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Research Article

Structural, Rheological and Calorimetric Properties of an Extruded Shrimp Feed using Glandless Cottonseed Meal as a Protein Source

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ABSTRACT

This study aimed to analyze the calorimetric, rheological, and structural characteristics of an extruded shrimp feed mainly made out of glandless cottonseed meal as protein source and nixtamalized maize a binder to understand its behavior under extrusion processing conditions to ensure proper structure and water stability. An optimal extruded shrimp diet was formulated using 44% of glandless cottonseed meal as the main protein source. Extruded shrimp diet showed the following composition: dry matter: 95.1%, total digestible nutrients: 81.0%, crude protein: 50.5%, crude fat: 8.8%, ash: 8.9%, digestible energy: 4.0 Mcal/kg, and metabolizable energy: 3.6 Mcal/kg. The rheological analyzes showed an increase in the viscosity of non-extruded optimal shrimp feed and a decrease in the viscosity of extruded optimal shrimp feed. Scanning electron microscopy showed that starch granules were present in the optimal treatment before and after extrusion processing. Different geometries can be appreciated, but mostly spherical and ellipsoidal starch granules of ~50 µm and flat and round particles that could be proteins from glandless cottonseed meal of ~150 µm of length and ~50 µm of thickness. Confocal microscopy showed the presence of chlorophylls and significant amounts of carotenoids. Fluorescence microscopy showed darker sections of the samples that made most contact with the inner surface of the extruder's barrel due to the Millard reaction and caramelization of carbohydrates. Differential scanning calorimetry suggests that due to an exothermic transition observed, covalent cross-links are formed in these systems, along with a swelling of starch granules and the formation of physical entanglements. The obtained results suggest that using glandless cottonseed meal as the main protein source in extruded shrimp might be a reasonable option to decrease feeding costs while showing an appropriate structure, stability, and high protein content.

Keywords: Cottonseed meal; Extrusion; Shrimp feed; Microstructure

INTRODUCTION

Global production of fish, crustaceans, mollusks, and other aquatic animals continued to grow and reached a record of 178.5 million tonnes in 2018, with an increase of 3.4 percent compared to 2017. Aquaculture production peaked at 82.1 million tonnes in 2018, with a total first sale value estimated at USD 205 billion. In 2018, Penaeus vannamei world aquaculture production was 4,966,241 tons [1]. Shrimp farming is a growing industry whose main setback is the expenses related to feeding purchase or manufacture. Commercial shrimp feeds are usually composed of 30 to 50% of crude protein, mostly of animal protein meal products made out of fish and squid. Fishmeal is arguably the most important ingredient in formulated aqua feed, specifically in shrimp feed, typically above 25% of the composition. It is mainly used due to its high-quality protein, oil content, and highly unsaturated fatty acids. However, its accessibility is limited due to its high cost and limited availability [2]. This restriction leads to exploring cheaper alternatives while fulfilling shrimp nutrient requirements. A feasible option is to use glandless cottonseed meal as a protein source due to its composition: 55.7% available protein (dry matter, DM), 6.11% total ω6 of fatty acids (sample basis). The majority of commercial shrimp feeds are obtained through pelletizing processes [3], although extrusion could be an alternative to manufacture such products. Extrusion

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of proteinaceous plant materials is used to transform them into higher nutritional value and enhanced consumer acceptability. Compared to pelleting, extrusion is characterized by higher levels of moisture, heat, and pressure. Therefore, extruded feeds are more firmly bound due to the almost complete gelatinization of the starch, resulting in higher water stability and nutrient digestibility, thus decreasing sediment disposal of feed particles, leading to an increase of microorganisms' proliferation that might affect water's quality and shrimp health. Glandless cottonseed meal is available at a lower cost than marine animal proteins, but its utilization in shrimp feeds still requires more information available to ensure its feasibility. This research aimed to investigate the calorimetric, rheological, and structural characteristics of an extruded shrimp feed mainly made out of glandless cottonseed meal as protein source and nixtamalized maize a binder to understand its behavior under extrusion processing conditions to ensure proper structure and water stability.

