Effect of Rennet Casein and Whey Protein Concentrate on Extrusion Behavior of Maize Flour

T. R. Thirumuruga Ponbhagavathi¹*, Ashish Kumar Singh¹, P. Narender Raju¹ and Neelam Upadhyay¹

¹Dairy Technology Division, ICAR-National Dairy Research Institute, Karnal, 132001, India.

ABSTRACT

The present study was carried out to find out the effect of blending of maize flour (MF) with varying levels of Rennet casein (RC 6, 8%) and Whey protein concentrate-70 (WPC-70 4, 8%) at different feed moisture content (12,14%) on the physico-chemical characteristics (pasting properties, colour, expansion ratio, bulk density, hardness, water absorption and water solubility index, resistant starch), sensory properties of extrudates and their changes in physico-chemical (TBA and Free Fatty acids) as well as sensory properties of extrudates during storage. Physical parameters like, bulk density, WAI, WSI, expansion ratio, texture profile (hardness and crispiness) shown significant (p<0.05) variation among the treatments. Addition of RC and WPC-70 increased the setback viscosity and decreased the peak, breakdown and final viscosity. The hunter L* value, a* value and b* value were significantly (p< 0.05) affected by the type and level of addition of protein. Incorporation of RC and WPC-70 upto 8% to maize flour was more suitable for protein enriched snack base with good overall acceptability of product. The developed extrudate was packaged in metallized Low Density Polyethylene (LDPE) pouches and stored under 25±1°C and 37±1°C for 1 month and monitored at weekly interval. The resistant starch content of extrudates increased from 45 to 128 mg/ 100 g and 132 mg/ 100 g of sample stored at 25°C and 37°C at the end of storage.
Changes in TBA value was non-significant and FFA value increased significantly (p<0.05) due to hydrolytic rancidity. The sensory scores obtained for color and appearance, texture and overall acceptability did not have any significant variation (p>0.05), however the flavour scores decreased significantly (p<0.05) which can be correlated with FFA and TBA value.

Keywords: Milk protein-maize based extrudates; resistant starch; green food processing; hydrolytic rancidity; texture profile.

1. INTRODUCTION

“Green food processing” is based on the discovery and design of technical parameters which will reduce energy and water consumption, allows recycling of by-products through bio-refinery and ensure a safe and high quality products [1]. Among the currently available technologies, probably the extrusion processing fulfills the criteria laid down for the green technologies. Extrusion processing is a High temperature Short Time process (HTST), encompasses number of unit operations including mixing, cooking, conveying, texturization, drying and formation [2]. Enormous range of food materials can be processed by extrusion processing to develop the newer range of processed products to meet the consumer’s demand of novelty. The extrusion processing technology has been employed to produce wide range of processed foods including snacks, breakfast cereals, analogues, Texturized Vegetable Proteins (TVP), modified starches, pre-cooked mixes, pet foods and certain food ingredients [3].

Extrusion processing makes it possible to apply different sources of ingredients for the enrichment of cereal-based extruded [4]. Cereal grains are preferred raw material for extrusion processing, however milk proteins could also be processed in combination with cereal products to produce wide range of processed foods. Maize has wide adaptability over various agro-climatic conditions. The production of maize is growing at an average annual rate of 3.46%. Maize contains 7-13 g of protein/100 g dry matter [5]. It contains roughly 1 percent lysine, whereas 5 percent is required for optimal human nutrition [6]. Milk proteins have high nutritional and therapeutic value [7]. Whey protein has a Protein efficiency ratio (PER) of 3.2 and rennet casein having PER of 2.6 have long been used in food product development. Patel et al., 2016 attempted to develop a extruded snack base using tapioca starch, sorghum flour and casein. Hence, maize lacking in essential amino acids like lysine and tryptophan, can be incorporated with milk proteins, which are rich in lysine to produce health foods [8].

Day and Swanson [9] revealed the scientific literatures on functionality of protein-fortified an extrudates and concluded that protein fortification may improve nutritional quality, digestibility, textural, sensory and physical properties. However, extend of beneficial effects depend on type, amount of protein, composition and moisture in feed, temperature and pressure. Hence, incorporation of milk protein to maize extrudates has a great potential to improve the nutritional quality. Therefore, the present study was undertaken for incorporation of rennet casein and WPC-70 to maize flour and investigate the effect of this blending on the quality attributes of extrudates. Further, it also focuses on evaluation of physico-chemical and sensory property changes in optimized product for their stability during storage.

