tunisia). Feeds primarily on planktonic crustaceans and other larger planktonic animals.

Systematic. Kingdom *Animalia*
Phylum Chordata
Subphylum Vertebrata
Superclass Osteichthyes
Class Actinopterygii
Order Clupeiformes
Family Clupeidae
Genus Sardina
Species *Sardina pilchardus*

Acquisition of sardine co-products. Co-products refer to by-products, by-catches, discards, unsold items. These are fish or parts of fish not consumed (skin, bone, head, viscera) but which are recoverable and usable [7]. The origin of these co-products are the processes of processing fishery and aquaculture products, for example, filleting, gutting, heading, washing, thawing or cooking raw products.

In our case, Capten el Mersa provided us with the sardine co-products and damaged parts (after sorting). So our raw material is not just left over from the fish. The co-products are transported in freezer bags and a cooler and then placed in a freezer until the day of use.

Fishmeal production. The meal fish manufacturing operation was carried out at the level of the CNRDPA national fisheries and aquaculture research and development center in the region of Bou Ismail, wilaya of Tipaza. the raw material is thawed and weighed to record the initial weight.

Experimental material. To make the fish meal, we used the following equipment:

- Receiving bins - dryer
- Steam boiler - sifters
- Spoons - gloves
- Cookers - weighing scales (precision 10^{-2})
- Conservation boxes - electric crushing (500 W)
- Centrifuge

Fish meal making process. We have adopted the manufacturing principle of Ifremer [7]. This is the same process that results in the manufacture of both fish meal and fish oil.

Fishmeal is made by cooking, pressing, drying, and grinding of fish or fish waste into a solid. Most of the water and some or all of the oil is removed.

1. **Receipt** of sardine waste
2. **Cooking:** Steaming this waste at a temperature of 80-90 ° C so that the proteins coagulate, releasing the oils and water. The cooking time depends on the amount of fish waste. In our case, a period of 50 minutes was sufficient for cooking our co-products.
3. **Pressing:** pressing of the coagulated material to obtain a dry dough "the cake" which contains no more oil, and a liquid fraction "press juice". Water content is reduced from 70% to about 50% and oil down to 4%.
4. **Decantation and centrifugation** of the liquid material to remove impurities and separate the oils from the aqueous fraction (glue water). The oils are stored in bottles in the refrigerator.
5. **Drying** the cake in an electric dryer to remove the water to obtain a dehydrated protein paste at a temperature of 45 ° c. This step is very important because an underdrying may result in the growth of molds or bacteria; over-drying can cause scorching and reduction in the meals nutritional value.
6. **Grinding** this dried meal into a flour using an electric grinder and to remove any lumps or bone particles.

Nutritional quality of fishmeal. A sample of the meal from the sardine waste was sent to the laboratory of the Office National for Cattle's food "ONAB" for biochemical analysis, into a Cooperation between CNRDPA and ONAB for a food manufacturing project for a Saharan fish with high fish-breeding potential, Nile Tilapia "*Oreochromis niloticus*".

Microbiological quality of fishmeal. The microbiological analysis of fishmeal was carried at the laboratory of CNRDPA, for the detection of streptococci, staphylococci, and Escherichia coli.

RESULTS AND DISCUSSION

Biochemical analysis. The results of the biochemical analysis of sardine fishmeal are shown in the table below.

The nutritional quality is satisfactory, the fishmeal produced is rich in proteins, it contains 45.88% of proteins, 13.45% of the fat, 0.57 of the total sugars, 3.9% of the carbohydrates, and 26.98% crude ash.

The biochemical quality of the meal depends on the type of co-products and the species of fish used [7Fishmeal contain 60-72% protein, no more than 12% fat, and not maximum of 10% water to ensure product stability.

According to Ifremer, fishmeal from co-products has a lower market value than meal from whole fish, since it is rich in minerals and therefore lower in protein and lipids, since the co-products are composed of bones, this reasoning corroborates our results, in particular the crude ash content which exceeds 26% indicating a high mineral content and explaining the protein content obtained.

Fishmeal is made up essentially of 70% protein, 10% minerals, 10% fat, and 10% water [8]. A study of fishmeal composition determined a content of 55.69% and 63.73% of protein and 9.59% and 11.99% of lipids [9]. However, another study found 56.12% protein in the head and bones and 42% to 45% protein in fishmeal [10]. Generally, the biochemical quality of fishmeal differs in relation to the type of fish (lean, fatty, large and small.) and again in relation to the nature of the co-product, for example the flesh is richer in protein compared to the bones. The biochemical quality of our fishmeal is very satisfactory and can meet the needs of aquaculture fish.

The only type of fish processing that exists in Algeria is canning factories, mainly tuna or sardines. These factories generate a large quantity of co-products whose economic potential is not optimized. They pay taxes to dispose of fish waste which will have a negative environmental impact, especially if it were treated. The transformation of co-products indicates several advantages, in several fields: in human and animal food, in agriculture, as pharmaceutical and aesthetic products.

Several studies have been carried out on sardine S.pilchardus, one recent in 2019 based on the application of a static gravimetric method to determine the optimal water for the conservation activity of sardine heads [12]. This study highlighted the importance of using drying chambers (dryers) for better meal conservation.

TABLE 1
The nutritional value of the fish waste meal used.

Analysis	steamed	Not steamed	Standard of the method of analysis
Crude protein %	45,88	45.34	NA 652-1992
fat %	13,45	29.73	NA 654-1992
total sugars %	0,57	0.24	BERTRAND
Amidon %	0	0	AFNOR
Humidité	8,07	/	NA 1291-1992
Cellulose %	3,53	9.77	European directive
carbohydrates %	3,9	10.01	European directive
crude ash %	26,98	/	NA 650-1994
energetic value Kcal/100g	338,17	488.97	European directive

TABLE 2
Potential for the use of co-products [11]

Markets of valuation	derived products	Use
Agriculture	Fertilizers (silage), compost,pesticide	-Soil enrichment - Pest control
Energy	Biocarburant, comburant Biofuel, oxidizing	- energy production
animal feed	meal, oils, protein derivatives, silage, minerals	- Food -Food supplements
Nutrition (complements food)	Oils, protein derivatives, minerals, amino acids	-Food supplements -Sports nutrition
-Food supplements Sports nutrition	Whole or partial use of fish, minced meat, food pulp, gelatin, broth and fish sauce, liver oil	-Unprocessed products -Processed products
pharmaceutical industry	Omega 3, calcium, chondroitin sulfate, collagen, bioactive peptides	-Nutraceutical -Cosmetic -Biotechnology

Another study aimed to optimise the production of fish protein hydrolysate (FPH) by enzymatic hydrolysis of sardine solid waste using raw pepsin, and to intensify the process in a bioreactor coupled to an ultrafiltration product recovery unit. The results showed that crude pepsin could be used satisfactorily for enzymatic hydrolysis of solid fish waste [13].

The main objective of our study is to enhance the value of sardine waste in fish nutrition. We point out the importance of incorporating fishmeal in food for its richness in protein and amino acids. Nutrition and aquaculture researchers stress the importance of fishmeal in aquaculture [14], [15], [16].

Fish meal has traditionally been used as the main source of protein in the aquaculture food industry, due to its high protein content and balanced EAA profile. Fish meal is also an excellent source of essential fatty acids, digestible energy, minerals and vitamins [16].

Of the world's fish meal production, about 90% is produced from oily fish species, such as sardines, anchovies, capelin and menhaden, and less than 10% from white fish offal (frames), such as cod and haddock [17].

