Development of immune-boosting vegan sausage utilizing baby jackfruit (*Artocarpus heterophyllus*) by replacing carcinogenic curing salts with natural pigment source

Wijegunawardhana D.T, Madushani E.D.A, Gamage S.D.C
Faculty of Technology, University of Sri Jayewardenepura

Abstract: In recent decades the demand for processed meat-based products like sausages has been gradually enhanced. But most traditional meat sausages available in the local market may lead to a lot of non-communicable diseases due to high-fat content. Curing agents (nitrates and nitrite), which are used to impart cured meat color & preservation purposes, are considered carcinogenic. This study was carried out to develop healthy non-meat-based sausage by utilizing jack fruit, a seasonal, underutilized crop in Sri Lanka. Moreover, some other tomato paste ingredients were added to replace the nitrate salt and spices, emulsion & composite flour mixture. The developed product has the potential to be preserved for more than four months without adding any preservatives.

Index Terms: Jack fruit, Curing agents, Cooking loss, Lycopene, Vegan sausage

1 INTRODUCTION

The word sausage is derived from the word "Salsus" in the Latin language, which is the meaning of preserved by salting[1] or "Salsicia," meaning something salted. Generally, this term applied to cured or salted meat. In ancient times, there were no refrigeration facilities to preserve the meat; therefore, this sausage making is the way to solve this problem early. This sausage making is the very olden method recorded since the ancient Egyptians and American Indians [2]. Sausage is pre-cooked food consisting of ground meat, animal fat, spices, Salt, and packed within a casing. According to the USDA (united state department of agriculture), 100g of meat sausage contains fat (18%), Protein (22%), Saturated fatty acid (7.3%), Salt (2.3%), and Carbohydrate (0.9%) [3].

As shown above, meat sausage products contain high-fat content, which leads to more non-communicable diseases. In the present context, the dietary behavior of the Sri Lankan people is more inclined to the meat-based product rather than vegetables. These meat-based dietary habits are the primary reason for the development of obesity and cardiovascular diseases and scientifically proved that there is a positive relationship between dietary patterns and obesity-related diseases such as diabetes, heart disease, stroke, and some forms of cancers [4]. Furthermore, as a curing agent, Nitrates (potassium nitrate, sodium nitrate) and nitrites(potassium nitrite and sodium nitrite) were added to these meat products to gain the quality characteristics(microbial safety, preferred red color in meat) of the end-product. According to the commission regulations in the European Union, the amount of nitrate permitted for used in meat products is 150mg per 1kg of end product[5, 6, 7], but after the production of meat product,10%-20% of nitrites remains on the food as residual nitrites which mainly caused adverse health effects like cancers, heart failures, etc.[8].

Due to society's problems mentioned above, a vegetable-based sausage product was developed by using the underutilized vegetable raw material in Sri Lanka. Here the term vegetable-based sausage means the product is mainly based on the vegetables, and although the production steps are much similar to the meat sausages. Vegetable consumption may cause to minimize the Cholesterols in blood, obesity, blood pressure, Body Mass Index (BMI), easier digestion of foods, remove toxins from the body, boost immune boost, and reduce the

cancer risks the consumption of meat [9]. Therefore, as the main vegetable ingredient, *Artocarpus heterophyllus*, commonly known as jack fruit, belongs to the family Moraceae, which was used to develop this sausage product [10, 11]. This is a tropical, climacteric fruit that is native to the Western parts of India and common fruit in Asia, Africa, and some regions in South America [12]. Jack tree is a medium-sized evergreen tree that reaches the 8-25m in height [13]. A matured jack tree can yield 10-200 fruits for one yield time. Jack fruits are dicotyledonous compound fruit [14], which are cylindrical, 22-90 cm in length, and 13-50 cm in diameter. The weight of individual fruits may vary between 2-20 kg. This fruit has a green to yellow exterior rind that is consist of hexagonal carpel apices that cover with a thick rubbery whitish-yellow wall [15, 16, 17]. There is a large number of bulbs inside the fruit which have high nutrient value. Depending on the variety, this bulb's color can be varied to white, light yellow, yellow, deep yellow, and orange [18]. The composition of the jack fruit is given in the Table 1.