2. MATERIALS AND METHODS

2.1 Sample Preparation

Maize grains and Milk protein sources (Rennet Casein and WPC-70) were procured from Karnal Farmer and Modern Dairies Ltd., Karnal, Haryana, India respectively. According to the preliminary studies based on the sensory scores, level of milk proteins in formulation was adjusted by using requisite quantity of RC/WPC-70. Maize flour and milk proteins viz., WPC-70/RC were dry-blended by passing through sieve (2mm) and calculated water was sprayed over it to adjust 12, 14 and 16% moisture in pre-mix. The mixture was again passed through 2 mm size sieve and blended for 15 min to obtain a uniform mixture. The pre-mix was packaged in 1 kg LDPE bag and stored overnight for equilibration of moisture.
### Table 1. Formulation for the preparation of composite extrudates

<table>
<thead>
<tr>
<th>Treatments</th>
<th>Protein source</th>
<th>Level of addition (%)</th>
<th>Feed moisture content (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>C0812</td>
<td>Rennet casein</td>
<td>8</td>
<td>12</td>
</tr>
<tr>
<td>C0612</td>
<td>Rennet casein</td>
<td>6</td>
<td>12</td>
</tr>
<tr>
<td>W0814</td>
<td>WPC-70</td>
<td>8</td>
<td>14</td>
</tr>
<tr>
<td>W0412</td>
<td>WPC-70</td>
<td>4</td>
<td>12</td>
</tr>
<tr>
<td>C0814</td>
<td>Rennet casein</td>
<td>8</td>
<td>14</td>
</tr>
</tbody>
</table>

#### 2.2 Extrusion Cooking

A twin screw extruder (Basic Technology Pvt. Ltd., Kolkata, West Bengal, India) was used for the preparation of extrudates. It consists of a feeder and extrusion cylinder having two heating zones. A circular die (4mm diameter) at the exit of the barrel was used for extrusion. The conditioned mixture was fed to feed hopper equipped with screw augers to load materials into the barrel at uniform rate. The extruder screw speed was set to 340 rpm throughout the experiment. The temperature of inlet and outlet cooking section was set to 40°C and 100°C, respectively. The plasticized mass was passed through 4 mm die and extruded samples were dried in a tray drier for 30 min at 50°C or till the final moisture reached in the range of 3-5% moisture. The dried samples were collected and stored in the appropriate laminated bags for further analysis.

#### 2.3 Storage Studies

Optimized product prepared by extrusion was packed in metallized LDPE pouches and they were kept in incubator at 25±1°C and 37±1°C for 30 days. The samples were drawn at an interval of 1 week for analysis of physico-chemical characteristics and sensory attributes.

#### 2.4 Quality Assessment

##### 2.4.1 Pasting properties

Pasting properties of extruded products were measured on Rapid Visco Analyser (RVA-4, Newport scientific Pvt. Ltd., Warriewood, Australia) with thermocline software (version 3.0) as per the method followed by Ortiz et al., 2010 with minor modifications. Three gram of extruded sample (14% moisture basis) was transferred into a sample canister and 25±0.1 mL distilled water was added. The slurry was held at 25°C for 2 min, then heated to 95°C in 5 min, held at 95°C for 3 min, and finally cooled to 25°C in 5min. RVA plot of viscosity (cP) vs. Time (s) was used to determine viscosity.

##### 2.4.2 Bulk density

Bulk density was measured by modified method of Hwang and Hayakawa [10]. Glass beads were poured into 250 mL glass cylinder up to 1 cm height. The content was tapped on the bench top for 10 times. Weighed quantity of extrudates was placed into a cylinder with simultaneous addition of glass beads up to a 250 mL marking. The whole content was tapped again as described above until the cylinder was filled to mark. The content was weighed and bulk density was calculated as below.

\[
\rho_{ex} = \frac{w_{ex}}{w_{msd}} \times \rho_{ms}
\]

\[
\rho_{ms} = \frac{w_{ms}}{V}
\]

Where,

- \( \rho_{ex} \) = bulk density of extrudates (g/cm\(^3\))
- \( \rho_{ms} \) = bulk density of glass beads (g/cm\(^3\))
- \( w_{ex} \) = Weight of extrudate (g)
- \( w_{msd} \) = Weight of glass beads displaced (g)
- \( w_{ms} \) = Weight of glass beads (g)
- \( V \) = Volume of glass cylinder (cm\(^3\))

