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Developmental Studies on Defatted Custard Apple Seed as a High-Moisture Meat Analogue Employing Extrusion Cooking

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Abstract:
The effect of animal meat consumption on the environment and also on the health due to the increasing awareness is a topic of discussion in the literate world. The changing definitions of the vegetarian foods have drifted the vegetarian population to vegan products that are totally devoid of any animal produce. Soy protein has emerged as an alternative option; still there are challenges of production and non-availability of variety. Looking into the need of high protein rich plant-based meat analogue, the present study investigates the defatted custard apple seed protein as a meat analogue product. After the extraction of the proteins from the seeds of defatted custard apple, twin screw co-rotating extruder was employed for the development of high moisture meat analogues. The developed protein meat analogue was analyzed for the rheological attributes, texture, thermal properties and colour. The findings provided an evidence of incorporation of defatted custard apple seed protein in the extrudate as a stable product with protein content ranging from 55.79% to 64.39%. The consistency and other attributes of the meat analogue were quite in the acceptable range. The viscosity of the final product was found to be 60 Pa.s, following a non-Newtonian nature of flow. The parameters like chewiness ranged from 7.77 N to 15.27 N, and the cohesiveness varied from 0.42 to 0.63. The developed meat analogue possessed the desired characteristics for a meat substitute advocated by the various characterization and evaluation parameters.

Keywords: Vegetarian meat; Extrusion; Meat substitute, High moisture meat

1. Introduction

Change in food habits has been considered as the most effective approach after comparing various strategies to improve the health of population around the globe by altering the dietary patterns such as reducing the intake of calories and minimizing products obtained from animals. For ensuring improved growth and development of human beings, most suitable and apt way is the well thought-out diet supplements based on plant source. And choosing such type of natural origin food can conjointly help in prevention as well as treatment of various chronic and persistent diseases. The likelihood of several diseases such as obesity, type-II diabetes, cancer and coronary heart disease can be lowered down by increasing the intake of plant foods and reducing intake of foods from animal sources and also expectations of a good life can be enhanced. The foods based on plant source are more eco-friendly and ecological than the products from animal sources. The risk of Greenhouse Gas Emissions (GHG) is considerably lesser with the plant based food products as compared to the animal derived foods. The main reason behind this are the sizeable carbon footprints produced due to the manufacturing of food from animal sources, mainly the beef. And also, the consumption of animal sourced food products by the people are the main reason behind the generation of their carbon footprints [1–3].

Meat analogs can be defined as the pre-packaged food products rich in macromolecules such as protein and have similar touch and makeup to that of the meat from animal source. Additionally, they are flavoured with artificial flavouring agents to provide them with a taste similar to that of chicken, beef, turkey or fish. It has also been noticed that in the past ten years, the selling of protein rich surrogates for meat has increased the marketing of meat analogs. Approximately, more than 33% of the Americans have been reportedly purchasing meat substitutes from the market instead of plain meat. Apart from their growing popularity and among vegetarians, non-vegetarians who plan to remove meat from their diet or those who pick the analogs as a healthy substitute or as part of more eco-friendly dietary supplement are also attracted towards the meat analogs [1,4]. Apart from providing required proteins and sufficient levels of dietary roughage, meat analogs possess additional qualities such as presence of natural anti-oxidants and phytochemicals and also lowering down the saturated fatty acid content and no cholesterol amount. Basically, the wheat or soy protein is the major components of meat analogs but some amount of mycoprotein, nuts, legumes or vegetables may also be present, while few of the meat analogs may also contain some ingredients from animal source like as egg or milk [5]. The composition of nutrients used and the effect of environment can be the factors for creating differences in the various protein food products based on animal source such as chicken, beef, pork etc. There is a scarcity of printed scientific data evaluating the environmental impact and nutritional benefits of meat analogs derived from completely different macromolecular sources. But there is consensus on the need of plant-based meat analogs for the people who are vegetarian and these analogs can be a nutritive and economic substitute for the majority of people [6,7]. The commonly known custard apple, with botanical name of *Annona squamosa L. (Annonaceae)*, is reported to be an endemic tropical flora distributed over Ecuador, Egypt, Mexico, India, parts of America, Peru, Bermuda, Brazil, Pakistan, and other tropical regions of the globe. In traditional medicine, *Annona squamosa* find its applications, and also its fruit is a good nutritional source. There are many scientific reports dealing with the protein content of the leaves and seeds of the plant, but no study has been traced for the meat analogue derived from the seed proteins [8]. Looking into the need of meat analogues and the non-availability of studies on non-soya meat analogues, it was envisioned to explore the potential of proteins extracted from deoiled custard apple seeds for the same [9].