Table 1. Composition of Jack fruit (100g edible portion) [15, 16, 19-22]

Composition	Young fruit	Ripe fruit
Water(g)	76.2-85.2	72.0-94.0
Protein(g)	2.0-2.6	1.2-1.9
Fat(g)	0.1-0.6	0.1-0.4
Carbohydrates(g)	9.4-11.5	16.0-25.4
Fibre(g)	2.6-3.6	1.0-1.5
Total sugars(g)	-	20.6
Total minerals(g)	0.9	0.87-0.9
Calcium(mg)	30.0-73.2	20.0-37.0
Magnesium(mg)	-	27.0
Phosphorus(mg)	20.0-57.2	38.0-41.0
Potassium(mg)	287-323	191-407
Sodium(mg)	3.0-35.0	2.0-41.0
Iron(mg)	0.4-1.9	0.5-1.1
Vitamin A(mg)	30.0	175-540
Thiamin(mg)	0.05-0.15	0.03-0.09
Riboflavin(mg)	0.05-0.2	0.05-0.4
Vitamin C(mg)	12.0-14.0	7.0-10.0
Energy(KJ)	50-210	88-410

Even though jack fruit contains lots of nutrients, it is considered an underutilized vegetable crop with excess production per year. But unfortunately, instead of processing that excess amount, they remove it as wastage [23]. Many countries develop more value-added products like jelly, jam, marmalade, and ice creams like products from the jack fruit puree to overcome this severe issue. In Sri Lanka, also there are some traditional cuisines made by jack fruit with different maturity stages. Mainly the raw jackfruit pieces (Sri Lankan term, "Polos") used to made curries. Therefore these raw pieces of jack fruit are the primary vegetable-based raw material used to develop this sausage product. Therefore, this study is carried out to develop vegetable-based sausage products using underutilized, highly nutritious raw material called *Artocarpus heterophyllus* (Jack fruit) as a useful alternative for meat sausages. Fig. 1 represent the different stages of the jackfruit which is used for this research.

Fig. 1. Jack fruits in different maturity stages and jack seeds
(a) Jack fruit tree with different sizes of fruits (b) Different stages of fruiting (c) Raw jack fruit pieces used for this product (d) Ripen edible jackfruit flesh (e) Jack fruit seed.

2 PROCEDURE

2.1 Process Description

Fresh, baby jack fruits *(Artocarpus heterophyllous)* were harvested from the trees in Sothern Province, Sri Lanka. The rest of all other ingredients (tomatoes, salt, sugar, black pepper, garlic, chili powder, rice flour, maize powder, milk powder, vegetable fat, non-fat milk powder) to prepare non-meat sausages were purchased from the local market.

2.2 Ingredients

- Jack fruits (*Artocarpus heterophyllous*) 500g
- Tomato paste 8g
- Salt 13g
- Sugar 4g

Spice mixture

- Garlic 4g
- Black pepper 3g
- Chilli powder 1.5g

Composite flour mixture

- Rice flour 17.5g
- Bread crumbs 17.5g
- Maize powder 8.7g

Emulsion

- Ice 4.3g
- Vegetable fat 13g
- Soy isolate 191g

2.3 Preparation of tomato paste

- Initially fresh, ripened tomatoes were selected by eliminating infected and bruised tomatoes
- Then tomatoes were thoroughly washed with chlorinated water
- After that tomato was cut into pieces & pulp was prepared by using a mixer
- Next pulp was strained & heated under low flame
- As the next step, tomato paste was removed from the flame at 42˚ Brix
- Finally, prepared tomato paste was filled into the sterilized glass bottle

2.4 Process flow diagram of preparation of non-meat jack fruit sausages

Schematic representation of the preparation of non-meat jack fruit sausages is depicted in Fig. 2, while Fig. 3 Show some pictures of non-meat based jack fruit sausage preparation.