##### 2.4.3 Expansion ratio

The ratio of diameter of extrudate and the diameter of die used for preparing extrudate was used to express the radial expansion of extrudate [11]. The diameter of extrudate was determined as the mean of 10 random measurements made with vernier calipers. The extrudate expansion ratio was calculated as follows:

\[
\text{Expansion ratio} = \frac{\text{Extrudate Diameter}}{\text{Die diameter}}
\]

##### 2.4.4 Water solubility Index (WSI) and water absorption index (WAI)

WAI and WSI were determined according to Anderson et al., [12] method. Milled extrudates (2.5 g) and 30 mL of water was vigorously mixed
in a 50mL centrifuge tube, incubated in a 37°C water bath for 30 min, and then centrifuged at 3500 rpm for 15 min. The supernatant was collected in a pre-weighed aluminum dishes and the residue with respect to the dry weight of extrudate powder used in the test was taken as water solubility index (WSI). The weight ratio of centrifuged precipitate to the dry weight of extrudate powder used in the test was taken as the water absorption index (WAI).

\[ WAI = \frac{WP}{Wd}; \quad WSI = \frac{Wds \times 100}{Wd} \]

Where,

- \( WP \) = Weight of precipitate
- \( Wd \) = Weight of dry solids
- \( Wds \) = Weight of dissolved solids in supernatant

2.4.5 Texture profile analysis

Texture profile of the extrudate was determined with the TA-XT2i (Stable Micro systems, UK). Texture analyzer was fitted with a 25 kg load-cell. Test conditions used for determining hardness and crispiness were pre-test speed 2.0 mm/sec, test speed 1.0 mm/sec, post-test speed 2.0 mm/sec. The probe used was HDP/BSW (Warner-Bratzler Blade) to determine hardness which is the peak force during compression of the product and crispiness which is the total number of positive peaks. A force-distance curve was recorded and analyzed using the Texture Exponent 32 software program. Ten replications were conducted for samples from each treatment.

2.4.6 Colour analysis

Hunterlab Colorflex Colormeter (Hunter Associated Laboratory Inc., USA) was used to measure the colour of the extrudate. Finely ground sample was taken for measurement in the glass sample holder up to 2 cm height and it was tapped for ten times on bench-top. Before the test, the instrument was calibrated with standard black and white tiles as specified by the manufacturer. The light source was dual-beam xenon flash lamp. 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 international (CIE) colour system.

2.4.7 Resistant Starch Content

Resistant starch content in the product was determined using ‘Resistant starch assay kit’ supplied by Megazyme. It is based on AOAC Official Method 2002.02.

2.4.8 Thiobarbituric acid (TBA) value

TBA value is used to measure the extent of oxidation of fat in the extrudates during storage. The extraction method of Strange et al., [13] was followed with slight modification. 2 gram sample of milled extrudate was taken and 50 mL of 20% of TCA was added to it. Add 50 mL of distilled water and left undisturbed for 10 minutes. Then the contents were filtered through Whatman No.1 filter paper. 5 mL filtrate was pipetted out from it in the test tube and added with 5mL of 0.01M 2-Thiobarbituric acid. The tubes were incubated in a boiling water bath for 30 min at 100°C. Color was developed and the contents were cooled to room temperature. Absorbance was determined at 532 nm and blank determinations were made using distilled water in place of sample. TBA was expressed as absorbance at 532 nm.

2.4.9 Free fatty acid value

The extent of lipolysis was measured by using FFA Value by the modified method of Deeth and Gerald [14]. 3 g of milled extruded sample was taken in a glass stoppered 60 mL test tube and 5mL of distilled water was added to it. 10 mL of extraction mixture containing (40 i-propanol: 10 petroleum ether: 1 4N H₂SO₄) was added and mixed thoroughly. 6 mL of petroleum ether and 4mL of distilled water was added to the content and left it undisturbed for 30minutes. After 30 minutes, 5mL upper layer was withdrawn and transferred to 50 mL conical flask. 2 drops of 1% methanolic α-naphtholphthalein indicator was added to it and titrated against 0.002N methanolic potassium hydroxide solution. Blank solution was also prepared without sample.

\[ FFA (\mu\text{eq}/\text{mL}) = \frac{\text{T} \times \text{N} \times 1000}{\text{P} \times \text{V}} \]

- \( T \) = net titration volume.
- \( N \) = normality of methanolic KOH.
- \( P \) = proportion of upperlayer titrated.
- \( V \) = weight of sample.