MATERIALS AND METHODS

Formulation of diet

A previously designed optimal shrimp diet [4] was used with the following components: 2% fish oil ([FOC] Grizzly Salmon Oil, Grizzly Pet Products LLC, Woodinville, WA, 98072, USA), 25% wheat starch (LifeSource Foods LLC, Louisville, KY, 40299, USA), 4% dicalcium phosphate (United Pharmacal Co. Inc., St. Joseph, MO 64503, USA), 2% sodium alginate (Modernist Pantry, York, ME, 03909, USA), 2% potassium chloride (Myron L Company, Carlsbad, CA, 92010, USA), 44% glandless cottonseed meal (CSM) (Cotton Inc., Cary, NC, 27513, USA), 1% calcium carbonate (Now Foods, Bloomingdale, IL, 60108, USA), and, 20% isolated soy protein (ADM, Protein Specialties Division, Decatur, IL, 62525, USA).

Extrusion processing

Diets were processed using a Brabender laboratory simple-screw vented extruder model 2523 (Duisburg, Germany), ¾" L/D - 25:1 RATIO (05-11-000) with the following characteristics: three heating zones (90, 100 and 130°C, respectively), screw compression ratio 1:1, screw speed: 180 RPM, and exit die: 3 mm I.D. before extrusion, formulate mixtures were prepared, and moisture content was adjusted at 14%. The desired moisture level was adjusted by spraying distilled water onto the mix of ingredients, which was hand-mixed for 15 min and conditioned for 12 h in closed plastic containers at 4°C. Extruded samples were cooled down at room temperature for 1 h and stored in sealed polyurethane bags at 4°C for further analyses.

Chemical composition

Fat (920.39), moisture (930.15), protein (990.03), and ash (942.05) of the raw materials and optimal extruded diet were determined following AOAC standards [5]. Digestible energy (DE) and metabolizable energy (ME) were obtained according to NRC (2001).

Viscosity profiles

Viscosity evaluation was performed on the optimal diet before and after extrusion, using the technique for mixtures with starch content in a Bohlin Gemini Rototenic 2 Drive rheometer. 2 g samples were sieved with a 60 mesh and adjusted to a weight of 14 g using distilled water. Evaluated samples were kept under constant stirring and were then heated to 45°C for 2 min, increasing temperature until 95°C was reached, with a heating ramp of 5.5°C/min. This temperature was kept constant for 5 min, and it was then cooled to 45°C. At the end of the process, maximum viscosity (V_{max}), minimum viscosity (V_{min}), and final viscosity (V_{fin}) were determined. Based on the obtained data, retrogradation viscosity (V_{retro}) was calculated according to Equation 2 [6]:

$$VRT = V_{fin} - V_{min}$$

Scanning electron microscopy, confocal microscopy, and fluorescence stereomicroscope

Scanning electron microscope (SEM) model S-3400N Type II (Hitachi High-Technologies Corp., Pleasanton, CA.) was used to analyze optimal non-extruded and extruded samples. The samples were placed on aluminum plates (12 mm diameter) previously prepared with carbon double face conductive tape and colloidal silver glue, which were later covered with the ionized gold coating with a Sputter Coater Denton Desk IV. For tridimensional visualization, TCS SP5 II Broadband confocal microscope supported by a sample preparation equipment model CM1850 cryostat microtome and Stadie-Riggs tissue slicer for specialized applications were used. A model M165FC fluorescence stereomicroscope (Leica Microsystems) was used. A Three-centimeter-wide field of view for spatial resolution was used with 0.63X and 2.0 objective lenses, along with a CCD color camera (model DFC 310 FX) operating through Leica Application Suite imaging software.