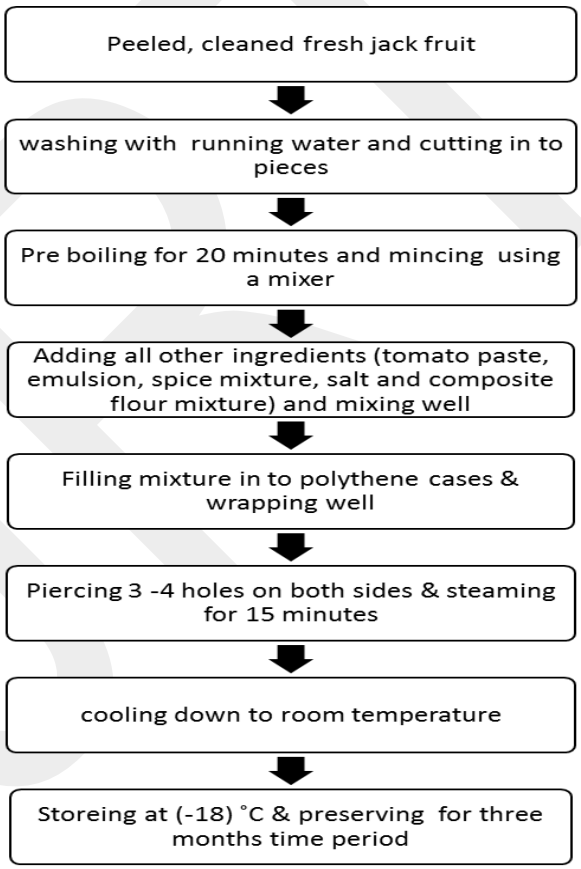

Fig.2. Non-meat Jack fruit sausages production process

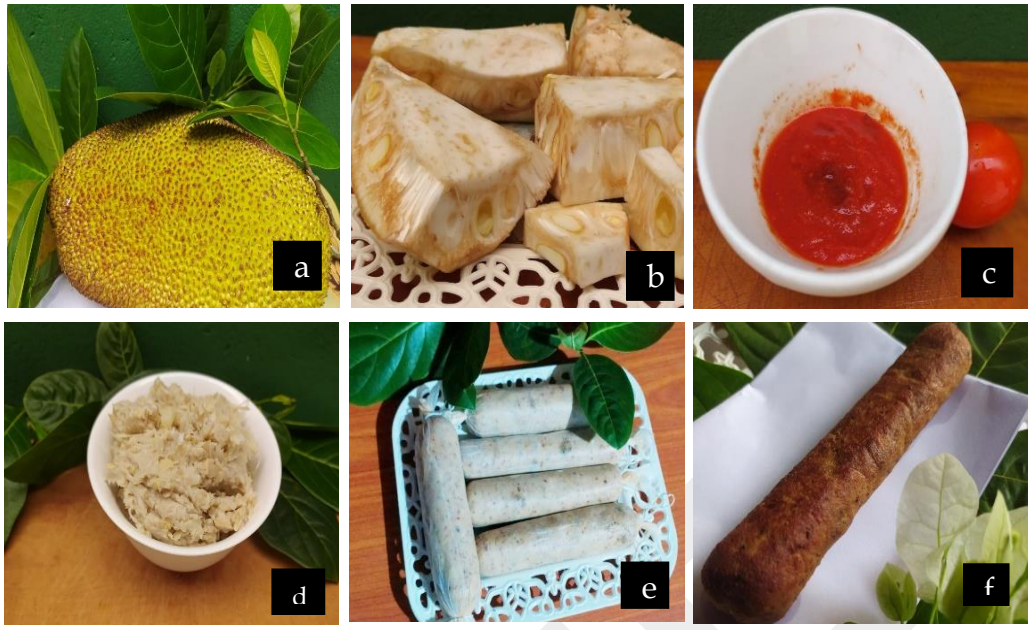

Fig. 3. Non-meat Jack fruit sausages production

(a) Raw jackfruit, (b) Peeled, cleaned jackfruit pieces, (c) Tomato paste, (d) Minced pre boiled jackfruit, (e) Jack fruit sausages before freezing, (f) Fried Jack fruit sausage