2. Materials and Method

2.1. Raw Material

Source of the custard apple seeds (*Annona squamosa L.*) was the local market of Sailu, Distt. Parbhani (Maharashtra). Fully matured fruits with opened eyes with off white and green color were screened. Emphasis was made on the selection of seeds without any mechanical injury and visual marks of damage. To harvest the seeds from the food pulp, manual separation was done [9].

2.2. Extraction of Proteins from the Custard Apple Seeds

A detailed method of the extraction of the proteins from the seeds was carried out as described by Kumar and Sharma, 2016 [9]. A response surface methodology was employed to find out the optimal conditions for the extraction of the proteins from the seeds. The conditions were found to be the use of 0.6% w/w of sodium hydroxide in a solution with pH of 11 and the flour to solvent ratio of 1:60. The optimized method fetched with the protein content of 68.07% and protein yield of 18.69%. 2.3. Moisture Determination

To determine the moisture content of the protein powder, slightly modified AACC method, i.e., 44-15A was employed. In brief, the protein powder was weighed (3-5 grams), the powder was placed in a ceramic container and was placed in an oven, maintained at 103 °C. The powder was placed there for a period of 16 h, till no change in the mass was observed. The moisture content was reported as the mass difference w.r.t. to the initial mass. The extruded products were harvested from the extruder, allowed to equilibrate with the room temperature, sealed in air-tight plastic bags and deep freezed till usage. For measurement of the moisture the samples were cut into the pieces and executed for the moisture content determination after thawing [6,10].

2.4. Extrusion

A twin-screw extruder was employed (KETSE 20/40, M/s Brabender GmbH, Germany) to carry out the extrusion process. The previously reported method was employed for the extrusion [7,11]. For the extrusion, a total of 04 batches were studied, i.e., protein to water ratios of 90% w/w, 80% w/w, 70% w/w and 60% w/w. For feeding the appropriate amounts of protein concentrate and water to the feeder, the feeder was pre-calibrated. The extrusion conditions were fixed at the screw speed of 800 rpm and six temperature cycles as 40 °C, 70 °C, 130 °C, 150 °C, 140 °C and 140 °C. The mass flow rate of 95 g/min-1 and specific mechanical energy of around 100 kJ/kg was used. A short die of 2 mm diameter was used and the extruded product was dried at 40 °C for 120 minutes and further packed in air tight poly-bags and stored in deep freezer.

2.5. Composition of the Meat Analogue

The Association of Official Analytical Chemists (AOAC, 2000) methods of protein amount, moisture content and ash were employed. AOAC method (Method No. 978.04) was used to determine the lipid content using the Soxhlet apparatus. The percentage of carbohydrate was determined by mass balance [12,13].

2.6. Texture Profile Analysis (TPA)

TA.XT2i texture analyzer (Stable Micro Systems Ltd., UK) with 36 mm diameter cylindrical probe was employed to study the texture profile of the samples. The previously reported standard procedure for texture analysis was followed. The meat analogue was rehydrated in tap water till no traces of dry material were observed in the core of the sample. The samples were cut in the pieces of 20 mm x 5 mm. Various textural parameters were reported by the software employed in the equipment [7].