The biochemical quality of fishmeal depends on the nature of the co-products used and the species of fish as well as handling during the manufacture of the meal. Variations in fishmeal are largely attributed to species and seasonal differences with the production of fishmeal [18].

TABLE 3
Fish waste yield result.

	Initial mass	Mass after cooking	Cake mass	Mass of fishmeal	yield
Sardine waste's fishmeal	15 kg 320 g	13 kg 500 g	10 kg 310 g	4 kg	26,1%

By comparing the quality of our fishmeal with other studies, we can say that our fishmeal is of good quality and can meet the needs of many species of fish. Over 80% of the fishmeal samples were analyzed, came from India, Indonesia, Philippines, Thailand and Vietnam showing crude protein levels ranging from around 40% to 75%. The protein and amino acid content of fishmeal varied widely, with a CV recorded of 7-10% for CP and> 10% for amino acids [18].

The lipid content in the fishmeal passed through the steamed is lower compared to the not steamed meal. The lipids in fishes can be separated into liquid fish oils and solid fats. Although most of the oil usually gets extracted during processing of the fishmeal, the remaining lipid typically represents between 6% and 10% by weight but can range from 4% to 20% [19].

Yield of fishmeal. For the fishmeal yield result is shown in the following table:

An amount of 15 kg 320g of sardine waste was used to produce about 4 kg of the fishmeal with a yield of 26.1%. This rate is really satisfactory considering that we used only waste without flesh. So 1 kg of waste allows us to produce 261g of meal, which is really important.

According to a study, the yield by weight of the transformation of the raw material into the finished product is 20% if we treat headless and gutted fish, and 13% if we treat only the head plus the viscera [20]. The loss in weight is mainly due to the elimination of water which constitutes 75% of the fresh product and only 3% of the flour. Heading and evisceration can result in 25 to 35% waste, with filleting producing 50% or more depending on the species.

According to Ifremer, the yield strongly depends on the raw materials used and the season (on average 20% of the fishmeal and 7 to 8% for the fish oil). According to FAO, the yield of co-products is 25%.

According to an old study, a quantity of 100 kilos of fish waste that can be dried, ground and sold without degreasing (lean fish waste) provides 20 to 25 kilos of powder at 10% moisture and titrating 7.5 to 9.5% [10].

Microbiological quality. According to the contamination thresholds of the Algerian standard (NA 6115), we deduce the absence of all types of microbiological contamination since streptococci and staphylococci are lower than the thresholds set and E.coli is absent in our analyzed sample. This good result is due to the good handling and hygiene during the flour manufacturing process.

CONCLUSION

The valuation of sardine waste from a cannery was studied in this work. The co-products of S. pilchardus indicate good performance in fishmeal. The results of the biochemical analysis show 45.88% protein, 13.45% fat, 0.57% of total sugars, 3.9% of carbohydrates, and 26.98% crude ash with a yield of 26.1% and good microbiological quality. The valuation of the coproducts of canned food has socioeconomic, product quality and environmental importance. Sardine waste can be used as meal for fish and other animals, in medicine and aesthetics.

REFERENCES

[1] Djemaa, S. (2014). Study of the population structure and diet of the European anchovy (Engraulis encrasicolus) and the European sardine (Sardina pilchardus): relations with the environment. Doctoral thesis, Université du Littoral Côte d´Opale..

[2] CGPM. (2011). General Fisheries Commission for the Mid-Year, Report of the Thirty-Fifth Session, Rome.

[3] MPRH. (2015). Contribution of the fishing and aquaculture sectors to the development of competitive production in Algeria. MPRH, Palais des Nations, Club des Pins, Algiers.

[4] Chikhi, S.M.R. (2018). The maritime fisheries sector in Algeria: challenges and realities the maritime fisheries sector in Alegria: challenges and realities. Review of in-depth economic studies. 32.

[5] EUMOFA (2017). Canned sardines in Portugal. European Market Observatory for Fisheries and Aquaculture Products. 36.

[6] FAO (1985). Species catalogue Vol. 7. Clupeoid fishes of the world. (Suborder CLUPEOIDEI) An annotated and illustrated catalogue of the herrings, sardines, pilchards, sprats, anchovies and wolf-herrings. Part 1.Chirocentridae, Clupeidae and Pristigasteridae.Whitehead, P.J.P. FAO Fish. Synop. (125)Vol.7 Pt.1, 303.

[7] IFREMER (2010). Raw fish meal and oils. Library.
[8] Blanco, M., Sotelo, C.G., Chapela, M.J., Perez-Martin, R.I. (2007). Towards sustainable and efficient use of fishery resources; present and future trends. Trends in Food Sciences and Technology. 18, 29-36.
[9] Ponka, R., Goudoum, A., Tchungouelie, U.A.C., Fokou, E. (2016). Evaluation nutritionnelle de quelques ingrédients entrant dans la formulation alimentaire des poules pondeuses et porcs d'une ferme d'élevage au Nord- Ouest Cameroun. Int. J. Biol. Chem. Sci. 10(5), 2073-2080.
[10] Hinard, G. (1923). Treatment of fish waste and use of by-products. Monograph, Review of Works. 29
[11] Secretariat of the Pacific Community. (2014). Promotion of fish by-products. Guidance Note. 4
[12] Bahammou, Y., Lamsyehe, H., Kouhila, M., Lamharrar, A., Idlimam, A., Abdenouri, N. (2019). Valorization of co-products of sardine waste by physical treatment under natural and forced convection solar drying. Renewable Energy.142, 110-122.
[13] Benhabiles, M.S., Abdi, N., Drouiche, N., Lounici, H., Pauss, A., Goosen, M.F.A., Mameri, N. (2012). Fish protein hydrolysate production from sardine solid waste by pepsin enzymatic hydrolysis in a bioreactor coupled to an ultra filtration unit. Mater. Sci. Eng. C 32, 922e928.
[14] Trushenski, J. and Gause, B. (2013). Comparative Value of Fish Meal Alternatives as Protein Sources in Feeds for Hybrid Striped Bass. North American Journal of Aquaculture. 75, 329–341.
[15] Waring, J.J. (1968). The nutritive value of fish meal, meat-and-bone meal and field bean meal as measured by digestibility experiments on the adult colostomised fowl. British Poultry Science. 10, 1969 - Issue 2.
[16] El-Sayed, A.F.M. (2020). In Tilapia Culture (Second Edition), Chapter 7 - Nutrition and feeding, Editor(s): Abdel-Fattah M. El-Sayed. 135-172.
[17] Barlow, S.M. (2003). Fish meals have an amino acid composition that is very close to optimal for milk protein synthesis compared to other protein sources, such as soybean meal, linseed or peanut proteins. Encyclopedia of Food Sciences and Nutrition (Second Edition). 6000.
[18] Masagounder, K., Ramos, S., Reimann, I., Channarayapatna, G. (2016). Optimizing nutritional quality of aquafeeds. Aquafeed Formulation. 239-264
[19] Miles, R.D. and Chapman, A. (2019) The Benefits of Fish Meal in Aquaculture Diets. IFAS. Extension. University of Florida.
[20] Marthe, G. and Laurence, R. (1992). Analysis of the variation in the composition of the meal produced at a fish processing plant. Interpêche. 66.

