RESEARCH ARTICLE

Suitability studies of different milk proteins for supplementation in functional extruded snack

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Received: 14 June 2020 / Accepted: 23 July 2020 / Published online: 27 October 2020
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Abstract: Carbohydrate based snacks clubbed with frying have substantial dominance on Indian snack market, depriving nutrition. But awareness for alternative healthier snacks has already attracted market attention. In the present study, an effort was ventured by utilizing the combined benefits of high carotene sweet potato flour, barley flour, rice flour and milk protein (Whey Protein Concentrate (WPC)-70/ Skim Milk Powder (SMP)/ rennet casein) to design a low fat-high protein, crisp snack by extrusion cooking. The conditions employed for snack processing were the temperature of heater 1 (110°C) and 2 (40°C), screw speed (370 rpm) and die diameter (4 mm). The effect of different proteins and their levels on snack quality were evaluated by physical and sensory responses like hardness (N), L*, a* and b* color values, expansion index (EI) and sensory scores (color and appearance, flavor, texture and overall acceptability). The results revealed that addition of SMP at different rate did not showed significant ($p>0.05$) effect on expansion index, color & appearance and flavor score of the snack. Whereas, incorporating rennet casein as a source of quality protein, EI increased significantly ($p<0.05$) at 15% and there was a significant effect on the sensory parameters

with increasing rennet level. However, increasing WPC-70 addition, the EI and sensory scores declined, and the hardness of the snack increased. Highest EI (3.18) and sensory scores were observed at 15% level of casein. From quantified parameters, casein was deduced to be most acceptable milk protein source for the intended snack.

Keywords: Composite flour, Extruded snacks, Functional food, Milk proteins, Physical properties

Introduction

Extrusion is a HTST technology that combines several unit operations comprising mixing, cooking, kneading, shearing, shaping, and forming (Steel et al. 2012). This process gelatinizes starch, denatures proteins, modifies lipids, and inactivates enzymes, microbes, and antinutritional factors (Singh, Gamalath and Wakeling 2007). The worldwide demand of extruded snack foods is high as they are available in variety of shapes, textures, colours and flavours. However, extruded snacks are typically high in calories and fat with low content of protein, fibre, and perceived as unhealthy or junk food to many consumers (Korkerd et al. 2016; Basto et al. 2016). A possible solution to this problem is incorporation of alternative ingredients like fruits and vegetables, banana powder, legume flour, oats and whole grains etc. in extruded product preparations to enhance their nutritional quality (Sukumar and Athmaselvi, 2019; Oliveira et al. 2018). However, many researchers have observed that addition of dietary fibre often leads to lower expansion volumes, higher density, harder texture, less crispness, and thus reducing the acceptability of the extruded snack (Veronica et al. 2006; Brennan et al. 2008; Robin et al. 2011; Chanvrier et al. 2013; Chassagne-Berces et al. 2011).

Milk proteins are recognized for their nutritive and health benefits (Zimecki and Kruzel 2007). These proteins have many biological activities: cancer prevention, tumour cell vulnerability increase, antimicrobial activities and immunomodulation (Madureira et al. 2007). Milk proteins have good digestibility (Hambræus, 1992), as well as Protein Efficiency Ratio (PER), whey protein and caseins have a PER of 3.2 and 2.6, respectively (Walzem et al. 2002). Lately, milk constituents have been acclaimed as functional foods,

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as they impart numerous therapeutic benefits which improve health. Use of dairy proteins in cereal based products, improves the nutrient density of extruded snacks by increasing protein and mineral content (Brncic et al. 2011; Pordesimo et al. 2009). Attempts have been made to use whey protein concentrates or dried whey for extrusion purpose (Onwulata and Konstance, 2006; Onwulata et al. 2010; Fernandes et al. 2016; El-Ghany, et al. 2013; Chaudhari et al. 2020). The type and level of milk protein incorporated into an extrudate affects the water holding capacity, nitrogen solubility index and sorption characteristics of extruded products (Allen et al. 2007). This study was focused on combining different milk proteins (Skim milk powder/ whey protein concentrate-70/ rennet casein) with composite flour (sweet potato, barley and rice) to enhance the nutritional status of extruded snack and evaluate the effect of level and type of these milk proteins on physical and sensorial properties, that would ultimately have an effect on acceptability of the product by the consumer in market.