3 CALCULATIONS

3.1 Cooking yield

The cooking yield of developed non-meat jack fruit sausage was calculated using the method described by Oliveira et al. [24].

Cooking yield (%) = [(final weight of sample) / (initial weight of sample)] * 100 (1)

3.2 Cooking loss

Cooking loss of the product was calculated by using the following Equation 2 [25];

Cooking loss (%) = [(weight before cooking – weight after cooking) / (weight before cooking)] * 100 (2)

3.3 Thermal shortening

An original length reduction during the cooking process is indicated by thermal shortening, given by Equation 3.

Thermal shortening = [(length before cooking – length after cooking) / (length before cooking)] * 100 (3)

4 RESULT AND DISCUSSION

4.1 function of ingredients

- Jackfruit

The primary ingredient of produced vegan sausage is baby jackfruit, and as a percentage, it is 57%. As the main ingredient, it contributes to the structural development of the final product. Also, incorporate unique texture, flavor, and color to the final product.

- Emulsion

Water and oil are immiscible fluids in nature itself, and both are used for preparing sausage. The emulsion is used to mix the immiscible parts. It makes even the distribution of ingredients while holding a more considerable amount of oil gives a better appearance and great taste. The prominent role is to disperse oil in water and prevent oil droplets from coming together and enhancing the intimate holding of oil, fat, and water as a mixture. Vegetable fat, ice, non-fat milk powder, and fat content in jackfruit maintain the emulsion stable. Those are protein sources, and protein is needed to mix substances such as fat and water.

- Salt and sugar

The salt plays the role of enhancing the flavor, water absorption, protein solubilizing, and sausage preservation. In protein solubilizing, protein solubilizes in salt and water creates a liquid that coats each fat particle with a thin layer of soluble protein. The coated fat particles combine with water and oil result in creating an emulsion. Also, sugar enhances the brownish color through caramelization during the cooking operation.

- Tomato paste

Give cured color for the prepared sausage. Also, add flavor, texture, and act as a preservative agent due to antioxidant properties.

- Composite flour mixture

Composite flour mixture enhances the bulkiness and carbohydrate of the mixture while Bread crumbs and rice flour as binders and fillers. Moreover, Maize powder and soy isolates are some starch and protein sources. The overall flour mixture acts as a binder and filler and also contributes to enhancing the taste, texture, stability, water holding capacity, and slicing ability of prepared sausage.

- Spices mixture

Spices mixture consists of garlic, black pepper, and chili powder. Garlic consists of antimicrobial, antioxidant, antiviral, antifungal properties. Peperine is the primary compound of pepper, and it is rich in antioxidant properties, which can prevent oxidation. Chili powder enhances the red color and taste in sausage. As a mixture, this contributes to enhancing the final product's taste, aroma, and shelf life.

4.2 Principals of preservation

1. Boiling

The raw jackfruit was subjected to boiling around 1000C. When the increasing temperature of the medium, the chemical bonds in the enzyme structure began to breakdown result in the loss of the three-dimensional structure of the enzyme; because of that, the enzyme has no longer the ability to fit with target substrate molecules and destroy the function of an enzyme. Jackfruit also contains some enzymes such as Polyphenol oxidase etc. It destroys when subjecting to the heat treatment. It facilitates higher shelf life for the product and stable sensory characteristics for the product. Also, the high temperature reduces the microbial load due to the lethal effect on microorganisms that increased the product's shelf life.