Received: **09.12.2020**
Accepted: **22.05.2021**

CORRESPONDING AUTHOR

Faiza Alliouche
Formulation of Artificial Feeds for Species of Aquaculture Interest,
CNRDPA, 11 Boulevard Colonel Amirouche 42415 Bou Ismaïl – Tipaza

e-mail: alliouchefaiza@gmail.com

ANXIOLYTIC AND ANTIDEPPRESSIVE EFFECTS OF COOKED BEANS (*Phaseolus vulgaris*) AND SEROTONIN PRECURSOR DIETS FOLLOWING SCOPOLAMINE-INDUCED MOOD IMPAIRMENT IN CD1 MICE

Johnbull Martins Uket, Charles Cyprian Mfem, Udemeobong Edet Okon, Eme Efiom Osim*

Department of Physiology, Faculty of Basic Medical Sciences, College of Medical Sciences, University of Calabar, Calabar PMB 1115, Nigeria

ABSTRACT

The common African Bean (*Phaseolus vulgaris*) contains serotonin and its precursors among several other constituents. Serotonin is generally known to affect mood in humans. However, whether consumption of beans diet which contains serotonin will affect the mood notably, anxiety and depression in mice with scopolamine-induced mood impairment has not been previously ascertained. Therefore, the effects of consumption of cooked beans (*Phaseolus vulgaris*) as well as serotonin precursor diets on scopolamine-induced anxiety and depression in mice were studied. Sixty mice were randomly assigned into 6 groups namely; control, scopolamine only, 50% cooked beans diet + scopolamine, serotonin precursor (tryptophan) diet + scopolamine, 50% cooked beans diet only and serotonin precursor (tryptophan) diet only. The animals were granted access to clean water *ad libitum.* Light and dark box and the Elevated plus maze tests were used for evaluation of fear/anxiety. Forced swim test was used to assess for the depression in the animals. Results showed that the scopolamine only group had a significantly shortened light chamber duration compared to the control group ($p<0.05$). The light chamber duration of the other treated groups was significantly longer compared to the scopolamine only group. The open arm durations of the experimental diets treated groups were significantly longer compared to the scopolamine only group. The immobility duration of the scopolamine only group was significantly increased compared to control and other groups that received cooked beans or serotonin precursor diets ($p<0.05$). In conclusion, consumption of beans and serotonin precursor diets alleviated anxiety and depression in scopolamine-induced anxiety and depression in mice. The anxiolytic and antidepressive effects observed may be attributed to serotonin synthesized from tryptophan in beans.

KEYWORDS:
Beans, Anxiety, Serotonin, Scopolamine, Depression

INTRODUCTION

The common bean (*Phaseolus vulgaris)* is a dicotyledonous plant belonging to the pea family. It is a staple food for many people in different parts of the world [1]. It is a rich source of vitamins, minerals, carbohydrates, dietary fiber, protein and many phenolic compounds and is a very nutritious food [1, 2]. It has been reported that beans have anti-carcinogenic, anti-mutagenic [4], anti-inflammatory, antidiabetic, hypoglycemic, cardio-protective and antioxidant effects [5]. It has also been reported that it contains serotonin and its precursor 5-Hydroxytrytophan (5-HTP) [6]. Beans contain other chemical compounds including but not limited to saponins, tannins, glycosides, flavonoids. Aduema [7] reported that long term consumption of beans diet improves learning/memory in apparently normal mice.

Notable among the array of chemical constituents present in beans is serotonin which has been reported to influence neurobehavioural actions such as sleep, memory and learning [8]. Serotonin has been reported to act as neurotransmitter which modulate behaviour in response to changing cues, acting on both muscles and neurons to affect locomotion and learning [9]. It is also reported to have direct influence on mood [10].

Scopolamine is a drug of choice in inducing memory impairment in animals including mice. The cognitive dysfunction or memory impairment observed after this drug's usage is analogous to observations in demented patients. Scopolamine is a muscarinic receptor antagonist. It impairs long term potentiation which is responsible for long term memory [11]. It is also reported to be an anxiogenic agent used for the evaluation of anxiolytic effects of novel drugs. Scopolamine has also been shown to impair mood in animals [12].

Owing to the adverse effects of synthetic drugs [13], there is increased search for natural remedies that are safer a more effective. According to World Health Organization statistical report, 80% of the

world's population presently uses traditional medicine for some aspects of primary health care including mental health [14]. Hence, natural products may provide a novel source of beneficial neuropsychotropic drugs [13] provided they are scientifically validated and their mechanisms properly established.

Since beans contain serotonin that can potentially affect mood, it is, therefore, possible that the consumption of beans may influence moods such as anxiety and depressive strokes. Therefore, this research investigated the effects of consumption of common beans on anxiety and depression in mice with impaired mood, notably anxiety and depression using scopolamine.

MATERIALS AND METHODS

Experimental diets preparation. Preparation of beans diet. Twenty (20) cups of beans were bought from Marian market, a local market in Calabar, Nigeria. The beans were cooked, air dried and ground into powdered form using an electric blender. The powdered form weighed 1,560g.

One kilogram of powdered cooked beans was mixed with one kilogram of normal rodent chow making 50:50(w/w) % of beans diet. The constituent was blended in a bending machine for a uniform mixture.

Preparation of serotonin precursor diet. Serotonin precursor (5-Hydroxytryptophan) was procured from Sigma Aldrich, Germany for use in this study. The estimation and preparation of the powdered 5-Hydroxytryptophan content of cooked beans was according to the method of Feldman and Lee [15] as modified by Mosienko *et al*., [16]. The serotonin precursor diet was prepared by mixing 1.15 g of the precursor in 98.85 g of the feed so that the quantity of 5 HTP added was similar to that present in every 100 g of cooked beans given to the mice. An electric blender was used to blend the mixture to form the serotonin precursor diet.

Experimental animals and design. Sixty adults CD1 white mice weighing between 17 – 26 g were used for this study. The animals were randomly assigned into 6 groups of 10 mice each, namely; control, scopolamine only, 50% cooked beans + scopolamine, serotonin precursor diet + scopolamine, 505 cooked beans only and 5HT precursor only groups respectively. The animals in the control group were fed normal rodent chow only and administered normal saline (1ml/kg bodyweight intraperitoneally). The second group of mice were fed normal rodent chow and administered Scopolamine (1mg/kg bodyweight intraperitoneally). In the third group, the mice were fed cooked beans diet and administered scopolamine, while in the fourth group, mice were fed serotonin precursor diet and administered scopolamine. The fifth group comprised mice that were fed the cooked beans diet and administered with Scopolamine. The sixth group was fed serotonin precursor diet and administered normal saline. Scopolamine was administered once daily for the first week. In the subsequent weeks, Scopolamine was administered once every two days. The feeding and behavioural tests lasted for four weeks.

The experimental animals were maintained in a specific pathogen-free and well–ventilated housing unit. The housing room was illuminated on a 12-hour light-dark cycle with clean drinking water *ad libitum*. Approval for use of the animals was obtained from the College Ethical Committee of University of Calabar, Nigeria on the use of experimental animals. The rodents were handled in accordance with the internationally accepted principles for laboratory animal use and care as found in the European Community guide lines (EEC Directive of 1986; 86/609/EEC).

Behavioural Protocols. Light/Dark Transition Box test for anxiety. The light-dark transition box is a test used to assess unconditioned anxiety and exploratoion. It is based on the conflict between exploring in a novel environment and avoidance of bright light [17]. This box has two compartments of unequal sizes. It is made up of plywood. The small chamber painted black makes up 2/5 of the box while the larger chamber is painted white and makes up 3/5 of the box. Both chambers are connected by a door (7.5 x 7.5 cm) that is located at floor level in the centre of the wall separating the two compartments. The floor which is covered with Plexiglas is divided into 9 x 9 cm squares. The tests in this apparatus were conducted in a 2 x 5 m neurobehavioral laboratory which was lit by a 60 watts red lamp for background lighting. The mice were placed into the apparatus and allowed an exploration time of 5 minutes. The test sessions were recorded with the aid of a video camera. Light chamber duration, stretch attend posture, rearing frequency, grooming duration were the behaviours scored.