Materials and Methods

Raw Materials

Carotene rich variety of sweet potato, *Indramadhur* was purchased from Regional Centre of Central Tuber Crop Research Institute (CTCRI), Bhubaneswar, Odisha. Barley (DWRB-73 variety) was procured from the Directorate of Wheat Research (DWR), Karnal, Haryana. Paddy (PR-44) was procured from Indian Agricultural Research Institute (IARI- Regional Centre), Karnal, Haryana. Milk Protein Sources (WPC-70, Rennet Casein and SMP) were procured from M/s Modern Dairies Ltd., Karnal, Haryana. Sweet potatoes were washed, sliced, dipped in 0.1% KMS solution for 5 min and then dried in tray drier at 60°C for 4-5 hr. The dried slices were then ground to fine powder. Barley and paddy were de-husked, cleaned and then subjected to milling.

Proximate analysis of the raw material

Standard procedures as described in AOAC (2000) were used for proximate analysis of sweet potato, barley and rice flour and skim milk protein, casein, and WPC-70 (Moisture, protein, crude fat and ash content).

Preparation of composite extruded snack

Based on preliminary trials, the composite flour ratio (sweet potato, barley and rice flour) were fixed at 20, 25 and 30 parts, respectively. The three types of milk protein (WPC-70, SMP and rennet casein) used for the study were at proportion of 5, 10 and 15 parts. The methodology adopted was as described by Yadav et al. 2016. The ingredients were blended in a Hobart mixer at 60 rpm, moisture was adjusted to 12% and the blend was passed through 2 mm size sieve and again blended for 10 min. to get uniform mixing. The premix was packed in 1 kg PE bag and preconditioned overnight in a refrigerator before extruding using

twin-screw extruder. The speeds of feeder screw and extruder screw were kept constant at 40 and 370 rpm, respectively. The temperature of the two heating zones were 40°C and 120°C, respectively. As the material was extruded through 4mm die and was cut into pieces of desired length by a rotating-blade cutter at 10 rpm. Extrudates were then packed in metalized PE pouches and stored at 37°C until further analysis.

Sensory evaluation

Sensory evaluation was conducted by 10 trained panelists from NDRI, Karnal. Product evaluation was based on its color & appearance, flavor, texture, and overall acceptability on 9-point hedonic scale (1 = dislike extremely to 9 = like extremely) (Deshpande and Poshadri, 2011).

Hardness (Texture Analysis)

Hardness is the peak force during compression of the product. It was determined using TA-XT2i (Stable Micro Systems, UK) Texture Analyzer fitted with a 25 kg load-cell and Warner-Bratzler Blade. The pre-test and post-test speed were 2.0 mm/sec, while the test speed was 1.0 mm/sec.

Expansion ratio

The ratio of diameter of extrudate and the diameter of die was used to express the radial expansion of extrudate (Fan et al., 1996). The extrudate expansion ratio was calculated as follows:

$$\text{Expansion ratio} = \frac{\text{Extrudate diameter (mm)}}{\text{Die diameter (mm)}}$$

Hunter color analysis

The color of the product was measured using a Hunter Lab Colour flex colourimeter (Hunter Associated Laboratory, Inc., USA) using the Universal software version 4.10. Measurement was made by taking finely ground sample in the glass sample holder up to 2 cm height followed by tapping for ten times on bench-top. Data were received through the software in terms of L* (lightness), ranging from zero (black) to 100 (White), a* (Redness), +60 (red) to -60 (green) and b* (yellowness), ranging from +60 (yellow) to -60 (blue) values of the international (CIE) color system.

Statistical analysis

The data obtained from the given experiments for milk protein sources incorporation in composite extruded snack were subjected to analysis using two-way ANOVA using SPSS Statistical tool (version 20, IBM Corp., New York, NY, USA) as described by Snecdecor and Cochran (1994). The data were presented as Means $\pm$ Standard Error (SE).

Results and Discussion

Proximate analysis

The results obtained for proximate analysis of raw material are presented in Table 1. It is evident, that rennet casein had highest crude protein content i.e. 80.16 %, followed by WPC-70 (69.70 %) whereas, sweet potato flour (SPF) had the least amount of proteins (1.05 %). The fat content in rice flour was least with the value being 0.45 % and highest fat content was observed in WPC-70 i.e. 4.66 %. Casein and SMP had high percentage of ash content (7.94 and 7.89 %, respectively) whereas barley and rice flours had lower levels (1.55 and 0.45%). The carbohydrate content of the flours was calculated by subtracting percentage of all other components from 100. Similar values of proximate analysis of milk proteins was reported by Ponbhagavathi et al. (2018)

Hunter color values of raw materials

The color of the raw material has a prominent effect on the appearance of the processed product, thereby effecting its consumer acceptability. The L* value was found to be maximum with Skim milk powder (92.03), thus indicating that SMP was the brightest and whiter among all the ingredients (Table 2). The lowest L* value was observed for SPF (72.61) but a* and b* values were highest for SPF indicating that it was darker compared to other raw materials.

