Journal of Emerging Trends in Engineering and Applied Sciences (JETEAS) 5(8): 206-209

jeteas.scholarlinkresearch.com

PROCESS AND MATHEMATICAL MODEL IN THE FORMULATION OF MICROBIOLOGICAL MEDIA FROM THE EXOSKELETON OF CRUSTACEANS

Egwari, L. O., and Oniha, M. I
Department of Biological Sciences,
College of Science and Technology, Covenant University,
Canaan Land, Ota, Ogun State, Nigeria.
Corresponding Author: Egwari, L. O

Abstract
Culture media are very central in the study of microorganisms. Most microbiological media are formulated with beef or soya as protein source. The need for cheaper source of raw material is exploited with the use of exoskeleton of crabs (*Callinectes amnicola*; shell only) and shrimps (*Penaeus notialis*; head, shell and appendages). The mathematical model for the formulation derived and the formulated media assessed for nutritional value and ability to support microbial growth. Proximate analyses of infusions from the nutrient sources revealed high contents of minerals especially Na^+, K^+, Ca^{2+}, Fe^{2+}, Mg^{2+}, SO_4^{2-}, NO_3^-, PO_4^{2-} and traces of glucose. Fats and proteins were higher in crab shell (25.39%, 12.36%) and shrimp exoskeleton (3.39%, 18.48%) respectively. Infusions from these nutrient sources separately or in combination supported good growth of bacteria, moulds and yeasts either as broth or as agar media. The pH changes as a result of microbial growth were minimal thus occluding the need for buffering. The diversity of microorganisms that grew on the media satisfies their requirement as general purpose growth media.

Keywords: crustaceans, exoskeleton, mathematical model, culture media, microorganisms

INTRODUCTION

The coastal waters of many Africa nations are rich in fisheries resources. The shrimp landing in Nigeria was estimated at 4,500-5,000t within the Exclusive Economic Zones (EEC) that covers an area of 210,900km^2 and estimated shrimp annual value of US$29.6M-$50M export earnings (Falaye, 2008; Raji, 2008). Shrimp fishery is also very lucrative in the marine and coastline of Angola, Gambia, Ghana, Guinea, Mauritania and Senegal (Falaye, 2008). The global report on Africa's marine fisheries is that approximately 4.6M tons of fish are harvested from the continent's marine waters each year, together with a total of 2.3M tons from inland fisheries and 0.7M tons from aquaculture (World Bank Group's Global Program on Fisheries; www.worldbank.org/fish).

Crabs, lobsters and shrimps are crustaceans with exoskeleton which is comprised of protein, chitin and calcium carbonate (Abdel-Salam, 2013). Ekpenyong *et al*. (2013) reported that in many communities in Nigeria and other parts of the world the exoskeleton and appendages of shrimps and crabs are not usually included in the diet despite the high nutrient contents and the fact that these parts constitute from 40-50% of the whole organism. These biomaterials are either discarded or have been processed as animal feed, or may find application in water purification, cosmetics or pharmaceuticals (Morganti *et al*., 2011; Abdel-Salam, 2013). The far reaching implication is the huge economic waste in situation where these parts are discarded in addition to the obvious environmental and health hazards their indiscriminate disposal constitute.

Ironically, over a century when bacteria were first grown on organic substrates, the nutrient source has remained food base; with beef and soya constituting the bulk. The diversion of these food sources to industrial use depletes the protein available from these sources and may signal protein malnutrition in many poor nations where drought, famine and war are prevalent. The justification for the present study is to replace beef and soya which are major sources of protein worldwide with biomaterials which are either waste or are not highly priced. In a series of study involving decapods we established infusion from the white sun-dried crayfish *Nematopalaemon hastatus* to support the growth of heterotrophic bacteria (Egwari and Otegbeye, 2000) and that crayfish chaff agar was suitable as a transport medium for clinical specimens for anaerobic study (Egwari *et al*., 2013). In the present study, we devised a mathematical model in the formulation of microbial growth media from the exoskeleton of crustaceans.