2. Freezing

Freezing of prepared sausage also contributes to preserving the product through enzyme and microbial inactivation. At significantly lower temperatures reduce the molecule movement leads to the reduction of enzyme-substrate collisions and facilitates decreasing enzyme activity. Moreover, at lower temperatures in freezing, a molecular motion decreased drastically as solid formation and formed as crystalline formations. Within these solid crystals, molecules have no more freedom to move than the same molecule in a liquid arrangement. As a result that enzyme-substrate collisions are infrequent result in the inactivation of enzyme activity. Also, during the freezing technique, the water in food is separated from other ingredients. It gets frozen lead to reduce the moisture content of the sausage increase the shelf life of the food due to a lack of water activity for microbial and enzyme activation. Lower temperatures slow down the rate of the different chemical reactions. As water converts to ice, other solutes may accumulate in remain water increase the solute concentration denatures the bimolecular. Ice can also rupture the cell membrane of microorganisms. During freezing, the temperature is led beyond the danger zone. It inhibits the microorganism increase the product's shelf life even though its effect on microorganisms and enzymes freezing does not affect the sensory quality and nutritional quality unfavorably.

3. Addition of spices mixture

Due to antimicrobial properties spices contribute to preserving the sausage by reducing the food bone pathogenic and spoilage microorganisms.

4. Method of packaging

The packaging method is one of the critical aspects of preserve any food for a more extended period. The produced sausage was vacuum packaged. Because in vacuum packages, no more oxygen is available; approximately availability of oxygen is lesser than 1%. In that condition, it creates an unfavorable environment for many microorganisms. Due to lack of oxygen, microorganisms cannot their metabolism, including reproduction, respiration, etc. result in longer shelf life for the product.

4.3 Importance of ingredients

Baby jack fruit

Jackfruit contains higher vitamin and mineral content than an apple, avocado and rich in antioxidants, including vitamin E, vitamin A, vitamin C, β-carotene, and selenium α-lipoic acid glutathione, which are effective in Myocardial infarction, coronary heart disease, hypertension, lung and prostate cancers. Also, contain carotenoids such as all-trans-β, α, lutein, neoxanthin, 9-cis-violaxanthin carotene effectively in cancers, inflammation, cardiovascular disease, cataract, age-related macular degeneration. Lignans, isoflavones, saponins, and niacin-like phytonutrients in jack fruit are effective in cancers, hypertensive, ulcer, aging, nerve function, and asthma, while phenolic compounds effective in chronic diseases and hyperglycemia. Jackfruit contains antiviral properties from jackfruit lectin to help to prevent HIV-1. Also, it is contributing to improving oral health and digestion.

Tomato

In tomato, lycopene is the main carotenoid, which acts as a vital antioxidant that helps fight against cancerous cell formation. Lycopene is a powerful antioxidant that contributes to flush out the free radicals in the human body, and it is not a naturally producing element in the human body. In addition to lycopene, tomato contain three other major carotenoid as alpha- and beta-carotene; lutein has both individual and synergy benefits to the human body, including reducing prostate cancer risk. Calcium, vitamin K, which contain in tomato, leads to strengthening and repairing bones and bone tissues. At the same time, chromium, like valuable minerals, works effectively on diabetics by keeping better blood sugar levels. Vitamin B and potassium in tomatoes reduce cholesterol levels, and blood pressure results in better heart health. Also, the risk of kidney stones is reduced by tomatoes.

Spices mixture

Garlic helps to increase cardiovascular wellness. It also inhibits LDL oxidation, lowers blood pressure, prevents blood clots, decreases homocysteine, and improves microcirculation, vital in diabetes. Garlic consists of anti-cancerous activities and boosts metabolism and the immune system. Garlic helps to aid poor digestion. The main compound of pepper, piperine, is considered an antioxidant that reduces the risk of chronic illnesses such as atherosclerosis, cardiovascular disease, and neurological conditions. Also, black pepper stimulates hydrochloric acid in the stomach to facilitate better digestion and nutrient absorption. Black pepper helps build a robust immune system that can fight diseases and boost the human body's white blood cells. Chili contains vitamin C, vitamin B6, vitamin K, vitamin A, copper, potassium, etc. It also contains capsanthin, violaxanthin, sinapic acid, and ferulic acid, which act as antioxidants.