Effect of incorporation of milk proteins on expansion index and hardness

A higher value of expansion index in extruded snacks represent a product with greater crispiness and less dense structure. The

composition of the raw ingredients has a significant effect on the expansion. During extrusion the high temperature and shear, gelatinize the starch forming a matrix with entrapped water molecules, due to sudden pressure drop at the exit, expansion of the extrudate occurs as the water molecules expand forming bubbles and creating a porous structure (Guy and Horne, 1988). When raw material have higher amounts of protein and/ or fibre, the starch level drops, resulting in lesser expansion and increased density. The values of expansion index and hardness are represented in Table 3. The values ranged from a lowest of 1.68 (15% WPC 70) to 3.18 (15% rennet casein). SMP did not have any significant difference with increase in level, while in case of WPC, the E.I. decreased tremendously with increase in level from 5 to 15%. Ding et al. (2005) reported values between 2.02 and 3.87 for rice-based extrudates. It is evident from the table that increasing level of SMP did not have any significant effect on E.I. of extrudates, whereas in case of rennet casein, E.I. increased significantly ($p < 0.01$) as the level increased from 5 to 15 %. Patel et al. (2016) also observed that the increasing level of rennet casein resulted in a significant increase in the expansion ratio ($p < 0.05$). This may be due to the large molecular structure of casein, hydrophobic properties and random coil conformation in comparison to whey protein concentrate and isolate (Onwulata and Konstance 2006). However, an opposite trend was observed in case of WPC-70, as the level of incorporation was increased, there was reduction in the expansion of the snack. Reduction in expansion with increasing whey protein concentration as a result of starch-protein interactions was reported by Allen et al. (2007). Another study conducted by Chinnaswamy and Hanna (1988) also concluded that there was decrease in the expanded volume of cereal flour-based snacks when level of lipid and protein is increased in the mixture.

Table 1 Proximate composition (g/ 100g) of raw materials used for extruded snack preparation

Constituent	Moisture	Crude protein	Fat	Carbohydrate*	Total ash
SPF [#]	5.94±0.36	1.05±0.45	0.69±0.05	88.93	3.38±0.03
Barley Flour	5.63±0.18	14.97±0.00	1.68±0.11	76.17	1.55±0.00
Rice Flour	11.22±0.22	8.40±0.45	0.45±0.19	79.48	0.45±0.042
Casein	8.90±0.04	80.16±0.44	0.96±0.04	—	7.94±0.021
WPC-70%	3.76±0.27	69.70±0.46	4.66±0.05	—	3.89±0.197
SMP	3.8±0.48	34.05±0.44	1.2±0.31	53.06	7.89±0.003

*By difference; [#] Sweet potato flour; Values are mean ± standard error (n= 3)

Table 2 Hunter color parameters (L, a, b values) of raw materials

Constituent	Hunter color parameters		
	L* value	a* value	b* value
SPF	72.61 ± 0.32	4.31 ± 0.09	20.65 ± 0.38
Barley flour	81.52 ± 0.14	1.43 ± 0.27	9.15 ± 0.04
Rice flour	86.71 ± 0.23	-0.01 ± 0.02	6.51 ± 0.02
Rennet Casein	83.03 ± 0.11	-0.64 ± 0.05	10.98 ± 0.02
WPC-70 %	84.55 ± 0.16	0.92 ± 0.13	20.68 ± 0.21
SMP	92.03 ± 0.01	-3.02 ± 0.04	16.71 ± 0.02

Values are mean ± SE (n=5)