MATERIALS AND METHODS

The exoskeletons of the crustaceans (shell of crab *Callinectes amnicola*; carapace, appendages and head region of the shrimps *Penaeus notialis*) were extracted, cleaned and air dried. These were crushed and ground into fine powder and infused in boiling water for 30 min and then infused in the cold at 4-6^o C for 48 h. Potassium hydroxide (0.1M) was added to the infusion before heating at 121^o C to precipitate phosphates while dilute acid was used for pH adjustment. The infusion obtained was concentrated over a burner heat and absorbed on a solid matrix (rice starch).

The process included determination of the gel property of starch of different starchy foods and other properties that make them suitable carriers for the infused nutrients; added to this was assessment of their hygroscopic property which was important in determining their contribution to the shelf-life of the final products. The

process also included in part steps in starch extraction from the starchy food (rice or potato) and the use of the starch extracted as carrier for the infusion to obtain dehydrated solid cakes or flakes which in the dry form is non hygroscopic. The dehydrated growth substrate (or media) was ground into fine powder and stored at ambient temperature (25-32^{o} C).

The optimum growth concentration (OGC) is the highest dilution of the infusion concentrate on a scale of 1 to 10 that yielded microbial growth of $\approx 10^6$ cells/ml after aerobic incubation at 37^0 C for 24h. The OGC was determined by turbidometry and was used to calculate the amount of media to be dispersed in 1L of water during preparation of agar plates.

The infusion concentrates from the aquatic animals were determined for nutritional composition (protein, fats, carbohydrates, ash and minerals) as described by Association of Official Analytical Chemists (AOAC, 2005) and the formulation was adjusted to determine the dilution factor by introducing mathematical equations to calculate OGC of the formulation that will support microbial growth (Equations 1-3). The medium formulated either singly or pooled was tested for ability to support the growth of heterotrophic bacteria and fungi.

RESULTS AND DISCUSSION

Figure 1 is a flow chart describing the processes in the production of growth infusion concentrate from parts of crustaceans used. Critical control points are weights of components used and the volume of the concentrate obtained from a determined weight. Thus in the flow chart, the weight of the whole fauna before extraction of exoskeleton, weight of component parts used and weights of the oven dried and ground portions were essential together with the volume of infused concentrate in deriving the formula for compounding the growth media and in the calculation of the amount of nutrient contained in a given amount of the dehydrated medium. The wet weight ratio of shrimps exoskeleton used to the whole fauna was 3:2 while crab shell to the whole organism was 2:1 (data not shown). This corroborated assertions that wastes generated from industrial shrimp processing in India ranges from 48 to 56% (Sachindra *et al*., 2006). The wastes are utilized in the production of animal feeds (Stevens *et al*., 1998) while Abdel-Salam (2013) is of the opinion that these may constitute useful nutraceuticals for animals. Their use in the present study for culture media formulation opens up new opportunities for further exploitation. A classical example of a food crop with enormous industrial application is the cassava plant with the root, leaves and waste from peels and pulp finding diverse applications that have revolutionized the agro-allied industry (Sriroth *et al*., 2000; Barros *et al*., 2013; Wongkongsooong *et al.*, 2013). The cassava phenomenon is a stitch in time and clarion call for investment in research outputs as the present study aptly present.

With respect to shrimp and crab, the emphasis was on the shell and appendages which in most places especially for commerce are not included as part of the edible portion sold to consumers as processed sea foods (Zhai and Hawkins, 2002). Therefore the sorting stage preferentially separated the edible portion and this did not constitute part of the formulation. This is important as the invention considered cost reduction in the formulation of growth media. In essence the food value of these sea foods were not reduced or hampered as a result of their use for this purpose. In an earlier described study with crayfish for same purpose, the de-shelling stage was not included because of the thin membrane that constitutes the exoskeleton (Egwari and Otegbeye, 2000). It is important to emphasize that crayfish can equally be part of this formulation as its use for culture media production will not significantly affect its food value for two reasons; it can be cultivated with ease and it has limited food use, mostly to spice up local diets.

