

PROTEIN ISOLATE USED TO FORM MEAT ALTERNATIVE

Dr. S.REGINOLD JEBITTA, P.R.DURGA DEVI, L.DEVA DHARSHINI,

T.NAGA SAI HARIKA, K.VIGNESH

Department of food technology

Kalasalingam Academy of Research and Education

durgadevi151099@gmail.com

ABSTRACT: The aim of the paper was to develop a new product from legume protein isolates. The legume protein isolates contains approximately 80-90% protein content so it can be a best meat replacer as the meat alternatives in the form of sausage (plant-based) along with starch based edible film with co-biopolymers and plasticizing agent like Sorbitol and glycerol. Several trial was done to optimize the final product and various analysis were taken to check the quality of sausage was physical analysis like (moisture content test, PH test and crude fiber test), Structural analysis like Texture profile analyzer (TPA), visual image microscope and sensory analysis for consumer acceptance.

KEY WORDS— co-biopolymers, plasticizing agent, Texture profile analyzer, visual image microscope

I. INTRODUCTION

Generally, Meat and meat by merchandise were thought of joined of the usually consumed food things in family's food basket. Many medical specialty studies are rumored that consumption of processed meat is related to many health problems like upset, diabetes, Cholesterol and numerous styles of cancers (Arihara 2006; Schulze et al. 2003). The existence of high amounts of saturated fatty acids (40–50%) particularly, Myristic and Palmitic acids and their prejudicial effects, oxidization of unsaturated fatty acids (PUFAs) throughout preparation in addition as exploitation artificial preservatives are documented because the major reasons for these adverse health effects (Schulze et al. 2003; Food and Agriculture Organization 1992). Thus

Majzoobi et al. (2017) developed the meat-free sausages exploitation unsmooth soy supermolecule, corn starch, edible fat, salt and soy supermolecule isolate. They rumored substantial quality loss like poor sliceability, high preparation loss and low water-holding capability once the meat was replaced by various ingredients. However, some parameters like change of state loss, water-holding capability and general satisfactoriness may be improved exploitation konjac mannan and K-carrageenan. To our

far, various tries are created so as to optimize the composition of meat merchandise and scale back the health problems exploitation plant extracts, fat and chemical group replacer (Arihara 2006; Yıldız-Turp and Serdaroglu 2008; Choi et al. 2009; Kurcubic et al. 2014; Amani et al. 2017). Hence, it are often inferred that the meat should be fully substituted by plant based ingredients in such the way that the resultant mimic product possesses the best similarity to the initial product with relation to textural and sensory satisfactoriness and with adequate organic process worth. However, holding the sensory and textural quality square measure accounted as huge challenges in production of meat analog merchandise. The feel and style (in explicit the appetisingness and tenderness) of meat square measure extremely valued by customers, whereas the non-meat ingredients might not possess an equivalent sensory attributes (Elzerman et al. 2011). to beat the mentioned issue, numerous plant proteins like legume, cereal, seed and soy proteins cluster (textured, flour, concentrate and isolate) are often used (Malav et al. 2015). These ingredients have applicable practical properties (e.g., emulsification, water and oil absorption capacity) in addition as high organic process values (e.g., well-balanced amino acids, essential fatty acids, vitamins and simple digestibility), that build them as distinctive meat substitutes (Malav et al. 2015; Asgar et al. 2010). To date, restricted analysis works are disbursed to develop meat analog-based merchandise.

information, few studies are rumored on formulation of meat analog merchandise, and there's lack of data during this regard, whereas it's a superb scope for food processors in addition as health-conscious customers. This study is an element of an attempt to introduce a brand new approach for producing meat-free sausages. Therefore, the particular aims of the current study were (1) to develop and optimized formulations of meatless sausages exploitation plant source;



(2) to Analyze the physical, organic process and sensory characteristics.