2.7. Viscosity

The American Association of Cereal Chemists (AACC) method was used to determine the viscosity of the developed meat analogue. Perkin Elmer viscometer was employed to determine this property. Around 3.5 grams of the product was weighed and mixed with 27 ml of water and allowed to absorb moisture. The sample was heated to 50 °C and then mixed for 10 s at 960 rpm. The mixture was maintained at 50 °C for 50 s. The temperature was increased at the rate of 12 °C/min and heated up to 130 °C. It was maintained at 130 °C for 2.5 minutes and then again cooled to the 50 °C [6].

2.8. Physicochemical and Functional Properties

Previously reported method was used to determine the vital properties like water absorption (WAC), bulk density (BD) and oil absorption capacities (OAC) [14]. For the determination of rehydration ratio (% RR), around 5 g of the meat analogue was placed in water (50 mL) for 60 minutes at the temperature of 20 °C. The equation employed for the determination of %RR was as follows:

$$\% RR = (X2 - X1)/X1 \times 100$$

where the mass of the sample after rehydration is represented as X2 and the initial mass is represented as X1. The specific volume of whole meat analogues was determined by rapeseed displacement AACC method 10-05.01.

2.9. Sensory Analysis

"A panel of 5 persons performed the task after one-time training of common edible products in terms of organoleptic attributes. The panelists evaluated the sample on an eleven point scale, ranging from 0-10. Verbal anchor points were used to calibrate the panel's understanding of the scale. Physical references were used for all the attributes, where plant-based extruded samples had to be compared with meat. Panel performance was validated using PanelCheck (version 1.4.1—Nofima Mat, Breivika, Ås, Norway). The list of the sensory attributes, definitions, and scale is reported elsewhere [7]. The chewiness of the sample was determined by chewing it till the mass is converted into a swalloable form by placing the sample between the teeth. A total of 5 chews were used as a reference. It was established that the scale had enough scale-points to distinguish the products, also to prevent the avoidance of scale-end anchors. For the descriptive sensory analysis, amounts of 5 g of rehydrated meat analogues (according to the times identified for the TPA) were put in a glass container, codified by a three-digit alphanumeric code, and then served to each panelist followed by Williams' Latin square design. Sensory analysis was replicated on two different sessions. Each panelist carried out the sensory evaluation in a sensory room according to the ISO 8589:2007 [7]."

2.10. Statistical Analysis

"The data were subjected to one-way ANOVA followed by Tukey's HSD (honestly significant difference) test. Significant differences were determined at $p \leq 0.05$ by Minitab 17 Statistical Software (Minitab, Inc., State College, PA, USA) [1]."

3. Results and Discussion

3.1. Extraction of Proteins from the Custard Apple Seeds

The protocol of the extraction of the custard apple protein has been already published and the response surface methodology was used to optimize the extraction process. A brief of the published results are presented here [9]. The conditions were found to be the use of 0.6% w/w of sodium hydroxide in a solution with pH of 11 and the flour to solvent ratio of 1:60. The optimized method fetched with the protein content of 68.07% and protein yield of 18.69%.

3.2. Moisture Content

The range of the moisture content of the extracted protein was 5.39% to 6.17% and the moisture content in the extruded meat analogue was in the range of 60.19 % to 76.28%. The moisture content of the extruded materials was falling in the desired range of moisture for the milk-like products, i.e., 70% to 75%, however, the product obtained with feed of 90% feed gave the lowest value of the moisture content, i.e., 60.19%, whereas other three batches exhibited the desired target product moisture value and the findings were in consonance with the similar studies, though involving different vegetarian protein bases [6].