4.4 Mechanism behind the properties of ingredients

1. Antioxidant property

As a result of the natural by-products of chemical processes such as metabolism, free radicals are formed. Namely, when a cell uses oxygen as a source to generate energy, free radicals are generated as a consequence of ATP (adenosine triphosphate) result in highly reactive oxygen molecules that contain higher energy levels make unstable oxygen molecules. Once that oxygen molecules become reactive, they take the ability to damage tissues and cell inside the body may result in various diseases including cancer, atherosclerosis, Alzheimer's

disease, Parkinson's disease, etc. free radicals always trying to become stable by gaining missing electron from any other molecule facilitate damage of the cell. Antioxidants do the role of donating that missing electron to the free radical and make it again into stable form result in the prevention of cell and tissue damages.

2. Antimicrobial property

Antimicrobial property kills bacteria through different methods, and the process depends on the type of bacteria. Most compounds kill bacteria immediately by causing the bacterial cell to explode. Also, sometimes the deterioration of the cytoplasmic membrane and subsequent leakage of compounds inside the cytoplasm leads to cell death. The cooking yield of prepared sausage is 88.44% reflect that sausage contains 88.44% usable product after cooking operation. Calculation of cooking loss is essential for the cost calculation of the final product. The cooking loss means a degree of shrinkage of sausage during cooking. The cooking loss of prepared jack fruit sausage is 4.33%. When compared with the commercially available meat sausage, cooking loss of prepared jackfruit sausage is significantly less. Cooking loss is lesser in jackfruit sausage means that shrinkage of jackfruit sausage during cooking operation is less. Thermal shortening of jack fruit sausage is 9.97% while commercially available is around 10%.

After freezing, jackfruit sausage was pale yellow with dull brown. But commercially available sausage contains reddish-brown color due to the addition of sodium nitrate. Sodium nitrate gives unique red meaty color to the meat sausage. But for preparing jack fruit based vegan sausage, utilize tomato paste instead of sodium nitrate like curing salts. As an alternative source for nitrate, tomato paste was used. When producing meat-based sausages, usually use curing salts such as nitrates and nitrites to preserve, the addition of flavor, texture, meat red color, and curing purposes. Also, it has some antimicrobial and antioxidative activity in processed meat products. Even though it benefits meat products exceed of standard amount, it will lead to carcinogenic effects on the human body. The reaction that happens inside of the product is as follows (Fig. 4).