Differential scanning calorimetry

Differential scanning calorimetry (DSC) of optimal extruded and non-extruded samples was performed. 4 mg were milled and screened through a 40 mesh and placed in 40 µL aluminum capsules, 16 mg of distilled water were added and sealed using a hermetic press. Capsules were heated from 22°C to 105°C. The heating chamber was ventilated with nitrogen at a 20 ml/min flow speed, using a heating ramp of 5°C/min. Enthalpy, initial, maximum, and final temperatures were determined using a Mettler-Toledo e822 calorimeter.

RESULTS AND DISCUSSION

Chemical composition

Optimal shrimp diet showed the following results: dry matter: 95.1%, total digestible nutrients: 81.0%, crude protein: 50.5%, crude fat: 8.8%, ash: 8.9%, digestible energy: 4.0 Mcal/kg, and, metabolizable energy: 3.6 Mcal/kg. Smith et al. reported that the protein requirement for *P. vannamei* shrimp is below 36%, although such requirements vary depending on protein quality and physiological state of crustaceans. Optimal extrudate has 50.5% protein. Regarding carbohydrates, in general, shrimp utilize complex starches better than glucose, allowing a lower optimal protein level. Standard wheat starch is generally the starch source used in shrimp feeds, whit an optimal dietary carbohydrate range of 20 to 40% [3]. The Carbohydrate content of the extruded optimal diet is 31.8%. Energy requirements reported for *P. indicus* are between 3.50 Mcal/kg - 4.00 Mcal/kg and are higher for species like *Penaeus monodon* (2.23 Mcal/kg to 3.71 Mcal/kg). Extruded optimal diet showed a

value of 4.00 Mcal/kg and 3.62 Mcal/kg for digestible energy and metabolizable energy, respectively.

Rheological analyzes

Figure 1 shows the behavior of complex viscosity against the temperature of non-extruded and extruded optimal shrimp feed, respectively. Figure 1 shows an increase in the viscosity of non-extruded optimal shrimp feed, which is common for both starch-rich and protein-rich materials. This may be attributed to network formation of the molecules in protein-rich material by cross-linking and in starch-rich materials by an entanglement of amylose and amylopectin chains [7]. Bartsch reports that the network formation may roughly be described as a first-order reaction. The viscosity increase is approximately proportional to the thus-formed concentration of cross-links or entanglements. Figure 1 shows a decrease in the viscosity of extruded optimal shrimp feed, which is affected by shear rate, temperature profile, cooking process, and activation energy [8], hence, the viscosity of the material may increase or decrease during extrusion. A small decrease in throughput (e.g. by a small increase of die resistance) may change the holdup, augment the residence time, and raise the viscosity. If this viscosity change strongly affects the pressure built up at the die, flow pressure will increase, and the throughput may further decrease, especially in an extruder with a soft material or insufficient grooves. This may lead to instabilities.

On the other hand, if the increase of viscosity affects the backflow most strongly, the process becomes more stable. Most cereal flours are transformed into viscoelastic dough's during extrusion, whose rheological properties depend on the extrusion variables and chemical composition. Starch conversion during extrusion is also affected by other components, such as fats and proteins. Lin et al. reported that fat interfered significantly with starch gelatinization: the degree of starch gelatinization has decreased from 100% for a dry pet food control without fat to 68.4% in the presence of poultry fat and 55% in the presence of beef tallow, at a fat addition of 75 g fat/kg in the extrusion mix, using a SS of 200 rpm. At higher SSs, the decrease in gelatinization was even more pronounced. Probably, due to the lubricating effect of fat during extrusion, which reduces friction and torque, and, consequently, mechanical energy input, also, it lowers melt temperature due to less viscous dissipation; as a result, less starch conversion takes place. According to Lin et al. it reasonable that fat is "coating" the starch granules with a hydrophobic layer, preventing moisture from being absorbed, thus interfering with the gelatinization process. The formation of starch-fat complexes [9] and starch-protein complexes [10,11] may be significant during the extrusion of cereal flours with complex composition. Such complexes affect the rheological properties of mixtures that include cereals and thus their expansion behavior. Experimental data suggest that amylopectin is more prone to macromolecular degradation than amylose, mostly due to its high molecular weight rather than its branched structure [12]. As a result of the applied shear, amylopectin molecules are broken mainly at the α-1:6 bonds [13-15], resulting in a degradation of the products with an average molecular weight in the range of 105 to 107 [9]. Generally, it was observed that less degradation occurs at higher MC (20% to 30%). The obtained results showed a decrease in viscosity after extrusion, which agrees with other, reports [16]. VRT of non-extruded and extruded optimal treatment were 83.89 and -15.24 Pas, respectively.