3.3. Composition of the Meat Analogue

The meat analogues were evaluated for various nutrients employing the standard protocols. The protein in the dry powder was 68.07% and the protein content in the four meat analogues ranged between 55.79 % to 64.39 %. The values of various nutrients have been reported in the Table 1. Lipids constituted the 7.23 % of the protein powder and from 5.49 % to 6.87 % of the meat analogues. In the modern meat analogue products the reported value of fats ranges from 3.47% to 20.0%, despite various processing steps [15]. The percentage of carbohydrate in the plain powder was 20.74 % and the ash value was 3.96 %. The carbohydrate content in the exudates was observed to be in the range of 27.27 % and 37.11% , whereas the ash content range was from 2.85% to 5.47% . The meat analogues offered good nutritive value, as in all of the compositions; the quantity of protein was higher than the 50% of the total mass of the product. The starch content was also good and iss desired in meat analogues as it helps in the extrusion process and also provide the fibrous texture to the meat analogues. The protein contents of similar marketed products range between 17.7 g/100 g and 25.0 g/100 g, advocating the present products offer good protein content than such products [15,16].

Table 1: The nutritional composition of the various products derived from defatted custard apple seeds

Component	**Pure Protein Extract**	**90% w/w Protein Extract**	**80 % w/w Protein Extract**	**70 % w/w Protein Extract**	**60 % w/w Protein Extract**
Protein (%)	68.07 ± 2.13	64.39 ± 3.09	60.37 ± 3.91	57.25 ± 4.20	55.79 ± 2.33
Lipids (%)	7.23 ± 0.69	5.49 ± 0.21	5.98 ± 0.17	4.76 ± 0.07	6.87 ± 0.41
Ash (%)	3.96 ± 0.21	2.85 ± 0.11	1.9 ± 0.07	2.53 ± 0.05	5.47 ± 0.37
Carbohydrates (%)	20.74 ± 1.93	27.27 ± 2.07	31.75 ± 0.09	35.46 ± 1.15	37.11 ± 1.13

Data expressed on dry matter. Different letters in the same row mean significant differences at $p < 0.05$. $n = 4$.

3.4. Texture Profile Analysis (TPA), Physicochemical and Functional Properties

The various parameters studied under the headings of TPA, physiochemical and functional properties have been represented in the Table 2. It is clearly evident that the values of hardness, cohesiveness, springiness, chewiness along with the values of bulk density, WAC, % RR and OAC were of highest magnitude for the crude protein powder. The data unequivocally advocates that the powder as such could not be regarded as the eatable option, however the extruded products offered substantial reduction in the values of hardness, cohesiveness, springiness, chewiness, indicating that they have the meat like/food like attributes. The 90% w/w product offered the maximum values of the TPA parameters over the extruded compositions, indicating a higher degree of muscle force and energy is required to chew this material. As the composition of water was increased these attributes decreased, indicating the ease of chewing increased. However, a notable fact is that the protein content also decreased in the same pattern [17].

After the incorporation of water and extrusion, the bulk density decreased in a chronological manner, as expected as the fluffiness due to fibr4es and carbohydrates might have increased. The water absorption and oil absorption capabilities coupled with the rehydration ration also decreased with the increasing water content due to the swelling of the fibres and development of a network matrix.

Table 2: Textural, physicochemical and functional characterization of the crude protein and meat analogues