Hardness is the peak force required for a probe to penetrate or break the extrudate (Ponbhagavathi et al. 2018). Expansion index and the cell structure of the extruded snack have an impact on the hardness. The values ranged from 21.51 N to 54.13 N, the highest value was observed for 15% WPC 70. When extrudate was made with incorporation of skim milk powder, the hardness of the product reduced from 25.64 N to 21.72 N. There was no significant change ($p > 0.05$) in the hardness values when extrudate was made with incorporation of rennet casein. It was reported by Voort and Stanley (1984), that there was no significant effect on hardness value of wheat flour extrudates by

incorporation of rennet casein (10 to 30 %). Another study concluded that rennet casein, tended to decrease the hardness at lower levels, but the effect was not significant at higher levels (Patel et al. 2016). When WPC 70 was added to the ingredient mix as the milk protein source, the hardness of the product increased significantly ($p < 0.01$) from 28.69 N (5%) to 54.13 N (15%). It is evident that hardness level increased with increase in level of WPC 70. Similar results were obtained by Yadav et al. (2013), in pearl millet-WPC extruded snacks. This may be due to fact that the protein competes with the starch for the water during extrusion, reducing the rate of gelatinization and thereby

Table 3 Physical properties of composite flour based extruded snack affected by different milk proteins

Physical Properties	Level (%)	Milk Protein Type [#]		
		SMP	Rennet Casein	WPC-70
Hardness	5	25.64 ^{ab} ± 0.55	22.27 ^{aA} ± 0.73	28.69 ^{cC} ± 1.53
	10	27.35 ^{cB} ± 0.09	21.51 ^{aA} ± 1.08	35.22 ^{bc} ± 2.61
	15	21.72 ^{aA} ± 0.12	22.26 ^{aA} ± 0.13	54.13 ^{cB} ± 1.42
Expansion Index	5	2.403 ^{aA} ± 0.21	2.91 ^{aB} ± 0.04	2.91 ^{cB} ± 0.19
	10	2.49 ^{aA} ± 0.12	2.87 ^{aB} ± 0.02	2.32 ^{bA} ± 0.13
	15	2.39 ^{aB} ± 0.10	3.18 ^{bc} ± 0.18	1.68 ^{aA} ± 0.02
L value	5	62.25 ^{aA} ± 1.58	65.46 ^{bB} ± 0.30	63.79 ^{aB} ± 0.02
	10	64.55 ^{bB} ± 0.13	64.05 ^{aA} ± 0.14	65.62 ^{bc} ± 0.29
	15	64.98 ^{bB} ± 0.09	64.45 ^{aA} ± 0.12	68.79 ^{cC} ± 0.23
a value	5	8.18 ^{aB} ± 0.56	5.59 ^{aA} ± 0.67	7.60 ^{cB} ± 0.31
	10	7.40 ^{aB} ± 0.31	7.71 ^{bB} ± 0.57	5.69 ^{bA} ± 0.73
	15	7.54 ^{aB} ± 0.22	7.52 ^{bB} ± 0.50	4.71 ^{aA} ± 0.30
b value	5	25.02 ^{aA} ± 0.33	25.25 ^{aA} ± 0.51	24.93 ^{cA} ± 0.54
	10	24.79 ^{aB} ± 0.14	25.05 ^{aB} ± 0.56	23.37 ^{bA} ± 0.44
	15	25.63 ^{bB} ± 0.18	25.501 ^{aB} ± 0.63	20.34 ^{aA} ± 0.25

[#]Milk Protein Type: SMP- Skim milk powder, WPC-70- whey protein concentrate

abc; ABC Means ± S.E. (n=15) with same superscripts in a row (A, B, C) or in a column (a, b, c) do not differ significantly ($P > 0.01$).

Table 4 Sensory scores of composite flours based extruded snack affected by different milk proteins

Sensory Attribute*	Level (%)	Milk Protein Type [#]		
		SMP	Rennet Casein	WPC-70
Color & appearance	5	5.93 ^{aA} ± 0.58	6.87 ^{bB} ± 0.08	6.49 ^{cAB} ± 0.06
	10	6.69 ^{aB} ± 0.27	6.43 ^{aB} ± 0.04	5.84 ^{bA} ± 0.10
	15	6.80 ^{aB} ± 0.44	7.50 ^{cC} ± 0.20	4.90 ^{aA} ± 0.13
Flavor	5	5.98 ^{aA} ± 0.51	6.83 ^{aB} ± 0.14	6.23 ^{bAB} ± 0.43
	10	6.44 ^{aA} ± 0.17	6.26 ^{aA} ± 0.41	5.78 ^{bA} ± 0.49
	15	6.73 ^{aB} ± 0.36	7.67 ^{bc} ± 0.08	4.36 ^{aA} ± 0.32
Texture	5	5.65 ^{aA} ± 0.78	7.03 ^{bB} ± 0.19	6.29 ^{cAB} ± 0.53
	10	6.77 ^{bB} ± 0.19	6.77 ^{aB} ± 0.11	5.47 ^{bA} ± 0.18
	15	6.93 ^{bB} ± 0.04	7.54 ^{cC} ± 0.09	4.05 ^{aA} ± 0.41
Overall Acceptability	5	5.88 ^{aA} ± 0.77	6.83 ^{bA} ± 0.06	6.17 ^{cA} ± 0.40
	10	6.64 ^{abB} ± 0.06	6.53 ^{aB} ± 0.13	5.49 ^{bA} ± 0.18
	15	6.86 ^{bcB} ± 0.12	7.32 ^{cC} ± 0.10	4.12 ^{aA} ± 0.16