Scanning Electron Microscopy

Figure 2 shows microscopy analyses performed on non-extruded and extruded optimal treatment to observe structural changes caused by the cooking extrusion process. SEM (Figure 2) showed that starch granules were present in the optimal treatment before and after processing. Figures 2A and 2B show non-extruded optimal treatment. Different geometries can be appreciated, but mostly spherical and ellipsoidal starch granules of ~50 µm and flat and round particles that could be proteins from CSM of ~150 µm of length and ~50 µm of thickness. The geometry and size of starch granules depend on the plants' biological origin [17-19]. These differences are important for functional and physical properties because small-size granules can absorb higher amounts of water and absorb heat more quickly due to a higher surface area [20,21]. Figures 2C and 2D show extruded optimal treatment. Similar geometries can be appreciated in Figures 2A and 2B, although some appear to be fractured. During the extrusion cooking process, protein bodies separate from starch, losing their structure due to heating denaturalization and starch gelatinization, affecting their integrity and structure. The discontinuous structure was found probably because of protein-starch-lipids interactions. A semi-

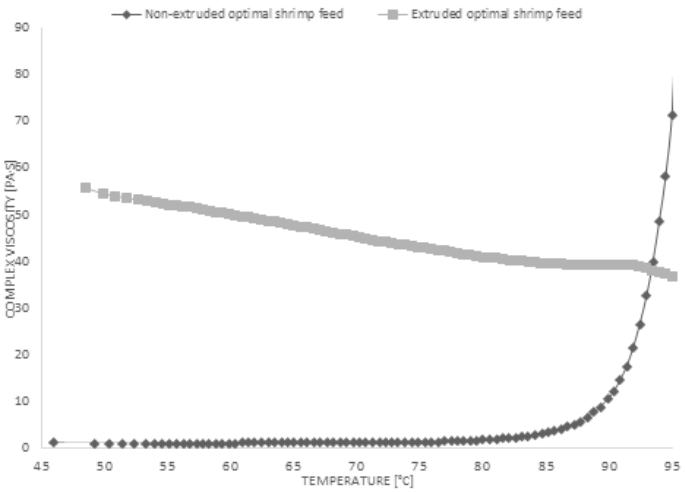

Figure 1: Complex viscosity profile of extruded and non-extruded optimal shrimp feed.