TPA, Physicochemical and Functional Properties	**Protein**	**90% Protein analogue**	**80% Protein Analogue**	**70% Protein Analogue**	**60% Protein Analogue**
Hardness (N) *	34.68 ± 3.93	23.53 ± 2.72[a]	17.19 ± 3.27[ab]	14.14 ± 2.15[ab]	13.93 ± 1.50[ab]
Cohesiveness *	1.23 ± 0.07	0.63 ± 0.02[a]	0.54 ± 0.01[ab]	0.44 ± 0.03[abc]	0.42 ± 0.01[ab]
Springiness *	1.19 ± 0.09	0.81 ± 0.06[a]	0.73 ± 0.03[ab]	0.64 ± 0.02[abc]	0.57 ± 0.03[abcd]
Chewiness (N) *	18.09 ± 1.17	15.27 ± 2.63[a]	13.76 ± 1.52[a]	8.29 ± 0.64[abc]	7.77 ± 2.15[abcd]
BD (g mL^{-1}) **	0.81 ± 0.05	0.63 ± 0.03[a]	0.45 ± 0.02[ab]	0.37 ± 0.01[abc]	0.29 ± 0.03[abcd]
WAC (g water g^{-1}) **	5.19 ± 0.08	3.19 ± 0.02[a]	2.85 ± 0.13[ab]	2.25 ± 0.08[ab]	1.92 ± 0.01[abcd]
RR (%) **	423.08 ± 18.75	323.47 ± 13.33[a]	291.96 ± 19.17[ab]	215.14 ± 10.98[abc]	204.49 ± 7.53[abc]
OAC (g oil g^{-1})	2.23 ± 0.17	1.45 ± 0.19[a]	1.37 ± 0.09[ab]	1.21 ± 0.18[abc]	1.05 ±