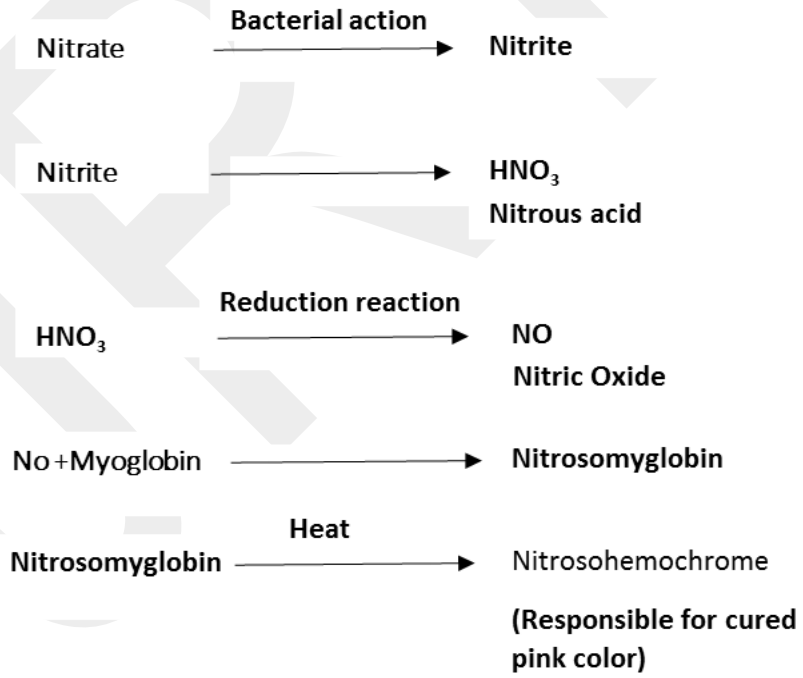

Fig. 4. List of reactions happen inside the product

If any residual nitrate is remained in meat after happening this series of reactions, it will act as a nitro sating agent in nitrosamine formation. Namely, meat is a rich source of protein hence amines. This residual nitrite can react with amine and form carcinogenic compounds. Because of that harmful effect, tomato is used as an alternative source for making sausages.

Obtaining a unique brownish red meat color is one of the main targets of nitrate use, like curing salts in the production of meat sausages. The jack fruit sausage produced by replacing the nitrate with tomato paste is pale yellow/brown. The main reason for that is the lycopene content of the tomatoes. For making this jackfruit, sausage-tomato paste, which contains 56.82 µg/g, was used. If it is used, tomatoes that have higher lycopene content, more red-colored sausage may obtain. Lycopene is the predominant pigment out of other carotenoid pigments responsible for the red color of tomatoes. Usually, in Sri Lankan tomatoes, lycopene content is lower compared to other countries.

5 CONCLUSION

As a meat replacer, jackfruit has a considerable possibility to produce non-meat sausage with enhanced nutritional value. Production of vegan sausage as a value-added product of jackfruit maintains availability throughout the year. Although there are a few drawbacks in characteristic color formation, lycopene pigment in tomato can impart natural color to the non-meat sausage. All added ingredients enhance the quality and preservation action of the final product. Cooking yield and cooking loss of vegan jackfruit sausage is 88.44% and 4.33%, respectively. The product can be preserved for four months without artificial preservatives. Formulating sausage utilizing jackfruit together with the mixture of spices, maintain the powerful immune-boosting ability.

REFERENCES

[1] R. Liyanage, I. Wickremasinghe, Development of Non-Meat Vegetable Based Sausage Using Locally Available Raw Materials, SLASS,2015.

[2] A.H. Varnam and J. P. Sutherland, Meat and Meat Products: Technology, Chemistry and Microbiology, Springer US, First Edition,429,1995.

[3] F.B.Hu, M.J. Stampfer, J.E. Manson, A. Ascherio, G.A. Colditz, F.E. Speizer, Dietary Saturated Fats And Their Food Sources In Relation To The Risk Of Coronary Heart Diseases In Women. American Journal Of Clinical Nutrition, Vol.70,1001-1008,1999.

[4] J.S. Zheng, X. Hu, J. Zhao, J. Yang and D. Li, Intake of fish and marine n-3 polyunsaturated fatty acid and risk of breast cancer: a meta-analysis of data from 21 independent prospective cohort studies. British medical journal, Vol.346, 2013.

[5] M. Govari, A. Pexara, Nitrates and nitrites in meat products, Journal of the Hellenic veterinary medical society, Vol 66,127-140,2015.

[6] E.De Mey, K. De Klerck, H. De Maere, L. Dewulf, G. Derdelinckx, M. C. Peteres, I. Fraeye, Y. V. Hyden and H. Paelinck, The occurrences of N-nitrosamine, residual nitrite and biogenic amines in commercial dry fermented sausages and evaluation of their occasional relation, Journal of meat science, Vol 96(2),821-828,2014.

[7] M. Eichholzer and F. Gutzwiller, Dietary nitrates, nitrites, and N-nitroso compounds and cancer risk: A review of the epidemiological evidence, Nutrition Reviews, Vol. 56,95-105,1998.