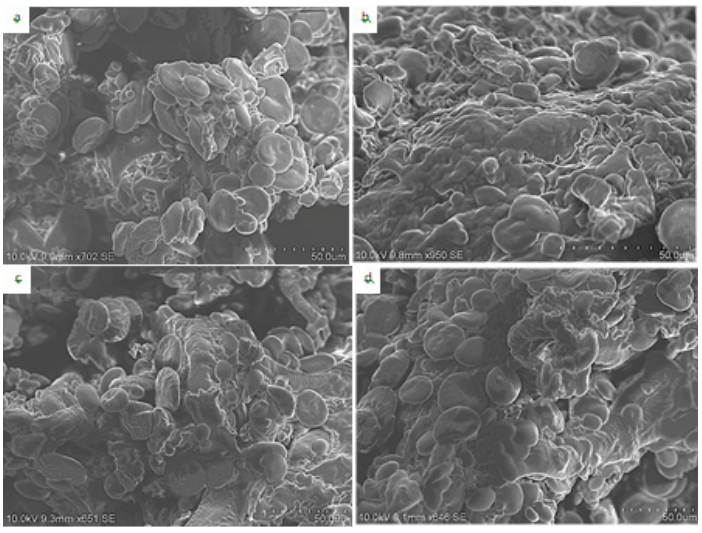

Figure 2: Scanning electron microscopy of optimal treatment: a) Non-extruded b) Non-extruded c) Extruded d) Extruded.

continuous matrix with scattered disruption was spotted in some areas of the samples. Cottonseed meal appears as semispherical bodies embedded in structures containing gelatinized starch and lipids (Figures 2C and 2D).

Confocal Microscopy

Figure 3 shows the optimal treatment images: A) Non-extruded; and, B) Extruded, C) Extruded and D) Extruded. Figures 3B, 3C and 3D showed the presence of chlorophylls, characteristic of chloroplasts. Figure 4B showed reduced levels of chlorophyll and significant amounts of carotenoids. Red spots on Figures 3B-3D corresponds to carotenoid fluorescence emitted from 500 to 600 nm. Chlorophyll fluorescence is shown in green and the carotenoid is shown in red; the overlay of these two components produces an orange overlay. All isolated plastids were spherical; this may be because of stromules [22], microfilaments, and microtubules control plastid morphology [23]. It has been demonstrated that chlorophyll breakdown products do not accumulate in higher plants [24], and they are rapidly metabolized into non-fluorescent compounds [25]. Carotenoid accumulation can be related to a very strong increase in the expression of phytoene synthase and phytoene desaturase genes [26,27].

Astaxanthin is the predominant carotenoid in penaeids [28], accounting for 86-96% of total carotenoids in the exoskeleton of *P. monodon*. Natural carotenoids such as dried *Spirulina* and carotenoid extracted from *Dunaliella* improved coloration in shrimp and are potentially cheaper than synthetic products such as astaxanthin and β-*carotene* [29]. To ensure a good color at harvest, the finisher diet should include 50-100 ppm (mg/kg) of astaxanthin [30].

Fluorescence Microscopy

Figure 4 shows the extruded optimal treatment: A) With no filter; and B) With a violet filter. Figure 4A shows darker sections of the samples that made most contact with the inner surface of the extruder's barrel. These darkening sections occurred due to the Millard reaction and caramelization of carbohydrates, resulting in a higher brown tone than other sections. Figure 4B shows a pale ellipsoidal pattern around the extrudate. This might have occurred due to the characteristic ellipsoidal movement of the sample inside the barrel during processing, resulting in areas with higher starch gelatinization and further developed Millard reaction.