Generally the soy protein isolates and pea protein isolates (approx 85% of protein) were prepared in the method of Acid/alkaline extraction or Iso-electric precipitation used in the centrifuge equipment @8000rpm, because it has less amount of Antinutrient content. It was purchased in the Amazon, India. Canola oil was used in tenderness and juiciness of the product. It was purchased from local retailer, Krishnankoil, India. Carboxymethylcellulose (CMC) from Amazon, India. it was in powder form and also used as a binding agent. Salt, potato starch, baking soda and seasoning blending (spices) from local retailer in Krishnankoil, India. Tapioca starch, carragennan, it was used as film forming agent in casing material were purchased from Amazon, India. Sorbitol and glycerol, used as plasticizing agent were purchased from Madurai, India. These are the ingredients we were used to formulate the meat alternatives and starch based edible film formation, respectively.

III. PREPARATION OF SAUSAGE

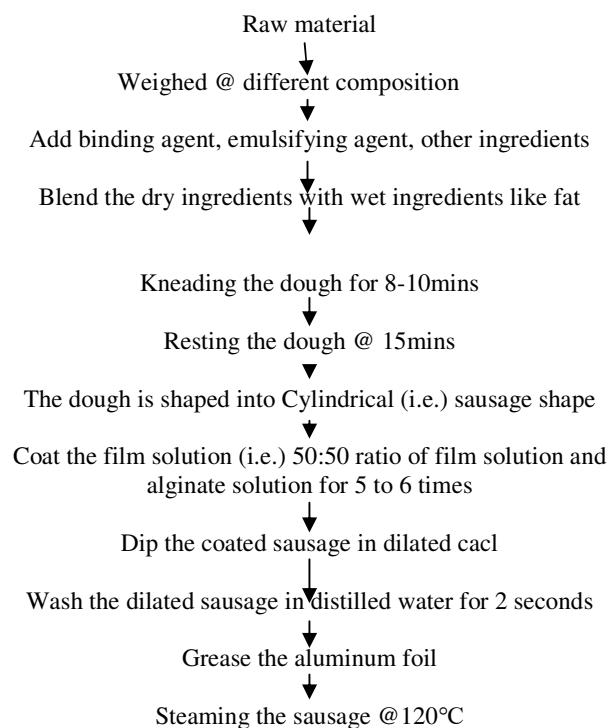
Four sausage (meat alternative) formulations were prepared and these are the three independent variables of each formulation were prepared on different days. (In table 1) S1, S2 and P1, P2 represent the sausage containing Soy protein isolates and other ingredients containing binding agent, emulsifying agent and flavoring agent respectively. The sausage (meat alternative) was processed as follows. First, our major ingredients like soy protein isolates and binding agents were weighed at different compositions. Next, Blending the dry ingredients with wet ingredients like fat (canola oil), Spices, salt and sugar. Now the dough was kneaded for 8-10 minutes and resting these dough was 15 minutes and then our dough were shaped into cylindrical (i.e.) sausage shape. Then we were coated 50% film solution + 50% alginate solution (i.e.) 50:50 ratio and spray the calcium chloride solution on the sausage and wash the sausage on the distilled for 2 seconds. and finally we were grease the aluminum foil and wrapped the sausage (meat alternative) at room temperature. Finally we were steamed the sausage @ 120°C for 25 minutes.

II. MATERIALS AND METHODOLOGY



FIG: 1. Meat texture using soy protein isolates and other ingredients.