2.5 Sensory Evaluation

Experimental samples of extrudates kept for storage study was evaluated for sensory
parameters like flavour, colour and appearance, texture and overall acceptability using 9-point hedonic scale by a panel of ten semi-skilled panelists selected from Dairy Technology Division, NDRI-Karnal.

2.6 Statistical Analysis

The influence of independent formulation and processing variables on quality characteristics of extrudates was analyzed using one-way analysis of variance. Significant differences were defined at p<0.05. All the analyses were performed using IBM SPSS Statistics 20.

3. RESULTS AND DISCUSSION

3.1 Pasting Properties

Pasting properties of extrudate is an indicator of extent of cooking during extrusion and RVA is effective equipment for exploring the degree of alteration of starch in food product. The pasting properties of milk protein-maize based extrudates are shown in Table 2. At 12% feed moisture, the cold peak (initial viscosity) significantly (p<0.05) increased in all samples. The control sample had a significantly (p<0.05) higher raw peak viscosity (383 cP) than the milk-protein maize composite based extrudates. Addition of WPC – 70 showed significantly (p<0.05) reduced raw peak viscosity than the RC. Lim et al., [15] also found that the protein content had a negative correlation with the raw peak viscosity of the starch paste. Decrease of peak viscosity is correlated to effect of extrusion on higher degradation and gelatinisation of starch [16]. The hold viscosity reflects the minimum viscosity, which is attained due to continued stirring and heating more starch during heating cycle, granules are disrupted which results in the lowest viscosity [17]. The control maize extrudate had higher hold viscosity of 260 cP and decreased by the addition of milk protein to maize composite. Breakdown viscosity reflects the stability of the paste during cooking [18]. Lowest values of breakdown viscosity was noticed for the extrudates containing 8% WPC-70 and conditioned to 14% moisture content.

The retrogradation behavior of starch gels are reflected by the final and setback viscosities. Addition of milk proteins decreased final viscosity and increased setback viscosity which indicates the rapid tendency of retrogradation and more syneresis is likely to take place except the sample incorporated with WPC-70 at 8% with 14% moisture.

3.2 Physical Characteristics

Physical parameters such as bulk density, expansion index, WAI, WSI and textural properties such as hardness, crispiness of extrudates prepared from maize – milk protein blends are presented in Table 3.

3.2.1 Bulk density

Bulk density of a extrudates indicate the volume occupied by it per unit mass and in determining the post processing requirements such as packaging material and storage space. The bulk density of the extrudates varied in the range of 0.06 to 0.09 g/ cm³. The control maize extrudate had a higher bulk density of 0.08 g/ cm³ and the addition of milk protein significantly (p<0.05) decreased the bulk density to 0.06 g/ cm³ due to lower FMC (< 20%) and increased casein content [19]. Our results are similar to the reported behavior of rennet casein, tapioca starch on sorghum flour extrudates bulk density [20].

3.2.2 Expansion ratio

The expansion index of the control maize extrudate was 2.62 which reduced significantly by the addition of milk proteins. Addition of WPC-70 to maize flour reduced significantly (p<0.05) the expansion ratio to 2.24 in extrudates. RC addition also decreased the expansion in extrudates, however it was not statistically significant from control. Addition of milk proteins in formulation might have promoted the interaction between maize flour constituents particularly proteins and lipids with milk proteins resulting decreased starch swelling and thus reduced expansion (Korkerd et al., 2016). Variation in expansion ratios of RC and WPC-70 addition of extrudates could be related to the difference in their nature and composition. Moraru and Kokini [21] reported that higher concentration of milk protein affects the water distribution in starch matrix and increases the number of sites during extrusion for cross-linking and thus reduces the starch matrix extensibility resulting in less expanded products.
Table 2. Pasting properties of milk protein-maize composite based extrudates