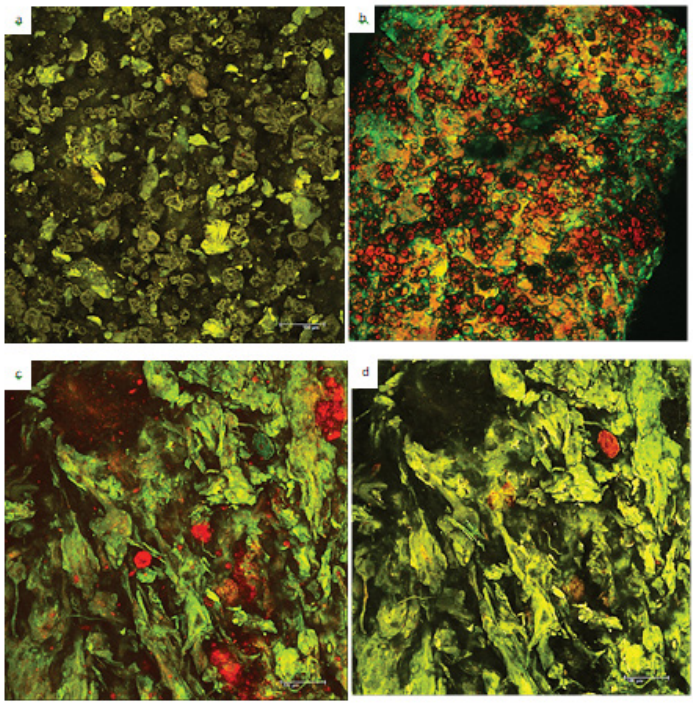

Figure 3: Confocal microscopy of optimal treatment: a) Non-extruded b) Extruded c) Extruded d) Extruded.

Differential Scanning Calorimetry

Figure 5 and Figure 6 show thermograms of non-extruded and extruded optimal treatment, respectively. DSC provides further evidence on the network structure whether the cross-links formed during extrusion cooking are noncovalent or covalent. The mechanism of network formation is a key consideration in developing a dough rheological model regarding the determination of whether or not covalent cross-links are formed during extrusion. Studies have indicated that bonds are noncovalent [31-33], while other reports have reached the opposite conclusion [34,35].

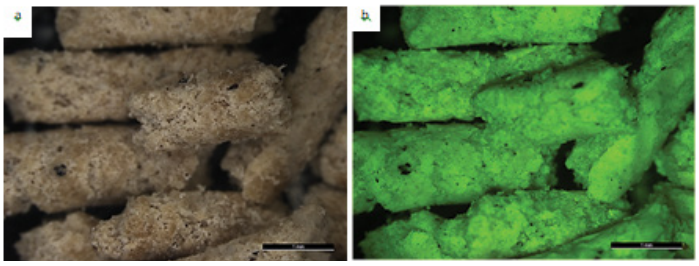

Figure 4: Fluorescence microscopy of extruded optimal treatment: a) No filter; and, b) Violet filter.

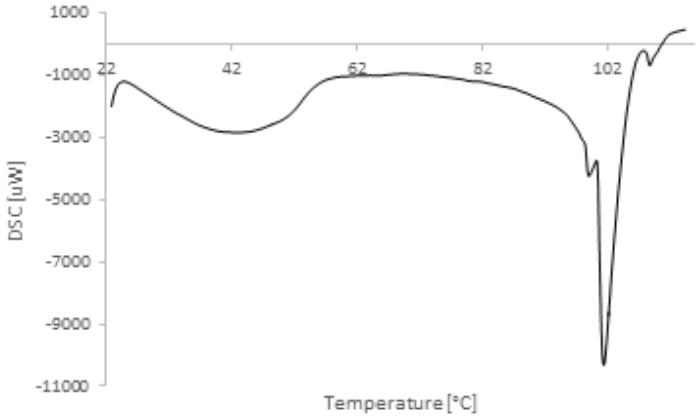

Figure 5: Thermogram of non-extruded optimal treatment.

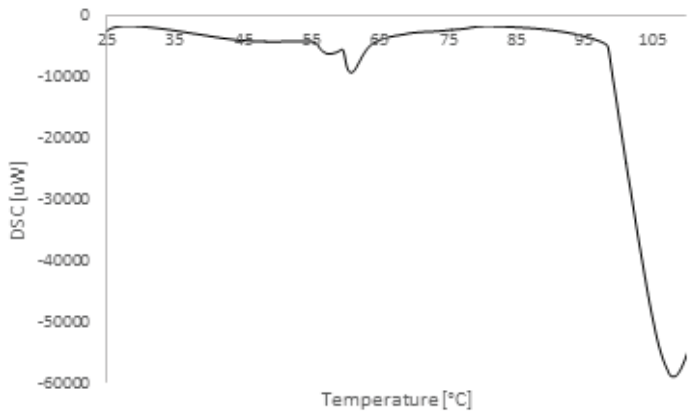

Figure 6: Thermogram of extruded optimal treatment.