Elevated plus maze test for anxiety. The Elevated plus Maze built according to the description of Lister [18], has two open arms ($45x5cm^2$) with 0.25cm high edges and two closed arms ($40x5cm^2$) with 15cm high walls radiating from a central square (5x5cm). There is a slight ledge of about 4 mm open arms have a slight ledge (4 mm high) to prevent the mice from slipping and falling off the edge. The closed arms provide a sense of safety because they are enclosed like most test of anxiety. This task exploits the conflict between the natural tendency of mice to explore the novel areas and fear of open spaces. The index of open arm avoidance also gives a measure of anxiety.

Before each test, the arm's surfaces and closed

sides were cleaned with Methylated spirit to remove olfactory cues and to eradicate fecal boli and urine. Mice were placed in the central square of the plus maze such that the mice faced an open arm away from the experimenter upon placement. After placement, a stop watch was started and the animals allowed exploring the apparatus for 5 minutes. The test sessions were recorded and video-taped. Increase in the time spent by the mice in the open arm as well as head dips indicate an antianxiety-like behaviour [19].

Forced swim test for depression. This test is a behavioral test used for evaluation of antidepressant drugs and its efficacy as well as assessing the experimental manipulations that are targeted towards alleviating depressive-like states. Mice were placed in an inescapable transparent tank that was filled with water and their escape related mobility behavior was measured. Successful implementation of the forced swim test requires adherence to certain procedural details and minimization of unwarranted stress to the mice [20]. The water tank was a cylindrical tank (30 cm height x 20 cm diameters) constructed of transparent Plexiglas. The water level was at 15 cm from the bottom and marked on the tank to ensure that the volume of water was consistent across mice used. The dimensions of the tanks were selected in a way that the mice were not able to touch the bottom of the tank, either with their feet or their tails, during the swimming test. The height of the tank was high enough to prevent the mice from escaping from the tank.

Statistical Analysis. Data obtained were analyzed using analysis of variance (ANOVA) followed by a post hoc test (least square difference (LSD) test) to determine significant difference between means. The analysis was done with an SPSS 18 statistical package. The values were presented as mean ± SEM and considered significant at $p<0.05$.

RESULTS

Behaviours scored in light/dark transition box. Stretch attend posture (SAP). Figure 1 below shows the comparison of frequencies of SAP in the light and dark transition box test of the experimental animals. The SAP of the scopolamine only group was significantly increased compared to the control ($p<0.05$). The SAP of the other treated groups were significantly lower compared to the scopolamine only group ($p<0.05$).

Light chamber duration. While the scopolamine group was observed to have a significantly shortened light chamber duration compared to the control group ($p<0.05$), the light chamber duration of the other treated groups was significantly longer compared to the scopolamine group (Figure 2).

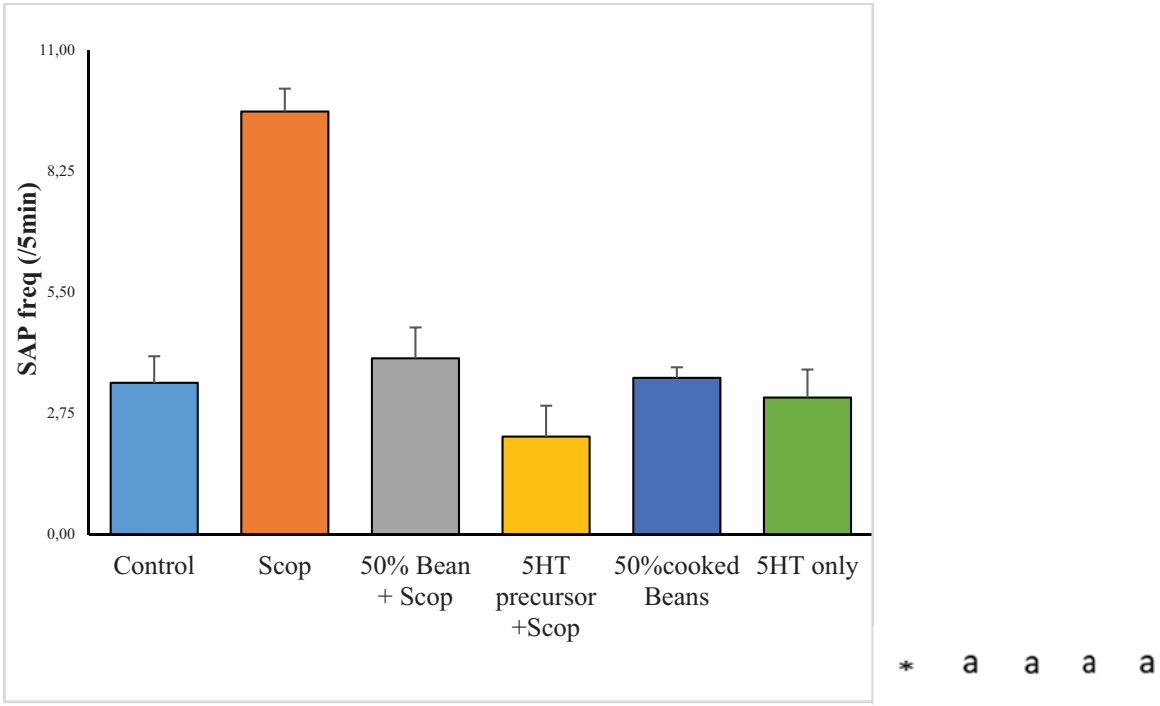


FIGURE 1
Comparison of the Stretch Attend Posture in the light and dark transition box test of the experimental animals.

Value are expressed as mean ± SEM, n = 10.
* = $p<0.05$ vs control;
a = $p<0.05$ vs scopolamine