IV. FLOW CHART OF MEAT ALTERNATIVES (SAUSAGES)



V. PREPARATION OF STARCH BASED EDIBLE FILM

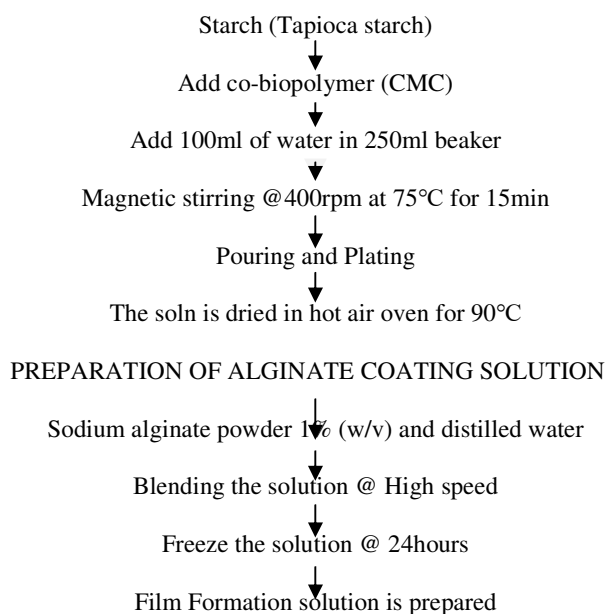
To make the starch based edible film these are ingredients are Tapioca starch, carragennan, sorbitol, glycerol and water. Next we were weighed the ingredients at different ratio and Mixing the all ingredients in a beaker with the help of Magnetic stirrer at 400 rpm for 15 minutes



S.L.N	INGREDIENTS	S1 (SOY)	S2 (SOY)	P1 (PEA)	P2 (PEA)
1	Protein isolates	5g	2.5g	3g	4.5g
2	Carragennan	4g	2g	2g	3g
3	Baking soda	2g	1g	1g	1.5g
4	Potato starch	2.5g	1.5g	1.5g	2.5g
5	Seitan	5g	1.5g	2g	2.5g
6	Maida	5g	1.5g	2g	2.5g
7	Chunks	6g	2g	2.5g	3g

@90°C in a hot water bath. Finally we were poured the solution in Petridis with 1cm thickness and we were dried the solution in hot air oven @60°C for 8 hours. Next, we were prepared the alginate solution, First the alginate powder were taken at 1%W/V and distilled water (30ml) at high speed then we were blended the solution at high speed. Finally we were stored the solution at 4°C For 24hours.

VI. FLOW CHAT OF STARCH BASED EDIBLE FILM



S1 – soy protein isolates , P1 – pea protein isolates
 Table 1 Formulation sausage with different
 Composition of sausages

VII. PHYSICAL ANALYSIS

The proximate composition of the Meat alternative (sausages) was determined per the Association of Official Analytical Chemists (AOAC, 2005). The moisture, ash, and crude fiber were determined following the AOAC Official Methods 920.151, 940.26, and 985.29, respectively.

VII.i. MOISTURE CONTENT

Moisture content is determined based on the loss of weight in the sample during heating/ drying. 2 g of sample was taken and the weight of sample before and after drying in hot air oven was weighed.

$$\text{Moisture content} = \frac{W1 - W2}{W2} \times 100$$

VIII. ii. ASH CONTENT

Ash content is determined to analyze the amount and type of minerals in the sample 2 g of sample was weighed in a crucible and kept in the muffle furnace for 4 hrs at 550°C.

$$\text{Ash content} = \frac{W2 - W}{W1 - W} \times 100$$

VII. iii. CRUDE FIBER

Crude Fiber for different sample was determined is to find the amount of fiber present in this sample. 2 g of sample was weighed in a crucible and kept in the muffle furnace for 4 hrs at 550°C.

$$\text{FORMULA} = \frac{(W2 - W1) - (W3 - W1)}{\text{Weight of sample}} \times 100$$

VIII. iv. PH VALUE

PH is used to measure of acidic/basic water. The range of PH is to 0 to 14, with 7 being neutral. pHs of less than 7 indicate acidity, whereas a pH of greater than 7 indicates a base. Take 2g of sample diluted with distilled water kept aside at room temperature. It was determined by digital potentiometer.