Luxenburg et al. observed that covalent cross-links were formed when exothermic transitions were seen. The increase in viscosity seen in Figure 1, along with the results shown in Figure 5 and Figure 6, suggests that due to an exothermic transition observed, covalent cross-links are formed in these systems, along with a swelling of starch granules and the formation of physical entanglements.

CONCLUSION

This research analyzes the extrusion processing potential of glandless cottonseed meal in shrimp feed. The extrusion process increases protein denaturation since heating and shearing in the extruder may increase the bioavailability of nutrients. The obtained results suggest that using glandless cottonseed meal as the main protein source in extruded shrimp might be a reasonable option to decrease feeding costs while showing an appropriate structure and stability as well as high protein content. Further studies are required to analyze the digestibility and acceptability of the shrimp extruded feed.

REFERENCES

1. Rahman MM, Mahejabin A, Al-Mamun M. Rural Development Academy [RDA] 2021; Bogura, Bangladesh.
2. Lim C, Persyn A. Practical feeding—penaeid shrimps. In nutrition and feeding of fish 1989; 205-222. Springer, Boston, MA, USA.
3. Solomon SG, Ataguba GA, Abeje A. Water stability and floatation test of fish pellets using local starch sources and yeast (*Saccahromyces cerevisae*). Int J Latest Trends Agr. Food Sci. 2011; 1:1.
4. Delgado E, Valles-Rosales DJ, Flores NC, Reyes-Jáquez D. Evaluation of fish oil content and cottonseed meal with ultralow gossypol content on the functional properties of an extruded shrimp feed. Aquacult Rep. 2021; 19:100588.
5. Horwitz W. Association of Official Agricultural Chemists, Official methods of analysis. Association of Official Analytical Chemists (AOAC). 1975; 1: 700. Washington D.C., USA.
6. Zeng M, Morris CF, Batey IL, Wrigley CW. Sources of variation for starch gelatinization, pasting, and gelation properties in wheat. Cereal Chem. 1997; 74:63-71.
7. Bartsch J. Extrusion cooking. Herausgegeben Von C. Mercier, P. Linko und JM Harper, 471 Seiten, zahlr. Abb. U. Tab., American Association of Cereal Chemists, Inc., St. Paul/Minnesota, USA, 1989, PREIS: USA.
8. Remsen CH, Clark JP. A viscosity model for cooking dough. J Food Process Eng. 1978; 2:39-64.
9. Van LB. Influence of extrusion processing on in-line rheological behavior, structure, and function of wheat starch. In Dough rheology and baked product texture 1990:421-471. Springer, Boston, MA, USA.
10. Madeka H, Kokini JL. Changes in rheological properties of gliadin as a function of temperature and moisture: development of a state diagram. Water in Foods. 1994: 241-252.
11. Kokini JL, Ho CT, Karwe M. Food extrusion science and technology. Dry Technol. 1993; 11:417-418.
12. Della Valle G, Colonna P, Patria A, Vergnes B. Influence of amylose content on the viscous behavior of low hydrated molten starches. J Rheol. 1996; 40:347-362.
13. Davidson VJ, Paton D, Diosady LL, Larocque G. Degradation of wheat starch in a single screw extruder: Characteristics of extruded starch polymers. J Food Sci. 1984; 49:453-458.
14. Gomez MH, Aguilera JM. Changes in the starch fraction during extrusion-cooking of corn. J Food Sci. 1983; 48:378-381.
15. Gomez MH, Aguilera JM. A physicochemical model for extrusion of corn starch. J Food Sci. 1984; 49:40-3.
16. Mestres C, Colonna P, Buleon A. Gelation and crystallisation of maize starch after pasting, drum-drying or extrusion cooking. J. Cereal Sci. 1988; 7:123-134.
