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Application of Twin Screw Extrusion Technology for the Development of Ready to Eat Snack using Low Value Shrimp : An Overview

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***Abstract*— Rice and Lali (*Metapenaeopsis stridulans*) mixture were extruded using a co rotating fully intermeshing twin-screw extruder. The extrudates were studied and analyzed for protein solubility, WAI, WSI and fragmentation during twin-screw extrusion. Protein solubility significantly decreased with increase of process temperature. Phosphate buffer and urea mixture were found to extract more protein than plain buffer solution. SDS polyacrylamide gel electrophoresis indicated no fragmentation in protein part. Response surface methodology was used to determine the effect of barrel temperature (120ºC-165ºC) and feed moisture content (10%-20%) on the WAI and WSI of the extrudate. WAI decreases with increase of temperature from 120ºC -135ºC and increases further with increase in temperature from 135ºC -165ºC. Similarly, decrease in WSI values with increase of temperature and moisture was observed.**

I. Introduction

Snacks contribute an important part of many consumers' daily nutrient and calorie intake. Starchy materials are generally used for production of snacks. However, carbohydrate-fish protein mixtures are rarely explored for production of snack food by extrusion. Lali (*Metapenaeopsis stridulans*), a local variety of shrimp has been used as food items in the coastal areas of West Bengal, India. Lali contains approximately 20% protein, 1% fat, 76% moisture, 1.5 % ash and a good amount of minerals. (USDA National Nutrient Database for Standard Reference, Release 15, 2002).A large amount of lali is wasted every year due to lack of proper preservation measures. Considering advantages of twin screw extrusion process, coastal Lali and rice flour mixture were extruded to improve the nutritional quality of the local trash shrimp variety and to study the related functionality of complex carbohydrate and shrimp-protein mixture.

The objective of the present work is to provide an overview data on study for the effect of the extrusion variables on protein solubility, fragmentation pattern, water solubility and water absorption index of the extrudate.

II. Materials and Method:

Materials: Rice (*Oryza sativa L) and salt* procured from local market and Lali (*Metapenaeopsis stridulans*) collected from coastal areas of West Bengal and salt (Tata) from local market was used for control formulation and brought to laboratory in iced condition for study .

III. Preparation of Feed for extrusion:

Lali (shrimp) was collected from coastal area and brought to laboratory in iced condition and washed thoroughly in fresh water and the water was drained and then it was dried in an oven (800 W grill oven Sanyo, JP) at 60°C for 2 hours. Rice collected from local market was also washed and dried at the same condition. After the water was completely removed, dried shrimp and rice were finely ground into powder form with a blender (Mixer Grinder, Bajaj,GM-550) separately. Rice and shrimp flour thus prepared was mixed (5:1). The flour was sealed in polyethylene bags & stored at 4°C in refrigerator for conditioning.

IV. Extruder & Extrusion cooking:

A co rotating fully intermeshing twin screw Extruder was used (screw profile 12:1 barrel length 350 mm; barrel bore diameter 38 mm; screw diameter 37.8 mm; conveying angle 30°; intermeshing screws, 24 mm apart) for extrusion of the feed mixture using a 3 mm diameter die. The screw speed of the extruder was set at 475 rpm, while the feed rate was maintained constant at 28g/min. The extruder started functioning properly with said feed mixture at 110°C and at 11% feed moisture and stopped totally after 170°C and 19% feed moisture condition. Rice and shrimp flour mixture were extruded at four different process conditions. The temperature of the extruder barrel was thus maintained 120°C, 130ºC, 150°C &165°C respectively and the moisture was adjusted to 11%, 13%, 15% and 17% adding the required amount of water to the flour mixture and conditioned at room temperature for 48 hour. Extrudate viscosity, protein solubility, fragmentation, WAI and WSI were studied for the extrudate obtained at four process condition.

V. Moisture Content:

Moisture content of control and extruded products were measured using standard method 2002.

VI. Protein Solubility:

Protein solubility test was performed with both unextrudate material and extrudate products using the method described by Lowry O H,Roscbrough NJ et al, 1951.

VII. Sample Preparation for Water Absorption Index (WAI) & Water Solubility Index (WSI):

Water absorption index (WAI) and Water Solubility index (WSI) were determined using the method outlined by Anderson et al (1969).

VIII. SDS-Polyacrylamide Discontinuous Gel:

Polyacrylamide gel was prepared according to Laemmlie's protocol (1970) to study the protein band after extrusion.