[8] K.O. Honikel, The use and control of nitrate and nitrite for the processing of meat products, Journal of Meat Science, Vol 78,68-76,2008.

[9] A. Pietrobelli, S. Rugolotto, P. de Cristofaro and M. Malavolti, “Pediatric Obesity: Looking into Treatment”,Nutrients,Vol.1,197-209,2009.

[10] M.Y.M. Sim, M.N. Ahmed, Z.A. Aziz, C.P. Ju, and C.C. Cheen, “Classification of Artocarpus heterophyllus L.(jack fruit) Maturity Using Disposable Screen –Printed Strips base on Chemomeuic analysis, "Asian Conference on sensors,135-142,2003.

[11] S. Singh, S. Krisnamurthi and S. Katayal, Fruit culture in India, ICAR,1963.

[12] T. K. Bose, “Jackfruit”,in fruits of india:Tropical and subtropical,488-498,1985.

[13] O. Prakash, R. Kumara, A. Mishra and R. Gupta, “Artocarpus heterophyllus (Jack fruit):An Overview”, Pharmacognosy Reviews,Vol.3,353-358,2009.

[14] O.A. Alagiapillai, P.S. Kuttalam, V. Subramanian and M. Jayasekhar, "PPI-1 jack: A new high yielding, regular bearing jack variety for Tamil Nadu", Madras agriculture journal, Vol.83,310-312,1996.
[15] B.M.C. Reddy,P. Patil, S. Shasikumar and L.R. Govindaraju, Studies on physic-chemical characteristics of jackfruit clones of south Karnataka", Journal of agricultural science, Vol. 17,279-282,2004.
[16] M.R. Sharma, "Morphological and anatomical investigations on Artocarpus forest, the fruit", Proceedings of the Indian Academy of Sciences, Vol.60,380-393,1964.
[17] S. Mitra and D. Mani, "Conservation and utilization of genetic resources in jack fruit(Artocarpus heterophyllus lamk.)-a potential underutilized fruit", Acta Horticulturae,Vol.523,229-232,2000.
[18] Y. Selvaraj and D.K. Pal, "Biochemical changes during ripening of jackfruit (Artocarpus heteropyllus Lam", *Journal of food science and technology*, Vol.26,304-307,1989.
[19] H.P.M. Gunasena,K.P. Ariyadasa,A. Wickramasinghe and S.B. Rajakaruna,Manual of Jack cultivation in Sri Lanka, *Forest information, Department of forest publication*,1996.
[20] A.K. Azad, Genetic Diversity of Jackfruit in Bangaladesh and development of propagation methods, Thesis for an award of doctor philosophy at the University of Southampton, UK,2000.
[21] W.R. Arkroyd, C. Gopalan, and S.C. Balasubramaniyam, "The nutritive value of Indian food and the planning of satisfaction diet", *Indian council of medical research*,1996.
[22] N.R. Chrips,R.G.S. Balasingh and C. Kingston, "Nutrient Constituents Of Neglected Varieties Of *Artocarpus Heterophyllus* Lam From Kanyakumari District, South India", *Journal of basic and applied biology*, Vol 2,36-37,2008.
[23] S.L. Jagadeesh, B.S. Reddy, L.N. Hegde, G.S.K. Swamy and G.S.V. Raghavan, "Value Addition in Jack Fruit(Artocarpus heterophyllus lam)".in proceedings of the ASABE meeting presentation,2006.
[24] J.M.G. Oliveira, R.L. Salgado, B.R.C. Lima, C.A. C. Junior, Washed cashew apple fiber (Anacardium occidentale L.) as a fat replacer in chicken patties. Vol .71, 268-273, 2016.
[25] J. Pereira, Guang-hong Zhou, wan-gang Zhang, Effects of rice flour on emulsion stability, organoleptic characteristics & thermal rheology of emulsified sausage. Journal of Food and Nutrition Research, Vol .4, 216-222,2016.,