VIII COLOR

The color was measured by Hunter lab color-flex using LAB value. The values of L* (luminosity), a* (negative-green; positive-red), and b* (negative-blue; positive-yellow) were measured.

$$E = a^2 + b^2 + L^2 \times 1/2$$

VIII. STRUCTURE ANALYSIS: VISUAL EXAMINATION AND IMAGE RECORDING BY A CAMERA



Samples were compound by hand, peeling on the direction of fiber orientation. The compound samples were examined visually for the degree of fiber formation. Their pictures were additionally taken by a high resolution camera hooked up to a laptop and recorded digitally.

IX. TEXTURE PROFILE ANALYSER:

A two-cycle compression take a look at was performed up to five hundredth strain compression of the first portion height employing a steel probe. A time of 5s was allowed to glide by between the 2 compression cycles. Force–time deformation curves were recorded at a crosshead speed of fifty mm/min. All four samples area unit take away 2cm diameter and 1cm height then we've to weight the every sample. It had been determined by Texture profile instrument. Finally the viscousness, cohesiveness, hardness

S/N O	PARAMET ERS	S1(SO Y)	S2(SO Y)	P1(PE A)	P2(PE A)
1	Moisture content	32%	50%	43%	50%
2	Ash content	18%	13.9%	16.7%	9.5%
3	Crude fiber	19%	21.6%	24.3%	35.5%
4	PH	7.0	6.9	6.6	6.8

were measured.

X. SENSORY ANALYSIS:

Sensory analysis was performed on streamed plant primarily based sausages in keeping with the strategy of Savadkoochi et al. (2014). The sausages were assessed by ten trained panelists, comprising of under-graduate students (4 males, four females; aged between twenty and twenty three years) at KARE (Krishnankoil, India), at temperature. The samples were ready for every critic and were asked for his or her opinions on color and look, odor, taste, texture and overall satisfactoriness. Scores were assigned employing a 9 purpose hedonistic scale as follows: Like extremely—9; Like terribly much—8; Like moderately—7; Like slightly—6; Neither like nor dislike—5; Dislike slightly—4; Dislike moderately—3; Dislike terribly much—2; Dislike extremely—1

XI. RESULT AND DISCUSSION

XI. i. PROXIMATE ANALYSIS:

The physiochemical analysis of sausage resolves by Association of official analytical chemistry (AOAC) methodology. Moisture content, Ash content, crude fiber were measured by AOAC methodology severally. In table

one shows the wetness content values of A, B, C, D samples was thirty second, 50%, 43%, 50%. The wetness content of the P1 and P2 sample was over S1 and S2. Sample A, B was represents to soy supermolecule isolates and C, D represents the pea supermolecule isolates. For wetness content, we have a tendency to dry our sample at 105°C for hrs. During this paper here it's compared wetness content with sausage and chicken sausage P1, P2, P3, P4 and terminated that P1, P2 sample was having higher wetness content than P3, P4. P1 wetness 84%, and P2 73%

XI. ii. ASH CONTENT

Ash content was refers to the inorganic residues remains organic content. It verifies the mineral content gift within the food. We have a tendency to determined four samples ash content of A, B, C, D. Sample A&B area unit soy supermolecule isolates and Sample C&D area unit pea supermolecule isolates. A,B contains eighteen and thirteen.2% and C,D Contains sixteen.7% and 9.5%.The () paper we have a tendency to referred was mentioned Ash content of sausage and chicken sausage. It ranges from nine.6% to 14.5%.pork sausage has least ash content than chicken sausage. As like our samples P1, P has low ash content S1 and S2 have high ash content worth.

XIiii. CRUDE FIBER

Crude fiber was wont to verify the fiber content within the food samples. Low crude fiber indicates the sustainable grains. High crude fiber indicates the high fiber content in food samples. Legumes area unit high fiber food we have a tendency to take two samples of S1 and S2 of soy supermolecule isolates and P1 and P2 area unit pea supermolecule isolates. A, B samples (S1, S2) contains nineteen, 24.3% C, D sample (P1, P2) Contains twenty one.6%, 35.6%. S2 and P2 sample thought-about as high fiber samples thanks to the composition as we've taken.