*On a 9-point hedonic rating scale: 9-like extremely; 1-dislike extremely.

[#]Milk Protein Type: SMP- Skim milk powder, WPC-70- whey protein concentrate

abc; ABC Means ± S.E. (n=10) with same superscripts in a row (A, B, C) or in a column (a, b, c) do not differ significantly ($P > 0.01$).

decreasing expansion and increasing hardness of the product. In addition, the higher amounts of protein may also result in variation in temperature and pressure inside the extruder, affecting the degree of expansion and textural properties (Anton et al. 2009; Sumargo et al. 2016). Anton and Luciano (2007) also stated that the extrudates hardness depends on raw material composition, as well as feed moisture and extrusion conditions. Protein rich extrudates produce less expandable products and more rigid network resulting in higher resistance to shear and lower expansion (Li et al., 2005).

Effect of incorporation of milk proteins on colour parameters

Food acceptability is affected by color of the product. It acts as an indicator of quality, flavor expectation and commercial value (Fradique et al., 2010). Color of an extrudate is an indicator of thermal history and account of non-enzymatic browning resulting from maillard browning during extrusion process (Yadav et al. 2013). The L^* , a^* and b^* value of extruded snack as effected by type and level of milk protein is presented in table 3. The L^* values for extrudates ranged from 62.25 to 68.79. The L^* value was highest in case of WPC 70, indicting a lighter product in comparison to SMP and rennet casein. Positive b^* values indicate the yellowness of a sample and negative values are indicative of a blue color. As the extruded snack had sweet potato flour rich in soluble sugars, the b^* value was on higher side (20.34 for WPC to 25.63 for SMP). Non-enzymatic browning reactions like caramelisation and the Maillard reactions forms compounds that have higher a^* value (Ilo and Berghofer 1999). Melanoidins formed in the Maillard reaction impart a darker colour to extrudates. Process conditions during extrusion, high lactose content and protein content in whey powder also intensify the dark color of these compounds (Tamanna and Mahmood, 2015).

Effect of incorporation of milk proteins on sensorial properties

The consumer acceptance of any product depend on the sensorial properties of the product. The sensory properties like colour, flavour, texture, and overall acceptability were significantly ($p < 0.01$) affected by the level and type of milk protein incorporated (table 4). The lowest score (4.90) for colour and appearance was observed for 15% WPC 70 while highest (7.50) was observed for rennet casein 15 %. The scores improved with increase in level of SMP and rennet casein from 5 to 15 %, whereas the values decreased in case of increment of WPC 70. More or less similar trend was also noticed for flavour, texture and O.A. scores of the extruded snack. On comparison of different protein sources, it can be observed that rennet casein at 15% level, had significantly higher scores ($p < 0.01$) in all sensory parameters compared to SMP and WPC 70. Higher acceptability in case of rennet casein incorporation maybe attributed to higher expansion and crisp texture of the extruded snack.

Conclusions

The study indicated that types and levels of milk proteins had a significant ($p < 0.01$) effect on the hardness, expansion index and color values of the extruded snack. The sensory scores were significantly affected by the type of milk protein used to make high protein extruded snack. Rennet casein had better acceptability as depicted by the trained sensory panelist compared to product prepared with incorporation of WPC. The snack prepared using 5% level of WPC was acceptable but at higher levels (10% and 15%), the overall quality of the snack declined extremely. Increasing level of SMP didn't show significant effect on the sensory and physical parameters. Significant improvement in the expansion index and sensory scores of extruded snack with rennet casein was observed at all levels. Thus, a highly acceptable, high protein product with desirable physical, sensory characteristics could be prepared with rennet casein as a source of quality protein.

Acknowledgements

The corresponding author is thankful to The Director, National Dairy Research Institute for providing infrastructural amenities and Department of Science and Technology, New Delhi for economic assistance in the form of INSPIRE Fellowship for conducting the presented research work.

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