IX. Results and Discussion:

A. Protein solubility:

Table 1 represents the protein solubility data in different extracting solutions of unextrudate and extrudate. Extractable protein decreased in all solvents as the extrudate temperature increased. Protein solubility test was measured to investigate the forces that were responsible for stabilizing the extrudate during the extrusion. Moisture content of each sample was determined before protein extraction. The extractable protein decreased in all solvents after extrusion (Table-1) probably due to formation of some protein with new chemicals linkages , such as non-disulphide chemical bonds that were not disrupted by the solvents used for the formation of some polymers with very high molecular weight. The amount of protein extracted by phosphate buffer in both raw material and extrudate was significantly less than amounts extracted by other solvent because the phosphate buffer alone only dissolved protein molecule that were in native states. When a second solvent was combined with phosphate buffer, protein solubility increased, which suggest that the protein aggregated with more than one type of chemical bond. Phosphate buffer and urea is the highest extracting solvent indicating significant portion of protein was linked with hydrogen bonds. For the same solvent, protein solubility decreases with increase in extrudate temperature as indicated in table 1.

Another significant change with change of cooking temperature and moisture was noticed. The extractable protein shows low even no difference with change of moisture (data not shown) whereas cooking temperature shows decrease in extractable protein with increase in temperature. The ANOVA in table 2 indicated that cooking temperature had significant effect on protein solubility in all solvent ($p<0.05$), whereas moisture condition did not affect the amount in extractable protein (data not shown). With increase in temperature from 110°C-150°C the decrease of extractability of protein is due to cross-linking of protein structure. Extrudate processed at 165°C causes structural damage of the extrudate structure and it becomes rigid and thus decrease in extractable protein observed. The above result is validated with the result of Harvath and Czukor (1993). In their study, the cooking temperature significantly affected the amount of extractable protein below 20% moisture level and at lower moisture condition extrusion temperature is a more important factor than the extrusion moisture condition on the extrudate protein solubility.

X. Water Absorption Index (WAI) and Water Solubility Index (WSI)

WAI is used to give an indication of degree of cooking. WAI and WSI both can be used as an indication of degree of molecular damage i.e. these are two important parameter for defining the applications of extrudates as ingredients and in predicting the material behaviour if further processed (Sriburi & Hill, 2000).

Table 3 indicates the changes of WAI and WSI of rice and shrimp protein mixture at four different extrusion conditions. WAI increases with increasing added moisture. Increasing extrusion temperature from 110ºC-130ºC, WAI decreases which was probably due to an increase in starch degradation (Collonna & Mercier, 1983, Badrie & Mellowes, 1991b). The increase in WAI was observed when temperature increases from 130ºC -165ºC. This effect of extrusion variables on WAI is shown in Fig.1. The result indicated the significant effect ($p<0.05$) of extrusion variable. The said behaviour might have been caused by structural modification with carbohydrate – proteins interaction. Four different temperature & moisture range is described by fitting a quadratic, log linear regression model. The resultant polynomial for the variable is as follows:

$$Y=0.17\ x_2^{2}+0.003\ x_2\ x_1+0.005\ x_1^{2}-0.553\ x_2-1.331\ x_1+96.858$$

The reduction of WAI suggested starch degradation, and the further increase at higher shear condition was probably due to structural modification of fiber, as previously mentioned. Badrie & Mellowes (1991) reported changes from a more sheet like microstructure to a more disrupted appearance when shear increased.

WSI decreases with increase in added moisture and temperature. Table 3 indicated four different temperatures & moisture range as described by fitting a quadratic, log linear regression model. This effect of extrusion variable on WSI is explained in Figure 2. ANOVA indicated (not shown) significant ($p<0.05$) effect of two extrusion variable on WSI data. The resultant polynomial for the variable was as follows:

$$Y= 0.001\ x_1^{2}+0.005\ x_2\ x_1-0.005\ x_2^{2}-0.252\ x_1-0.686x_2+34.717$$

XI. SDS Polyacrylamide Gelelectrophoresis

SDS polyacrylamide gelelectrophoresis was used to study the state of fragmentation during extrusion. Figure 3 represented Sodium dodecyl sulphate polyacrylamide gel electrophoresis of column fraction of unextruded and extruded rice and Lali mixture where Column 1 represented banding pattern of unextruded raw material , Column 2,3,4 banding pattern of extrudate at 150°C and Column 5,6 shows banding pattern of extrudate at 130°C & 7,8 represents banding pattern at 110°C. Almost no difference in the polypeptide-banding pattern of unextrudate and extrudate was observed. Polypeptide of 100, 70, 40, 25 kDA ranges were obtained. Most protein was recovered. Absence of new polypeptide provides evidence of no covalent breakage after extrusion.