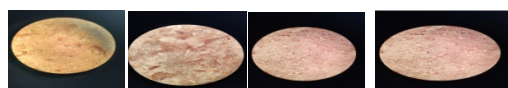
Table 2: The proximate analysis of cooked sample followed AOAC method

XIII. VISUAL IMAGE

Visual Examination was performed on dissected the samples. The corresponding digital camera was shown. The total magnification was 40x-1600x. The light source used is electric light source (LED or HALOGEN).Suitable for research experiments in biology, bacteriology and medicinal chemistry etc. Konus offers a child-friendly digital unit with image magnifications from 100 to 450xs.The Microscopes head changes into web camera and comes complete with



USB cable and computer software allowing picture resolutions up to 640*480 pixels. It provides a high resolution images once only available to researchers and scientists. In this paper all samples were placed on the light microscope and we had adjusted the magnification and then digital camera was connected to the computers. Finally the digital camera was captured by the internal structure of fiber samples with the help of light microscope.



(A) SOY PROTEIN FRIED (B) SOY SAMPLE STREAM (C) PEA SAMPLE FRIED (D) PEA STREAM

XII. SENSORY EVALUATION

FIG 1 shows the sensory evaluation of meat alternative (i.e.) Sausage which is close to meat (i.e.) myofibillar protein. The results of sensory assessment is there was no significant change ($p>0.05$) observed in the scores of appearances, color, flavor, taste and texture between sample A and sample B. However, the Sample A (i.e.) Soy protein stream showed the significant differences in terms of overall appearance, color, texture. The sample B(i.e.) soy protein isolates stream had a better overall appearance and texture as compared to sample C and sample D. No significant differences found in terms of color, taste, flavor amongst all samples.(i.e.) sample B showed the significant change in terms of color as compared to sample A. Porcella et.al(2001); reported that the incorporation of soy protein isolates(SPI) at 7.5 and 10% in raw sausage was detectable by panelists, while at 5% SPI no beany flavor was detected in the form of sausage.

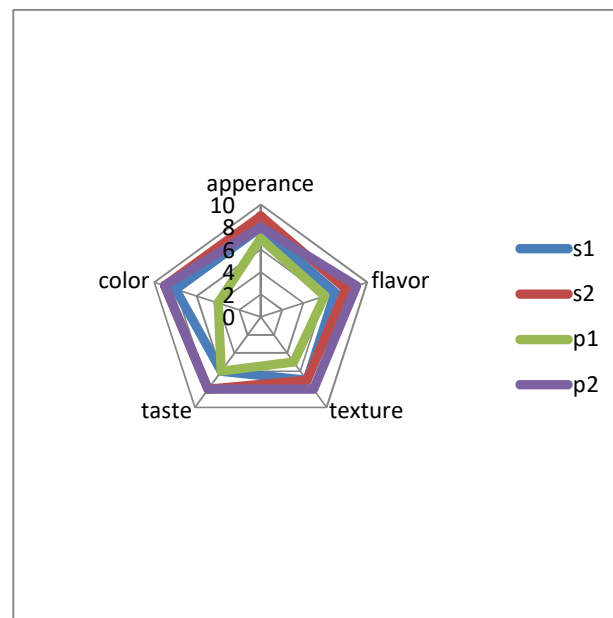


FIG 2: Sensory evaluation of sausage (meat alternative)

XIV. COLOR ANALYSIS

Color is regarded as first qualitative criterion in the meat and other meat by products. It play an important role in consumers and product acceptability (kanmani et.al (2017); According to the results showed that the values of L^* , a^* , b^* did not differ ($p>0.05$) among the sausage. The values of b^* did not differ ($p>0.05$) between sample B and D. But for Sample A and C were significantly ($p<0.05$) higher than the meat indicating a tendency towards Dark brown color. This might be because of pea protein isolates was highly grinded and blended on the canola oil. Consequently the meat alternative did not intensively influences the color, except b^* so which resulted in meat alternative sausage that were move brown.