The stages in the extraction of infusion from the dried, ground powder of the heat processed crustaceans followed conventional methods (boiling, cold infusion, sieving, and precipitation of phosphate at elevated temperature, filtration and evaporation). Adjustments of conditions at each of these stages may be peculiar to this formulation especially the process of concentrating the infusion. For agar formulation the infusion concentrate was absorbed at optimum growth concentration (OGC) unto solid matrix. In this case starch extracted from rice and or potato. Starch from these sources was selected because of their low gel capacity as was determined to be suitable for this purpose. The starch augmented the low calorie in the exoskeleton due to trace levels of glucose detected (0.91%) and as earlier reported; 1.17% and 0.63% in hard shell crab and soft shell crab respectively (Okuzumi and Fujii, 2000). The formulation however allows for the infusion concentrate to be absorbed onto any other carrier particle as may be required or considered economically viable.

Equation 1 was used to determine the amount of nutrient as contained in infused concentrate that will give the optimal microbial growth concentration per liter of solution. The OGC was the highest dilution of the infusion concentrate that yielded 10^6 cells/ml of culture (C^{-1}); the reciprocal of the dilution known as the dilution factor (C_1) was used for the calculation. V is the volume of infusion concentrate to be reconstituted based on the dilution factor per liter.

Therefore, $VC_1 = 1000ml$ Equation 1

Equation 2 was used to determine the amount of starch (carrier substance) that will absorb a known volume of the infusion concentrate at OGC. Y is the weight of the starch to be determined, C_2 is the absorbing capacity of the starch (i.e., reciprocal of the dilution) and V is the known volume of the infusion concentrate per liter of medium. From the formula $YC_2 = V$, the value for Y can be calculated. Thus, $Y = V/C_2$ Equation 2

Equation 3 was the formula used to calculate the amount of the dehydrated medium that was dispersed in 1L of solvent at OGC. Equation 3 was derived from equations 1 and 2.

Recall; $VC_1 = 1000$ 1

Therefore $V = 1000/C_1$

$$Y = V/C_2 \quad (2)$$

Therefore $V = YC_2$

If $V = 1000/C_1 = YC_2$

Therefore $YC_2 = 1000/C_1$

$$Y = 1000/C_1.C_2 \quad (3)$$

Table 1: Nutrient contents of exoskeleton of crab and shrimp

Nutrient (%) availability	Crab	Shrimp
Carbohydrates (glucose)	0.78	0.91
Fats	25.39	3.39
Protein	12.36	18.48
Minerals		
Na^+	0.54	0.56
K^+	0.51	0.50
Ca^{2+}	2.13	1.10
Fe^{2+}	+	+
Mg^{2+}	0.46	1.83
SO_4^{2-}	+	+
NO_3^-	+	+
PO_4^{2+}	+	+

+; detected qualitatively.

The formulated media grew well moulds (*Aspergillus*, *Penicillium*, *Rhizopus*, *Fusarium* and *Alternaria*), yeasts notably *Candida* and five genera of bacteria namely; *Escherichia*, *Klebsiella*, *Pseudomonas*, *Staphylococcus* and *Bacillus*. Table 1 highlights the nutrient composition of the crustaceans' exoskeleton with values comparable with those of Ekpenyong *et al*. (2013), Sudhakar *et al*. (2009) and thus attesting to the diverse industrial use of crab and shrimp exoskeleton.

CONCLUSION

The importance of microorganisms in health, food production, pharmaceuticals and biodegradation would have been obscured without first isolating and characterizing them. Though new technologies are emerging in understanding the physiology of microorganisms and also in improving their functions, the culture media remain the gold standard especially in harvesting microorganisms for industrial applications. Despite the huge demand for growth media across the globe, few companies have maintained monopoly of the market. This is connected with the huge investment in the raw materials that form the nutrient base of these media (notably beef or soya bean). Consequently, the cost of growth media is prohibitive; this justifies alternative sources of proteins for formulation of microbial growth media. This study which described the formulation and production of microbial culture media from exoskeletons of crabs (*Callinectes amnicola*; shell only) and shrimps (*Penaeus notialis*; head, shell and appendages) has the added advantages of low production cost as the technology is simple, the raw materials are available and very cheap. This makes it possible for small to medium scale enterprise to engage in media production for local consumption. The invention is environmentally friendly and of health benefit, as the sources of raw materials are wastes which when not properly disposed off will constitute public health risk. Further the invention if applied to industrial use has the potential of opening up other industries and creating new jobs. It will of particular expand shrimp and crab farming in Africa with the emphasis of harnessing the exoskeletons for industrial use. This invention when exploited has great economic gains, immense potentials for industrial diversification and it is both feasible and sustainable.