XII. Conclusion and Recommendation:

The extrusion process parameters (barrel temperature and moisture) markedly affects the protein solubility of rice and Lali mixture. This study also investigated the effect of extrusion condition on the protein solubility and fragmentation. Decrease of protein solubility with temperature revealed the presence of chemical bond in extrudate. Protein solubility data also predict the extent of soluble solid available after extrusion, which is also an important criteria for fabrication of new food product. WAI and WSI are also two important parameters for defining the applications of extrudates as ingredients and in predicting the behaviour of material for further processing. High WSI is related to stickiness of extrudate. Thus decrease of WSI made the product more acceptable at higher extrusion temperature. The benefit of using the trash and unutilized fish for twin screw extrusion process open up the new experimental approach for preserving and modifying (in terms of texture, flavour and consumer acceptability etc.) coastal resources in a proper manner.

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Samples	Protein Solubility (%)		
	PB	PB+Mercap-toethanol	PB+Urea
Unextrud-ate	0.958±0.004	2.98±0.004	3.87±0.003
A	0.667±0.002	2.678±0.003	3.17±0.003
B	0.332±0.003	2.123±0.002	2.87±0.002
C	0.228±0.002	1.923 ±0.003	2.85±0.001
D	0.112±0.001	1.123±0.002	1.87±0.003

Table-1 Protein solubility in different extracting solutions of unextrudate and Extrudate A: Extrusion at 120°C B: Extrusion at 135°C C: Extrusion at 150°C D Extrusion at 165°C PB: phosphate Buffer (pH6.9); **PB+Mercaptoethanol** 8(M) U: 8 molar urea

Source of Variation	*SS*	*df*	*MS*	*F*	*P-value*	*F crit*
Between Groups	15.87455	2	7.937274	20.43951	0.000137	3.88529
Within Groups	4.65996	12	0.38833			
Total	20.53451	14				

Table 2: ANOVA for protein solubility data

Sl. no.	*Temperat-ure (℃)*	*Moisture %*	*WSI (g/g)*	*WAI (g/g)*
1.	120	10	10.80±0.01	10.24±0.01
2.	120	15	9.56±0.03	11.56±0.02
3.	120	17	9.05±0.02	12.05±0.03
4.	120	20	8.07±0.01	14.07±0.04
5.	135	10	9.56±0.01	8.97±0.05
6.	135	15	9.12±0.005	9.23±0.03
7.	135	17	8.89±0.05	9.78±0.05
8.	135	20	8.04±0.04	10.12±0.06
9.	150	10	8.78±0.01	11.56±0.02
10.	150	15	8.45±0.02	12.08±0.01
11.	150	17	8.12±0.03	13.06±0.03
12.	150	20	8.06±0.01	14.05±0.04
13.	165	10	8.56±0.02	15.08±0.01
14.	165	15	8.45±0.03	15.67±0.02
15.	165	17	8.23±0.04	16.23±0.04
16.	165	20	8.10±0.06	16.78±0.01

Table 3: WAI and WSI data at different extrusion condition

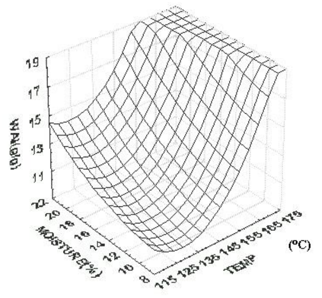

Figure 1: Effect of Extrusion variable on WAI

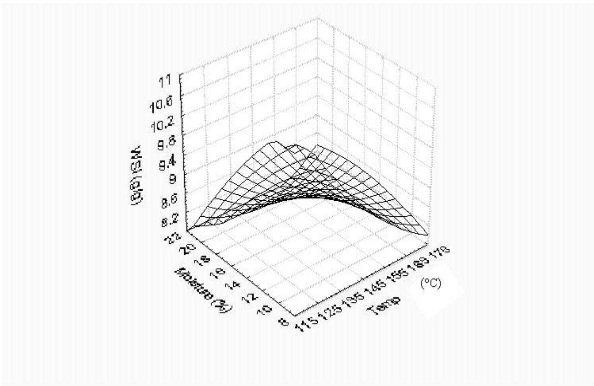

Figure 2:Effect of extrusion variable on WSI

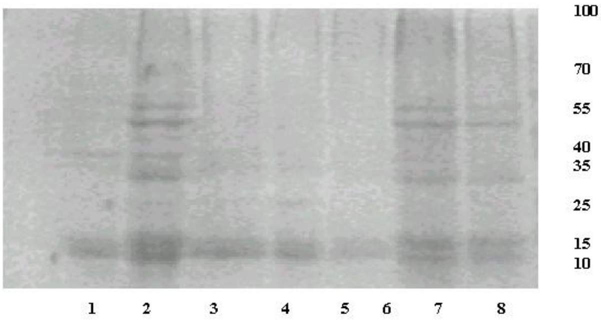

Figure 3: Sodium dodecyl sulphate polyacrylamide gel electrophoresis of column fraction of unextruded and extruded rice and Chapra mixture 1, unextruded 2, 3 & 4 extruded at 150°C 5,6 extruded at 135°C, 7,8 extruded at 120°C