Table 3: The color analysis of sausage (meat alternative)

PARAMETERS	L^*	A^*	B^*
S1	28	23	11
S2	67	15	35



P1	55	39	63
P2	28	21	38

XV. TEXTURE PROFILE ANALYSER

In meat systems, the structural integrity of the protein matrix and consumer desirability of the products is typically evaluated via TPA (Souissi et al., 2016). The hardness value was found to be significantly lower than the sausage containing meat.(i.e.) sample A .when chewiness the sample A required a greater force to chew than the Non meat(sample B).this is mainly because of myofibillar proteins Feng and xiong(2007) provide strong evidence regarding the interaction between plant protein and myofibillar. The sample B showed the lowest value of gumminess(0.12kgf) and springiness(1.99mm).These parameters are higher in sample A (i.e.) meat sample. The texture analysis showed that the presence of meat in samples associated with increase the value of stiffness so this value was not significant of stiffness sample A($p>0.05$).so overall, it can interpreted that the meat alternative (sausage) may result in a remarkable of decreases in the chewiness and consistency of final cooked sausage samples.

Table 4: Texture profile analyzer value of meat alternative (Sausage)

PARAMETERS	LOAD	PEAK LOAD
S1	5.985 kgf	9.667 kgf
S2	2.298 kgf	9.768 kgf
P1	0.194 kgf	1.213 kgf
P2	0.244 kgf	9.729 kgf

XVI. CONCLUSION

This study was dispensed to research the quality of plant proteins to substitute totally chicken meat in sausage.The results incontestable that the mixed plant ingredients Significantly SPI and super molecule improved the emulsion stability and reduced the shrinkage change of state and preparation loss. However, the non-meat proteins within the emulsion system resulted in a very poor folding/elasticity and gel quality in line with the results of sensory analysis, the quality of the meat-free sausages was a bit like the full-

meat sausage. Finally, the findings of this study advocate that the replacement of one hundred pc of plant proteins is gift in sausage formulation was promising. All the same any works unit required to enhance the gel-forming characteristics, that's that the foremost obstacle in manufacturing meat-free sausage.

XVII. REFERENCE

- 1) Peter H.G. and Carroll P. V. (2003) Legumes: Importance and Constraints to greater use.Plant Physiology.
- 2) Allen O. N. and Allen E. K. (1981) In leguminosae. A source book of characteristics, uses and Nodulation.
- 3) Duranti M. (2006) Grain legumes proteins and nutraceutical properties.Fitoterapia
- 4) Pitchford P. (1993) Healing with whole foods. 3rd edition, North Atlantic Books, California.
- 5) Khalil, I. A., & Durani, F. R. (1989). Nutritional evaluation of tropical legume and cereal forages grown in Pakistan.
- 6) Tropical Agriculture (Trinidad), 67,. Khalil, I. A., & Durani, F. R. (1990). Haulm and Hull of peas as a protein source in animal feed. Sarhad Journal of Agriculture, 6,. Khalil, I. A., & Manan, F. (1990). Chemistry-one (Bio-analytical chemistry) (2nd ed.). Peshawar: Taj kutab Khana. Khalil, I. A. (1994).
- 7) Nutritional yield and protein quality of lentil (*Lens culinaris* Med.) cultivars. Microbiologie Aliments Nutrition,. NRC (1980).
- 8) Recommended Dietary Allowance (9th ed.). Food and Nutrition Board NRC. Washington, DC, USA: National Academy of Sciences. Raghuvanshi, R. S., Shukla, P., & Sharma, S. (1994).
- 9) Nutritional quality and cooking time tests of lentil. Indian Journal of Pulses Research, Zarkdas, C. G., Yu, Z., Voldeng, H. K., & Minero-Amador, A. (1993). Assessment of the protein quality of new high protein Soybean Cultivar by amino acid analysis. Journal of Agricultural and Food Chemistry.
- 10) M.S.Butt, R.Batool; Nutritional and functional properties of some promising legumes protein isolates. Pakistan Journal of Nutrition, 9(4), 373-379 (2010).
- 11) N.J.Enwere; Foods of plant origin.Afro-Orbis Publishers, Nsukka 124-145 (1998).
- 12) N.J.Enwere, P.O.Ngoddy; Effect of heat treatment on selected functional properties of cowpea flour. Trop.Sci. 26, (1986).
- 13) Canella M, Castriotta G, Bernardi A (1979) Functional and physicochemical properties of succinylated and acetylated sunflower proteins. Lebens Wiss. University u-Technology 12: 95.
- 14) Horax R, Hettiarachchy NS, Chen P, Jalaluddin M (2004) Preparation and characterization of protein isolate from cowpea (*Vigna unguiculata* L. Walp.). J Food Sci 69: fct114-fct118.
- 15) Abbey BW, Ibeh GO (1988) Functional properties of raw and heat processed cowpea (*Vigna unguiculata*, walp) flour. J Food Sci.
- 16) Aluko RE & Yada RY (1993): Relationship of