BIOGRAPHY

Professor L. O. Egwari is a professor of Medical Microbiology at Covenant University with specialization in anaerobic bacteria in human infections. He is the Director of Research and Development in Covenant University and has published many papers in reputable journals. He is a member of Anaerobes Society of the Americas.

Miss M. I. Oniha is a Ph.D student in Covenant University working on the pathology of *Carica papaya* L fruits.

REFERENCES

Abdel-Salam, H. A. (2013). Assessment of biochemical compositions and mineral contents of carapace of some important commercial crustaceans and mollusks organisms from Egyptian and Saudi Arabia coasts as a new animal feed. Am J Biosci, 1(2):35-43.

AOAC (2005). Association of Official Analytical Chemists. Official methods of analysis (18th ed.). Arlington VA, USA.

Barros, F. F. C., Simiqueli, A. P. R., de Andrade, C. and Pastore G. M. (2013). Production of enzymes from agro-industrial wastes by biosurfactant-producing strains of Bacillus subtilis. Biotechnology Research International, 2013, ID 103960, http://dx.doi.org/10.1155/2013/103960.

Egwari, L. O. and Otegbeye, F. (2000). Crayfish broth for bacterial culture. East Afr Med J, 77(5): 264-267.

Egwari, L. O., Nwokoye, N. N. and Oniha, M. I. (2013). Evaluation of crayfish chaff charcoal agar as a transport medium foranaerobes. Scientific Research and Essays, 8(23): 1107-1111.

Ekpenyong, E., Williams, I. O. and Osakpa, U. U. (2013). Variation in the proximate, energy and mineral compositions of different body parts of Macrobrachium macrobranchion (Prawn). J Food Res, 2(2): 150-156.

Falaye, A. E. (2008). Illegal unreported unregulated (IUU) fishing in West Africa (Nigeria and Ghana). Marine Resources Assessment Group Report, January,2008; pp1-32MRAG Ltd, Queens Street, London.

Morganti, P., Morganti, G. and Morganti, A. (2011). Transforming nanostructured chitin from crustacean waste into beneficial health products: a must for our society. Nanotechnol Sci Appl, 4: 123-129.

Okuzumi, M. and Fujii, T. (2000). Nutritional and functional properties of squid and cuttle fish. 35th Anniversary of commemorative publication, pp:223.

Raji, A. (2008). The Nigerian Fisheries and the attainment of the Millennium Development Goals. Fish Network, 4(3): 28.

Sachindra, N. M., Bhaskar, N., Mahendrakar, N. S. (2006). Recovery of carotenoids from shrimp waste in organic solvents. Waste Manag, 26: 1092-1098.

Sriroth, K., Chollakup, R., Chotineeeranat, S., Piyachomkwan, K. and Oates, C. G. (2000). Processing of cassava waste for improved biomas utilization. Bioresource Technol, 71(1): 63-69.

Stevens, W. E., Cheypratub, P., Haiqing, S, Lertsutthiwong, P. and Chandrkrachang, S. (1998). Bioprocess Technology Program, Asian Institute of Technology, Bangkok Thailand, 5th Asian Fisheries Forum, ISBN 974-7578-02-6.

Sudhakar, M., Manivannan, K. and Soundrapandian, P. (2009). Nutritive value of hard and soft shell crabs of Portunus sanguinolentus (Herbst). Interna J Animal and Vet Adv., 1(2): 44-48.

Wongkogsoong, R., Iwai, C. B. and Ta-oun M. (2013).Effectof organic materials from agro-industrial wastes (molasses and distillery slop) on earthworms under vermicomposting using cassava waste. International Journal of Environmental and Rural Development, 4 (1): 95-99

World Bank Group's Global Program on Fisheries; www.worldbank.org/fish

Zhai, X., and Hawkins, S.J. (2002). Interactions of aquaculture and waste disposal in the coastal zone. J Ocean University of Qingdao, 1:8-12.