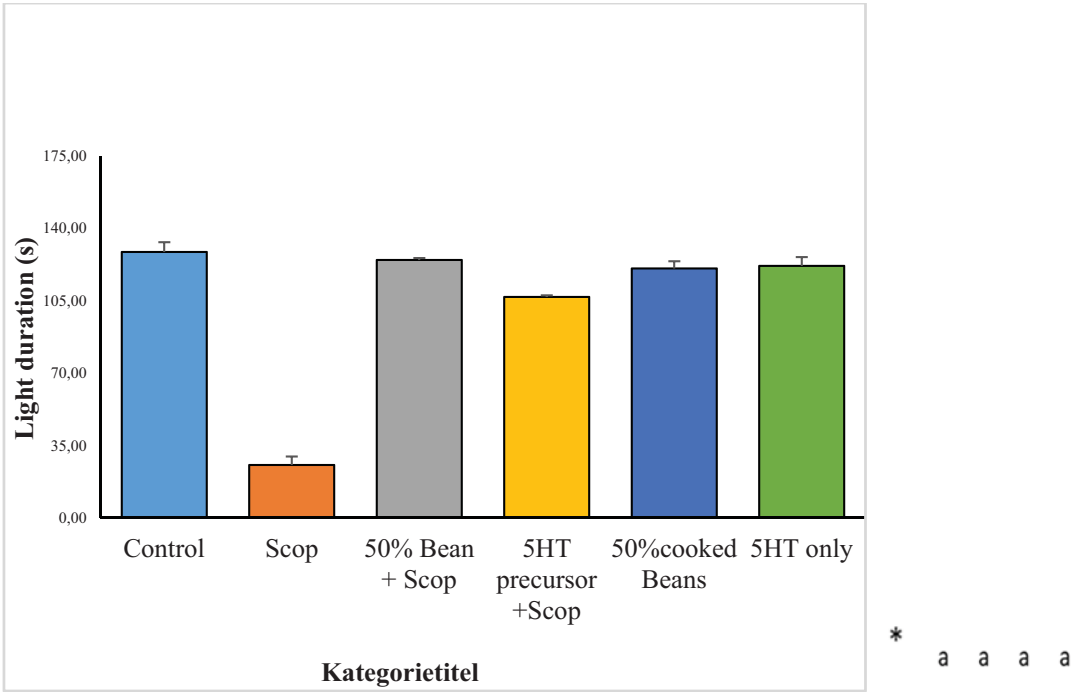


FIGURE 2
Comparison of light chamber duration in the light and dark transition box test of the experimental animals.

Value are expressed as mean ± SEM, n = 10.
* = p<0.05 vs control;
a = p<0.05 vs scopolamine

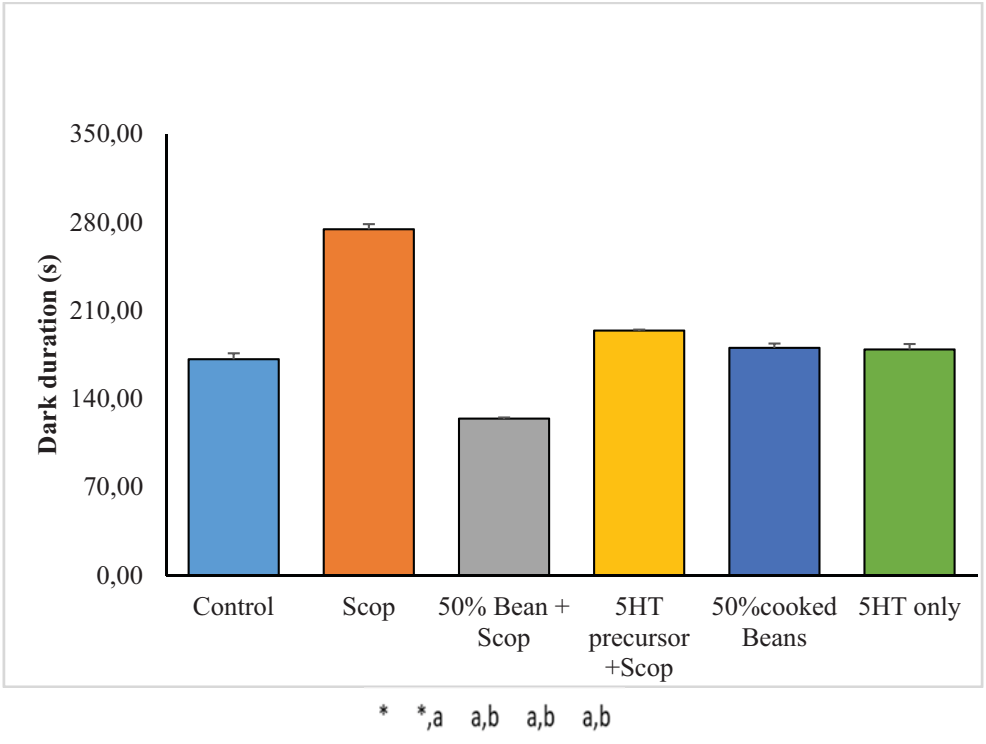


FIGURE 3
Comparison of dark chamber duration in the light and dark transition box test of the experimental animals.

Value are expressed as mean ± SEM, n = 10.
* = p<0.05 vs control;
a = p<0.05 vs scopolamine
b = p<0.05 vs 50% cooked beans + scopolamine

Dark chamber duration. The result showed that the dark chamber duration of the scopolamine only group was significantly longer ($p<0.05$) compared to the control group. The result also showed that the dark chamber duration of the 50% cooked beans diet + scopolamine group was significantly shorter compared to both the control and scopolamine only groups ($p<0.05$). While the dark chamber durations of the other treated groups were significantly shorter compared to the scopolamine only group, the values were significantly longer compared to the 50% cooked beans diet + scopolamine group ($p<0.05$) (Figure 3).

Behaviours scored in the elevated plus maze. Frequency of open arm entries. In Figure 4, the result showed that the scopolamine group had a significantly lowered open arm entry compared to the control. The values for the other treated groups were significantly higher compared to the scopolamine group ($p<0.05$).

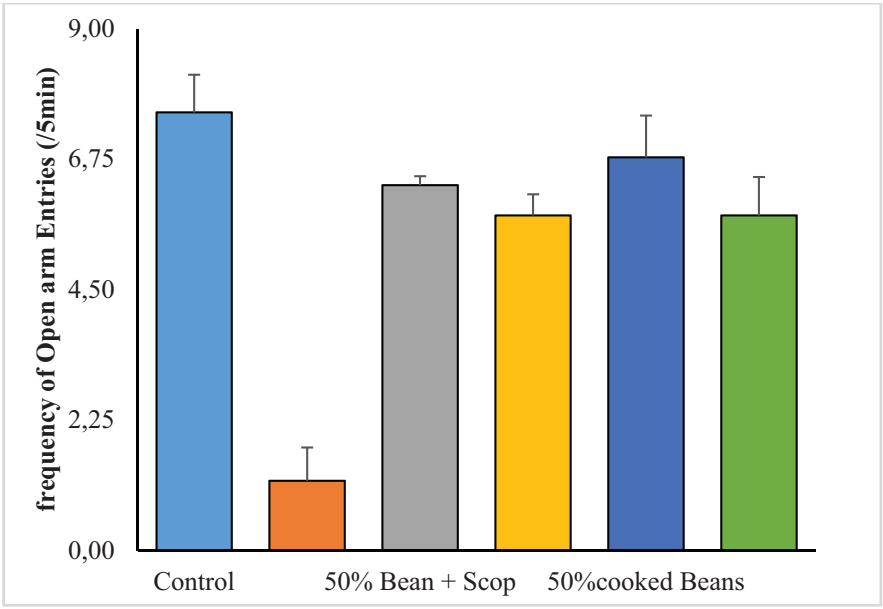


FIGURE 4

Comparison of frequency of open arm entries in elevated plus maze test of the experimental animals.

Value are expressed as mean ± SEM, n = 10.
* = $p<0.05$ vs control
a = $p<0.05$ vs scopolamine.