- hydrophobicity and solubility with some functional properties of cowpea (*Kgna unguiculata*) protein isolate. J. Sci. Food Agric.
- 17) Aluko RE & Yada RY (1995): Structure-function relationships of cowpea (*Kgna unguiculata*) globulin isolate. Influence of pH and NaCl on physicochemical and functional properties. Food Chem.
- 18) Demchenko AP (1986): Ultraviolet Spectroscopy of Proteins. Heidelberg: Springer.
- 19) Gibrat R & Grignon C (1982): Measurement of the quantum yield of 8-anilino-1-naphthalene sulphonate bonds on plant microsomes. Critical application of the method of Weber and Young. Biochim Biophys Acta 691.
- 20) S.S. Jha, D Ohri. [2002] Comparative study of seed protein profiles in genus *Pisum*. Biologia Plantarum.
- 21) Sammour R. H., [1991] Using electrophoresis techniques in varietal identification, biosystematic analysis, phylogenetic relations and genetic resources management. Journal of Islamic Academy of Sciences.
- 22) Arulsekar, S. and D. E. Parfitt, [1986] Isozyme analysis procedures for stone fruits, almond, grape, walnut, pistachio and fig. Hortscience.
- 23) Bradford, M.M. [1976] A rapid and sensitive method for the quantitation of microgram quantities of protein utilizing the principle of protein-dye binding. Ann Biochem.
- 24) Laemmli, U.K. [1970] Cleavage of structure proteins assembly of the head of bacteriophage T4. Nature.
- 25) Sambrook, J., E.F. Fritsch, and T. Maniatis. 1989. Molecular Cloning: A Laboratory Manual, 2nd ed., Cold Spring Harbor Laboratory Press, Plainview, NY.
- 26) Blum, H., Beier, H. and Gross, H.J. [1987] Improved silver staining of plant proteins, RNA and DNA in polyacrylamide gels. Electrophoresis.
- 27) Lecomte N, Zayas J, Kastner C. Soya proteins functional and sensory characteristics improved in comminuted meats. Journal of Food Science. 1993.
- 28) Can Karaca A, Low N, Nickerson M. Emulsifying properties of chickpea, faba bean, lentil and pea proteins produced by isoelectric precipitation and salt extraction. Food Research International. 2011.
- 29) Gupta R, Dhillon S. Characterization of seed storage proteins of Lentil (*Lens culinaris* M.). Annals of Biology. 1993.
- 30) Saharan K, Khetarpaul N. Protein quality traits of vegetable and field peas: Varietal differences. Plant Foods for Human Nutrition. 1994.
- 31) Osborne TB. The vegetable proteins. Monographs in Biochemistry. London: Longmans, Green and Co.; 1924.
- 32) Oomah BD, Patras A, Rawson A, Singh N, Compos-Vega R. Chemistry of Pulses. In: Tiwari BK, Gowen A, McKenna B, editors. Pulse Foods Processing: Quality & Nutritional Applications.
- 33) Hu HY, Pereira J, Xing LJ, Zhou GH and Zhang WG, Thermal gelation and microstructural properties of myofibrillar protein gel with the incorporation of regenerated cellulose. LWT - Food Sci Technology (2017).