ACKNOWLEDGEMENT: The work was partly supported with the research grant from the Covenant University Centre for Research and Development.

APPENDIX

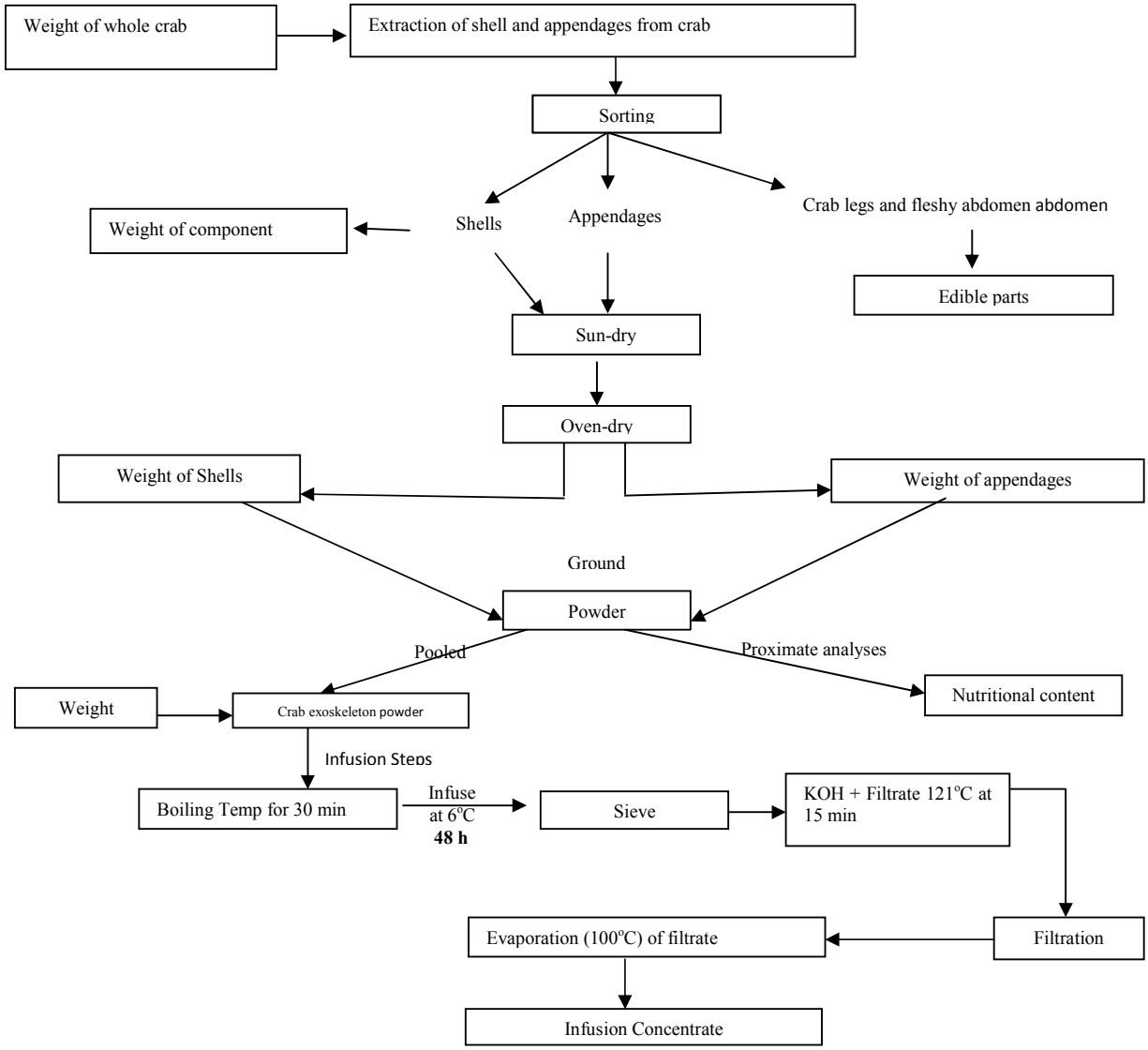

Figure 1: Production of growth infusion concentrates from parts of crustaceans