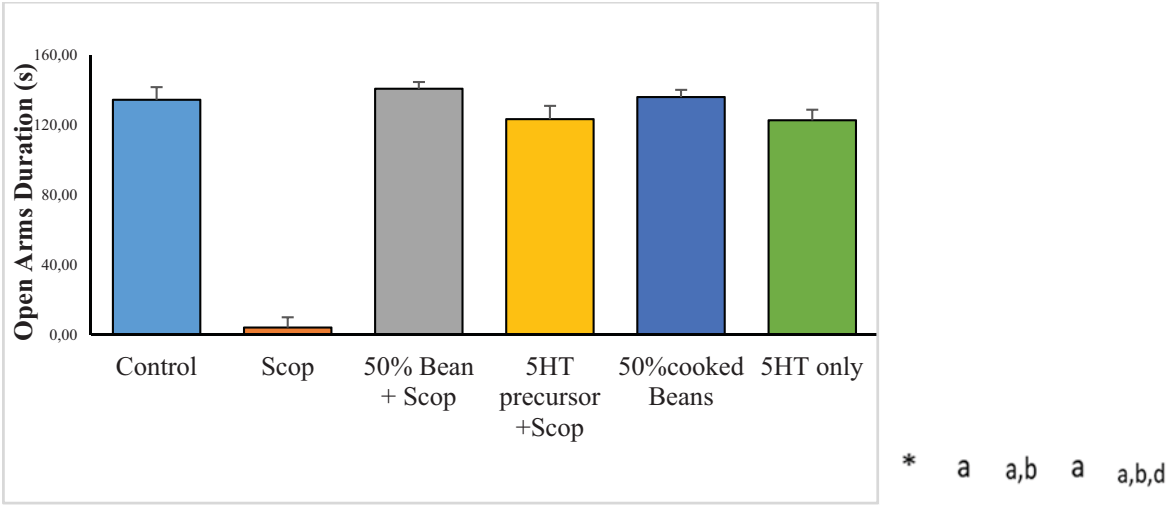


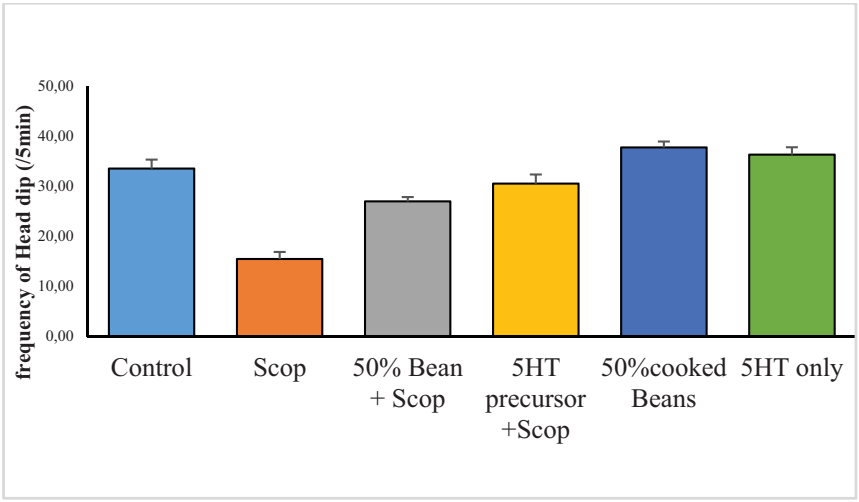
FIGURE 5

Comparison of open arm duration in elevated plus maze test of the experimental animals.

Value are expressed as mean ± SEM, n = 10.
* = $p<0.05$ vs control;
a = $p<0.05$ vs scopolamine
b = $p<0.05$ vs 50% cooked beans + scopolamine
d = $p<0.05$ vs 50% cooked beans

Duration of open arm entries. The duration of open arm stay of the scopolamine group was significantly shorter compared to the control ($p<0.05$). The values for the other treated groups were significantly longer compared to the scopolamine group ($p<0.05$). While the open arm durations of both the 5HT + scopolamine precursor and 5HT only groups were significantly shorter than that of the 50% cooked beans + scopolamine group ($p<0.05$), that of 5HT only group was significantly shorter than that of the 50% cooked beans group ($p<0.05$) (Figure 5).

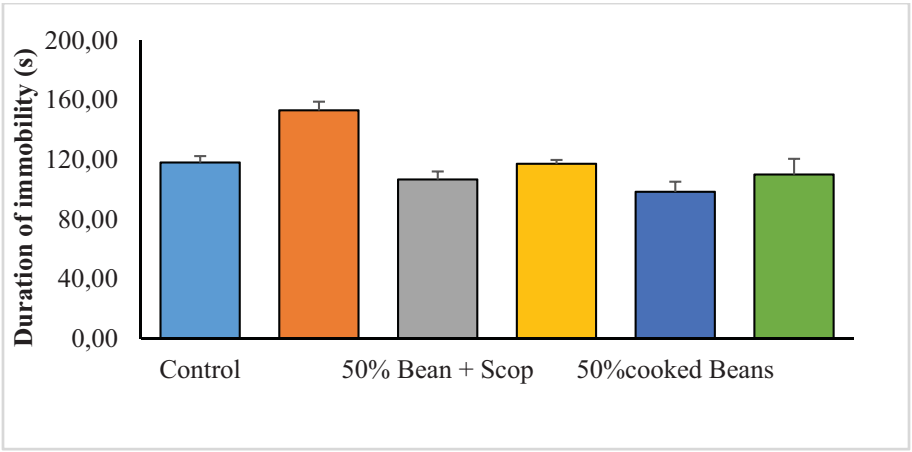
Frequency of Head dips. The result in Figure 6 showed that the frequency of head dips for the scopolamine, 50% Beans + scopolamine and 5HT precursor + scopolamine groups were significantly lower compared to control ($p<0.05$). The values for 50% cooked Beans and 5HT groups were significantly higher compared to the control ($p<0.05$). The frequencies of head dips of the 50% Bean + scopolamine, 5HT precursor + scopolamine, 50% cooked Beans and 5HT only groups were significantly higher compared to the scopolamine only group ($p<0.05$). The values for 50% cooked Beans and 5HT only groups were significantly higher compared to both cooked beans + scopolamine and 5HT precursor + scopolamine groups ($p<0.05$).



*,a *,a,b,c * *,a *,a,b,c

FIGURE 6
Comparison of head dips in elevated plus maze test of the experimental animals. Value are expressed as mean ± SEM, n = 10.

Value are expressed as mean ± SEM, n = 10.
* = $p<0.05$ vs control;
a = $p<0.05$ vs scopolamine
b = $p<0.05$ vs 50% cooked beans + scopolamine
c = $p<0.05$ vs 5HT precursor + scopolamine



* a a a a

FIGURE 7
Comparison of duration of immobility in force swim test of the experimental mice.

Value are expressed as mean ± SEM, n = 10.
* = $p<0.05$ vs control;
a = $p<0.05$ vs scopolamine

Forced Swim Test. Duration of Immobility in Forced swim test. Figure 7 the mean durations of immobility in force swim test of the experimental mice. The result showed that while the duration of immobility of the scopolamine only group was significantly increased compared to control ($p<0.05$), the values for other treated groups were significantly lower compared to the scopolamine only group.

DISCUSSION

The effects of consumption of cooked beans (*Phaseolus vulgaris*) as well as serotonin precursor diets on scopolamine-impaired anxiety and depression in mice were studied.

In the light-dark transition box tests, most mice naturally evince a preference for the dark chamber. Mice with less anxiety tend to transit more into the light chamber. The animals administered scopolamine only where observed to spend less time in the light chamber than they did in the dark chamber. This implied that scopolamine is anxiogenic. However, the animals fed the cooked beans or serotonin precursor diets were observed to spend more time in the light chamber and less in the dark chamber compared to scopolamine only treated mice. This implied that they had lower levels of anxiety. Furthermore, in the light and dark box environment, behaviours such as stretch attend posture frequencies in the light/dark chamber was observed to be less in groups of mice fed with cooked beans as well as serotonin precursor diets compared to scopolamine only treated group. This indicates the fact that there was a decreased level of anxiety in the group of mice fed with cooked beans as well as serotonin precursor diets.