- 34) Lin DQ, Zhang LT, Li RJ, Zheng BD, Rea MC and Miao S, Effect of plant protein mixtures on the microstructure and rheological properties of myofibrillar protein gel derived from red sea bream (*Pagrosomus major*). Food Hydrocolloids (2019).
- 35) Pan LH, Feng MQ, Sun J, Chen X and Xu XL, Thermal gelling properties and mechanism of porcine myofibrillar protein containing flaxseed gum at various pH values. CyTA - J Food (2016).
- 36) Zhao YY, Zhou GH and Zhang WG, Effects of regenerated cellulose fiber on the characteristics of myofibrillar protein gels. Carbohydrate Polym 209:276-281.(2019).
- 37) Siddiq M and Uebersax MA. Dry beans and pulses production and consumption—An overview. Dry Beans and Pulses Production, Processing and Nutrition(2012)
- 38) Paglarini CDS, Martini S and Pollonio MAR, Using emulsion gels made with sonicated soy protein isolate dispersions to replace fat in frankfurters. LWT - Food Sci Technology (2019).
- 39) Gao XQ, Zhang WG and Zhou GH, Emulsion stability, thermo-rheology and quality characteristics of ground pork patties prepared with soy protein isolate and carrageenan. J Sci Food Agriculture (2015).
- 40) Lee HC, Jang HS, Kang I and Chin KB, Effect of red bean protein isolate and salt levels on pork myofibrillar protein gels mediated by microbial transglutaminase. LWT - Food Sci Technology (2016).
- 41) Kaur M and Singh N, Studies on functional, thermal and pasting properties of flours from different chickpea (*Cicer arietinum* L.) cultivars. Food Chemistry
- 42) chicken meatballs during frozen storage. J Food Science Technology(2015).
- 43) Verma AK, Banerjee R and Sharma BD, Quality of low fat chicken nuggets: effect of sodium chloride replacement and added chickpea (*Cicer arietinum* L.) hull flour. Asian Australasian J Animal Science (2012).
- 44) Siddhuraju, P., Vijayakumari, K., & Janardhanan, K. (1996). Chemical composition and nutritional evaluation of an underexploited legume, *Acacia nilotica* (L.) Del. Food Chemistry, 57(3), 385–391.
- 45) Association of Official Analytical Chemists (1980). Official Methods of Analysis, 13th edn. Washing, DC.
- 46) Association of Vitamin Chemists (1966). Methods of Vitamin Assay, 3rd edn. InterScience Publishers, New York.
- 47) Dubois, M., Gilles, K. A., Hamilton, J. K., Rebers, P. A. & Smith, F. (1956). Anal Chem.
- 48) Hussain, M. A., Akinyele, I. O. & Omololu, A. (1984). Perceptions of mothers on medical problems associated with cowpea consumption. Pro. And Abstract Worm Cowpea Research Conf. HTA, pp.
- 49) Kitson, R. E. & Mellon, M. G. (1944). Colorimetric determination of phosphorus as molybdivanadophosphoric acid. Ind. and Eng. Chem., 16
- 50) Mehta, V., Jood, S. & Bhat, C. M. (1985). Effect of processing on flatus producing factors in legumes. J. Agric. Food Chem.
- 51) Vose, J. R. (1980). Production and functionality of starches and protein isolates from legume seeds (field peas and horse beans). Cereal Chemistry.