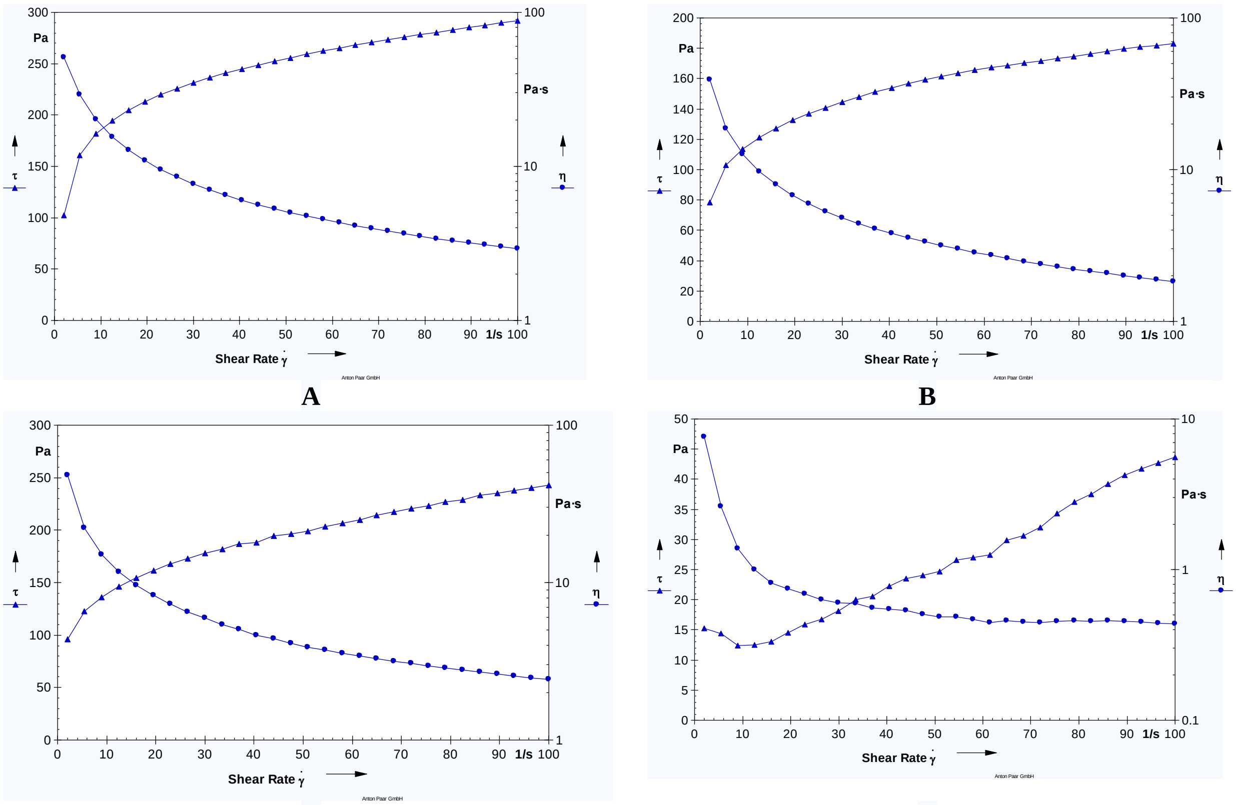
**					0.04^{abcd}

Different letters in the same row mean significant differences at $p < 0.05$. * $n = 15$; ** $n = 4$. BD: bulk density; WAC: water absorption capacity; RR: rehydration ratio; OAC: oil absorption capacity. "a" represents the statistical difference with the values of the row in the column 2, "b" represents the statistical difference with the values of the row in the column 3, "c" represents the statistical difference with the values of the row in the column 4 and "d" represents the statistical difference with the values of the row in the column 5

These studies have provided a proof of the concept that the meat analogues with acceptable physicochemical, functional and textural attributes can be developed with simple extrusion technique.

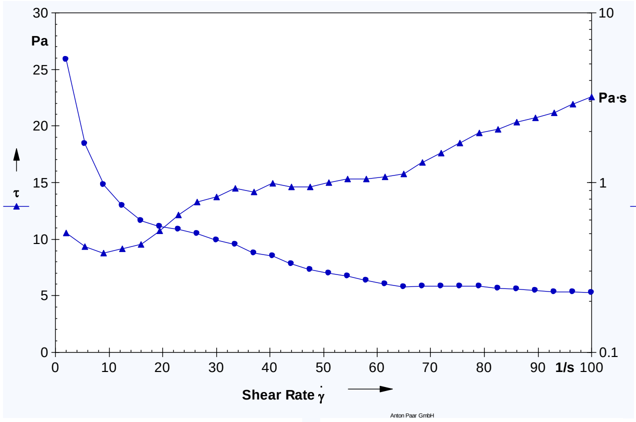
3.5. Viscosity

The results of the viscosity have been presented in the Figure 1. The findings of the rheology are also analogous to that of the texture analysis. The crude protein was highly viscous in nature and offered the viscosity of the order of 100 Pa.s, the extruded product with 90% w/w protein offered the apparent viscosity of around 80 Pa.s, which further decreased to 60 Pa.s for the 80% w/w protein batch and to 8 Pa.s for the 70% w/w batch. The lowest apparent viscosity was for the 60% w/w protein batch, i.e., of the order of 6 Pa.s. The findings show that the developed systems followed non-Newtonian kind of flow and the consistency of the extruded products, esp. the one with 80% w/w was in quite acceptable range [18,19].



C

D



E

Figure 1: The rheogram showing the change in viscosity and shear stress as a function of varying shear rate for the crude protein (A), the meat analogue containing 90% w/w protein (B), the meat analogue containing 80% w/w protein (C), the meat analogue containing 70% w/w protein (D) and the meat analogue containing 60% w/w protein (E)

3.6. Sensory Analysis

From the sensory analyses, the texture and color of the developed meat analogues resembled the meat and the fibrous nature was maintained. The product with 90% w/w protein content was relatively difficult to chew, whereas the one with 60% w/w was almost spongy in nature. The best chewability was offered by the two compositions, i.e., 80% w/w protein-based product and 70% w/w protein-based analogue. None of the products offered any obnoxious odour and the colour of the product was almost near to the flesh [20].

4. Conclusions

The extracted concentrate from the defatted custard apple seeds contained 68.07% of proteins with a protein yield of 18.69%. The three meat analogues developed were comprised of protein in the range of 55.79% to 64.39% and the textural properties along with the physicochemical characteristics of all the three compositions were in the acceptable range. The viscosity analysis of the three batches of the meat analogues followed non-Newtonian system of flow, with all the compositions acceptable with an edge for the composition comprising of 80% w/w protein concentrate. The findings provide a clear evidence for a nutritious and acceptable meat analogue derived from the seeds of custard apple and possess immense future promises.

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