Stretch attend posture is a risk assessment behaviour were rodents demonstrate forward elongation of the head and shoulder followed by retraction into its original position [21]. It is a behaviour displayed by mice introduced in a new environment and a measure of anxiety. Fear and anxiety are basically controlled by neutral circuitry involving the amygdala mostly and the hypothalamus. Stimulation of the amygdala electrically, is associated with fear and feeling of terror [22]. Beans are known to contain cardiac glycosides and the neurotransmitter, serotonin, which act by reducing depressive feelings and promoting the relaxation of skeletal muscle tone [23]. Hence, it is likely that the presence of these compounds and other constituents in the beans could be responsible for the anxiolytic property of beans which may have acted by decreasing the excitability of the amygdala through increase in the threshold of response of the cells of these nuclei, thereby reducing fear related behaviour in the mice [23].

This observation is supported by Young and Teff [24] who reported that increased level of brain serotonin enhances calmness and mellowing serotonin neural circuits frequently serve to counterbalance the arousing activating dopamine/noradrenaline circuit. Anxious, agitated emotion occurs when a person's dopamine/noradrenaline activity arousal circuits are functioning more strongly than the calming, mellowing serotonin circuits (as a compensatory counterbalance). It is likely that those rodents did not show anxiety and fear related behaviours because cooked beans may have increased their brain serotonin levels.

The elevated plus maze has two open and closed arms respectively in the shape of a plus. The open arms are aversive to mice because they are open and the maze is elevated [18]. The closed arms provide a sense of safety because they are enclosed. This test exploits the conflict between the natural tendency of mice to explore new areas and have phobia for open spaces.

Behaviours such as open arm activity and head dipping are considered exploratory and a greater frequency of these measures depicts a heightened level of boldness. Fear related behaviours typically include, closed arm activity, stretch attend posture; a greater number of these measures depict greater level of fear [18]. Another risk assessment behaviour is head dip. This also serves as an index of level of anxiety in a rodent [25]. Another behaviour that strongly correlates with anxiety, is the closed arm duration in the elevated plus maze. Close arm duration was found to be significantly longer in scopolamine only treated group compared to control. This result showed that the scopolamine only treated mice displayed increased level of fear and anxiety. On the other hand, the frequency of head dips for groups of mice fed with cooked beans as well as serotonin precursor diets were significantly higher compared to scopolamine only treated group. The open arms duration for groups of mice fed with cooked beans as well as serotonin precursor diets were also significantly higher compared to scopolamine only treated group. This depicted a lower index level of anxiety and fear. Longer open arms duration and higher frequency of head dips are both behaviours which point to decreased anxiety. These behaviours correlate strongly, and the higher their values, the less the anxiety level. Therefore cooked beans consumption may be reducing anxiety in mice.

The forced swim test in mice was developed to test rodents for immobility because it was discovered that rodents became immobile after an initial swimming activity in an inescapable situation. The duration of immobility is considered a measure of despair or depression. The forced swim test result shows that the group of mice treated with scopolamine only had increased significant level of immobility compared to control and other experimental groups. The result of this work shows that, cooked beans as well as serotonin precursor diets improves depressive symptoms in mice models of depression.

Cooked beans have been shown to contain ser-

otonin precursor (tryptophan) and antioxidants (flavonoids and polyphenols) [7] It is possible that cooked beans as well as serotonin precursor diets may have enhanced the biosynthesis of serotonin in the brain of the mice which could have caused reduced depressive activities in ours models [26]. Lee *et al.,* [27] reported that antioxidants improved stressful conditions in mice. The improved depressive conditions observed in this research work may be attributed to serotonin and antioxidants present in beans.

In conclusion, consumption of cooked beans and serotonin precursor diets displayed anxiolytic and antidepressive potentials in animals fed such diets following scopolamine administration. These potentials may be attributed to serotonin and its precursors present in cooked beans diet.

REFERENCES

[1] Wader, J.K., Telek, L., Vozari-Hampe, M., Saini, H.S. (1998) Antinutritional factors in Anasazi and other Pinto beans (*Phaseolus vulgaris*). Plant Foods for Human Nutrition. 51, 85-98.

[2] Shansuddin, A.M., Elsayed, A. (1998) Suppression of large intestinal cancer in F344 rats by inositol Hexaphosphate Carcinogenesis. 9, 577-580.

[3] Vanderpoel, A.F.B., Liener, L.E., Mollee, P.W., Huisman, J. (1990C) variation among species of animals in response to the feeding of heat processed beans (Phsaseolus vulganis.2) Growth and organ weights of chickens and rats and digestibility of rats. Livestock Production Sciences. 25, 137-150.

[4] Gref, E., Eaton, J.W. (1993) Suppression of Caloric Cancer by Dietary phyto Acid. International Journal of Nutrition and Cancer. 19, 11-19.

[5] Bennink, E., Maurice, O., Elizabeth, R. (2008) Beans & Health: A Comprehensive Review, Frazee, MN; 2008, Accessed from beaninstitute.com on March 1, 2021.

[6] Porta, H., Figueroa-Balderas, R.E., Rocha-Sosa, M. (2008) Wounding and pathogen infection induce a chloroplast-targeted lipoxygenase in the common bean (*Phaseolus vulgaris* L.). Planta. 227, 363-373.

[7] Aduema, W. (2016) Effects of Long-Term Administration of Cooked Beans (*Vigna unguiculata*) Diet On Learning and Memory in Mice. International Journal of Food Sciences. 1, 1-15.

[8] Patras, A., Tiwari, B.K., Brunton, N.P. (2011) Influence of blanching and low temperature preservation strategies on antioxidant activity and phytochemical content of carrots, green beans and broccoli. LWT-Food Science and Technology. 44, 299-306.

[9] Daniel, L.C., Michael, R.K. (2007) Biogenic amine neurotransmitter in *C.elegans*. Panadesa: Wormbook. 1-15.

[10] Young, S.N., Leyton, M. (2002) The role of serotonin in human mood and social interaction: Insight from altered tryptophan levels. Pharmacology Biochemistry and Behavior. 71, 857–865.

[11] Ovsepian, S.V., Anwyl, R., Rowan, M.J. (2004) Endogenous acetylcholine lowers the threshold for long-term potentiation induction in the CA1 area through muscarinic receptor activation: in vivo study. European Journal of Neuroscience. 20, 1267-1275.

[12] Hasselmann, H. (2014) Scoplamine and depression: A role for muscarinic antagonism. CNS & Neurological disorders- Drug targets. 13, 673-683.

[13] Johnson, W.C., William, O.W. (2002) Warfarin toxicity. Journal of Vascular Surgery. 35, 413-431.

[14] WHO. (2003) Traditional Medicine, World Health Organization, Geneva.

[15] Feldman, J.M., Lee, E.M. (1985) Serotonin content of foods: effect on urinary excretion of 5-hydroxylindole acetic acid. The American Journal of Clinical Nutrition. 42, 639-643.

[16] Mosienko, V., Bert, B., Beis, D., Matthes, S., Fink, H., Bader, M., Alenina, N. (2012) Exaggerated aggression and anxiety in mice deficient in serotonin. Translational Psychiatry. 2, 122-124.

[17] Bourin, M., Hascoët, M. (2003) The mouse light/dark box test. European Journal of Pharmacology. 463, 55-65.

[18] Lister, R.G. (1990) Ethologically-based animal models of anxiety disorders. Pharmacological Theory. 46, 321-340.