17. Jiménez-Hernández J, Salazar-Montoya JA, Ramos-Ramírez EG. Physical, chemical and microscopic characterization of a new starch from chayote (*Sechium edule*) tuber and its comparison with potato and maize starches. Carbohydr Polym. 2007; 68:679-686.
18. Madsen MH, Christensen DH. Changes in viscosity properties of potato starch during growth. Starke. 1996; 48:245-249.
19. Svegmark K, Kidman S, Hermansson AM. Molecular structures obtained from mixed amylose and potato starch dispersions and their rheological behaviour. Carbohydr Polym. 1993; 22:19-29.
20. Hernánde SL, Feria SJ, Montealvo MG, López PO, Pérez BLA. Isolation and partial characterization of Okenia (*Okenia hypogaea*) starch. Starke. 2002; 54(5):193-197.
21. Paredes-López O, Schevenin ML, Hernández-López D, Cárabez-Trejo A. Amaranth starch-isolation and partial characterization. Starke. 1989; 41:205-207.
22. Waters MT, Fray RG, Pyke KA. Stromule formation is dependent upon plastid size, plastid differentiation status and the density of plastids within the cell. Plant J. 2004; 39:655-667.
23. Kwok EY, Hanson MR. Microfilaments and microtubules control the morphology and movement of non-green plastids and stromules in *Nicotiana tabacum*. Plant J. 2003; 35:16-26.
24. Matile P, Hörtensteiner S, Thomas H. Chlorophyll degradation. Annu Rev Plant Biol. 1999; 50: 67-95.
25. Oberhuber M, Berghold J, Breuker K, Hörtensteiner S, Kraütler B. Breakdown of chlorophyll: A non-enzymatic reaction accounts for the formation of the colorless "nonfluorescent" chlorophyll catabolites. Proceedings of the National Academy of Sciences. 2003; 100:6910-6915.
26. Giuliano G, Bartley GE, Scolnik PA. Regulation of carotenoid biosynthesis during tomato development. Plant J. 1993; 5:379-387.
27. Ronen G, Cohen M, Zamir D, Hirschberg J. Regulation of carotenoid biosynthesis during tomato fruit development: expression of the gene for lycopene epsilon-cyclase is down-regulated during ripening and is elevated in the mutant Delta. Plant J. 1999; 17:341-351.
28. Okada S, Nur-E-Borhan SA, Yamaguchi K. Carotenoid composition in the exoskeleton of commercial black tiger prawns. Fish Sci. 1994; 60(2):213-215.
29. Supamattaya K, Kiriratnikom S, Rungsombat P, Boonyaratpalin M, Kliangpradit A. Effects of carotenoid sources on growth performance, blood parameters, disease resistance and stress tolerance in black tiger shrimp (Penaeus monodon Fabricius). Warasan Songkhla Nakharin (Sakha Witthayasat lae Technology). 2005.
30. Putra D, Mariana, Abidin M Sanani. Effect of Various Feeding of Live Feeds on the Growth and Survival Rate of Black Tiger Shrimp Larvae (Penaeus Monodon). In Proceedings of the 7th International Conference on Multidisciplinary Research (ICMR 2018) 128-132.
31. Baird DG. The effect of heat and shear on the viscoelastic properties of soy flour dough. J Food Process Eng. 1982; 5:231-245.
32. Catsimpoolas N. Sulfhydryl and Disulfide Groups in Glycinin. The Major Soybean Protein. 1977; doi:10.21236/ada042461.
33. Ewart JA. Re-examination of the linear glutenin hypothesis. J Sci Food Agric. 1977; 28:191-199.
34. Chronakis IS. Network formation and viscoelastic properties of

commercial soy protein dispersions: effect of heat treatment, pH and calcium ions. Food Res Int. 1996; 29:123-134.

35. Luxenburg LA, Baird DG, Joseph EG. Background studies in the modeling of extrusion cooking processes for soy flour doughs. Biotechnol Prog. 1985; 1: 33-38.