[19] Gilhotra, R, Goel, S., Gilhotra, N. (2015) Behavioral and biochemical characterization of elevated "I-maze" as animal model of anxiety. Beni-Suef University Journal of Basic and Applied Sciences. 4, 214–224.

[20] David, D.J., Renard, C.E., Jolliet, P., Hascoet, M., Bourin, M. (2003) Antidepressant-like effects in various mice strains in the forced swimming test. Psychopharmacology (Berl). 166, 373–382.

[21] Nilson, R. (1998) A qualitative and quantitative risk assessment of snuff dipping. Regulatory Toxicology and Pharmacology. 28, 1–16.

[22] Osim, E.E. (2008) Neurophysiology (3rd edition). Calabar-Nigeria. Glad Tidings Press. 2008.

[23] Adolphs, R., Gasselin, F., Buchanan, T.W., Tranel, D., Scgyns, P., Damasio, A.R. (2005) A Mechanism for impaired fear recognition after amygdala damage. Nature. 433, 68–72.

[24] Young, S.N., Teff, K.L. (1989) Tryptophan availability, 5-HTP synthesis and 5-HT function. Progress in Neuropsychopharmacology and Biological Psychiatry. 13, 373–379.

[25] Braun, A.A., Skelton, M.R., Vorhees, V., Williams, M.T. (2012) Comparison of the elevated plus and elevated zero mazes in treated and untreated male Sprague-Dawley rats: Effects of anxiolytic and anxiogenic agents. Pharmacology, Biochemistry and Behavior. 97, 406–415.
[26] Meltzer, H.Y. (1990) Role of Serotonin in Depression. Annals of the New York Academy of Sciences. 600, 486-499.
[27] Lee, D.S., Jo, H.G., Kim, M.J., Lee, H., Cheong, S.H. (2019) Antioxidant and Anti-Stress effects of taurine against electric foot-Shock-Induced Acute Stress in Rats. Advances in Experimental Medicine and Biology. 11, 185-196.

Received: **12.06.2021**
Accepted: **28.06.2021**

CORRESPONDING AUTHOR

Eme Efiom Osim
Department of Physiology,
Faculty of Basic Medical Sciences,
College of Medical Sciences,
University of Calabar,
Calabar PMB 1115 – Nigeria

e-mail: emeosim@yahoo.com

AUTHOR INDEX

SUBJECT INDEX

AFS– GUIDE FOR AUTHORS

General

AFS accepts original papers, review articles, short communications, research abstracts from the entire sphere of Foodchemistry,-biology,- microbiology,- technology, -biotechnology and-management, furthermore, about residue analysis/ and ecotoxicology of contaminants.

Acceptance or no acceptance of a contribution will be decided, as in the case of other scientific journals, by a board of reviewers.Papers are processed with the understanding that they have not been published before (except in form of an abstract or as a part of a published lecture, review or thesis);that they are not under consideration for publication elsewhere; that their publication has been approved by all co-authors, if any, as well as- tacitly or explicitly- by the responsible authorities at the institute where the work has been carried out and that, if accepted, it will not be published elsewhere in the same form, in either the same or another language, without the consent of the copyright holders.

Language

Papers must be written in English. Spelling may either follow American (Webster) or British (Oxford) usage but must be consistent. Authors who are less familiar with the English language should seek assistance from proficient colleagues in order to produce manuscripts that are grammatically and linguistically correct.

Size of manuscript

Review articles should not exceed 30 typewritten pages. In addition up to 5 figures may be included.

Original papers must not exceed 14 typewritten pages. In addition up to 5 figures may be included.

Short-Communications should be limited to 4 typewritten pages plus not more than 1 illustration.

Short descriptions of the authors, presentation of their groups and their research activities (with photo) should together not exceed I typewritten page.Short research abstracts should report in a few brief sentences (one-fourth to one page) particularly significant findings.Short articles by relative newcomers to the chemical innovation arena highlight the key elements of their Master and PhD-works in about 1 page.

Book Reviews are normally written in-house, but suggestions for books to review are welcome.

Preparation of manuscript

Dear Authors,

AFS is available both as printed journal and as online journal on the web. You can now e-mail your manuscripts with an attached file. Save both time and money.To avoid any problems handling your text please follow the instructions given below:

When preparing your manuscripts have the formula K/SS (Keep It Simple and Stupid) in mind.Most word processing programs such as MS-Word offer a lot of features. Some of them can do serious harm to our layout. So please do not insert hyperlinks and/or automatic cross-references, tables of contents, references, footnotes, etc.

1. Please use the standard format features of your word processor (such as standard.dotfor MS Word).
2. Please do not insert automatisms or secret link-ups between your text and your figures ortables. These features will drive our graphic department sometimes mad.
3. Please only use two fonts for text or tables
"Times New Roman" and for graphical presentations "Arial".
4. Stylesheets, text, tables and graphics in shade of grey
5. Turn on the automatic language detection inEnglish (American or British)
6. Please - check your files for viruses before you send them to us!!

Manuscripts should send to:parlar@wzw.tum.de
or:parlar@prt-parlar.de

Thank you very much!

STRUCTURE OF THE MANUSCRIPT

Title page: The first page of the manuscript should contain the following items in the sequence given:A concise title of the paper (no abbreviations). The names of all authors with at least one first namespelled out for every author.The names of Universities with Faculty, City andCountry of all authors.

Abstracts: The second page of the manuscript should start with an abstract that summarizes briefly the contents of thepaper (except short communications). Its length shouldnot exceed 150-200 words. The abstract should be as informative as possible. An extended repetition of the paper's title is not considered to be an abstract.

Keywords: Below the Summary up to 6 key words have to beprovided which will assist indexers in cross-indexing your article.

Introduction: This should define the problem and, if possible, the frame of existing knowledge. Please ensure that peoplenot working in that particular field will be able to understand the intention. The word length of the introductionshould be 150 to 300 words.

Materials and methods:
Please be as precise as possible to enable other scientiststo repeat the work.

Results: Only material pertinent to the subject must be included.Data must not be repeated in figures and tables.

Acknowledgements: Acknowledgements of financial support, advice orother kind of assistance should be given at the end of thetext under the heading "Acknowledgements". The names of funding organisations should be written in full.

References: Responsibility for the accuracy of references rests with the authors. References are to be limited in numberto those absolutely necessary.References should appear in numerical order in brackets and in order of their citation in the text. They should be grouped at the end of the paper in numerical order of appearance. Abbreviated titles of periodicals are to be used according to Chemical or Biological Abstracts,but names of lesser known journals should be typed in full.References should be styled and punctuated according to the following examples:

ORIGINALPAPERS:
l. Author, N.N. and Author, N.N. (Year) Full title of
the article. Journal and Volume, first and last page.

BOOK OR PROCEEDING:
2. Author, N.N. and Author, N.N. (Year) Title of the contribution.
In: Title of the book or proceeding. Volume (Edition
of klitor-s, ed-s) Publisher, City, first and last page

DOCTORALTHESIS:
3. Author, N.N. (Year) Title of the thesis, University and
Faculty, City

UNPUBLISHED WORK:
Papers that are unpublished but have been submitted to a journal may be cited with the journal's name followed by "in press". However,this practice is acceptable only if the author has at least receivedgalley proofs of his paper. In all other cases reference must be made to “unpublished work" or "personal communication".

Discussion and Conclusion: This part should interpret the results in reference to the problem outlined in the introduction and of relatedobservations by the author/s or others. Implications for further studies or application may be discussed. A conclusion should be added if results and discussion are combined.

Corresponding author: The name of the corresponding author with complete postal address