<table>
<thead>
<tr>
<th>Samples</th>
<th>Cold Peak (initial viscosity) (cP)</th>
<th>Raw Peak (cP)</th>
<th>Hold (Hot peak viscosity) (cP)</th>
<th>Breakdown (cP)</th>
<th>Final Viscosity (cP)</th>
<th>Setback (cP)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>265±2.65&lt;sup&gt;a&lt;/sup&gt;</td>
<td>383±1.53&lt;sup&gt;a&lt;/sup&gt;</td>
<td>260±2.31&lt;sup&gt;a&lt;/sup&gt;</td>
<td>123±2.52&lt;sup&gt;ab&lt;/sup&gt;</td>
<td>323±1.00&lt;sup&gt;a&lt;/sup&gt;</td>
<td>63±1.53&lt;sup&gt;a&lt;/sup&gt;</td>
</tr>
<tr>
<td>C0812</td>
<td>312±1.15&lt;sup&gt;a&lt;/sup&gt;</td>
<td>384±2.08&lt;sup&gt;a&lt;/sup&gt;</td>
<td>249±1.15&lt;sup&gt;a&lt;/sup&gt;</td>
<td>135±1.73&lt;sup&gt;a&lt;/sup&gt;</td>
<td>322±1.53&lt;sup&gt;a&lt;/sup&gt;</td>
<td>73±2.65&lt;sup&gt;a&lt;/sup&gt;</td>
</tr>
<tr>
<td>C0612</td>
<td>289±1.53&lt;sup&gt;b&lt;/sup&gt;</td>
<td>360±3.06&lt;sup&gt;b&lt;/sup&gt;</td>
<td>249±1.53&lt;sup&gt;b&lt;/sup&gt;</td>
<td>111±1.53&lt;sup&gt;b&lt;/sup&gt;</td>
<td>314±2.52&lt;sup&gt;a&lt;/sup&gt;</td>
<td>65±3.06&lt;sup&gt;a&lt;/sup&gt;</td>
</tr>
<tr>
<td>W0814</td>
<td>251±2.08&lt;sup&gt;cd&lt;/sup&gt;</td>
<td>310±1.15&lt;sup&gt;c&lt;/sup&gt;</td>
<td>213±2.08&lt;sup&gt;b&lt;/sup&gt;</td>
<td>97±1.53&lt;sup&gt;c&lt;/sup&gt;</td>
<td>264±2.65&lt;sup&gt;b&lt;/sup&gt;</td>
<td>51±2.08&lt;sup&gt;a&lt;/sup&gt;</td>
</tr>
<tr>
<td>W0412</td>
<td>319±1.53&lt;sup&gt;a&lt;/sup&gt;</td>
<td>360±2.52&lt;sup&gt;b&lt;/sup&gt;</td>
<td>242±1.73&lt;sup&gt;a&lt;/sup&gt;</td>
<td>118±2.08&lt;sup&gt;ab&lt;/sup&gt;</td>
<td>306±1.53&lt;sup&gt;a&lt;/sup&gt;</td>
<td>64±2.08&lt;sup&gt;a&lt;/sup&gt;</td>
</tr>
<tr>
<td>C0814</td>
<td>240±2.52&lt;sup&gt;d&lt;/sup&gt;</td>
<td>365±2.08&lt;sup&gt;b&lt;/sup&gt;</td>
<td>240±1.15&lt;sup&gt;b&lt;/sup&gt;</td>
<td>125±2.08&lt;sup&gt;ab&lt;/sup&gt;</td>
<td>322±0.58&lt;sup&gt;a&lt;/sup&gt;</td>
<td>82±1.53&lt;sup&gt;a&lt;/sup&gt;</td>
</tr>
</tbody>
</table>

<sup>a,b,c</sup> Means with different letter within column are significantly different (p<0.05); Mean ± S.E (n=3)
3.2.3 Water Absorption Index and Water Solubility Index

Water absorption index measures the volume occupied by granules or food polymer after swelling in excess water. Water solubility indicates the amount of food molecules solubilized in water, which is related to changes in starch and protein during extrusion [22].

The control sample of maize extrudate had WSI of 32.81% and WAI of 5.81 g g\(^{-1}\) and addition of milk proteins reduced both significantly (< 0.05). WSI decreased with increased level of whey protein and moisture in feed material [23]. WPC-70 added extrudates exhibited higher WAI values and it was not significantly varied with control. Matthey and Hanna [24] suggested that WPC decreased starch degradation with higher WAI.

3.2.4 Hardness

The hardness of extruded product is a sensory perception of the humans and is associated with the expansion and cell structure of the product. The hardness is the average force required for a probe to penetrate the extrudate. The range of hardness is the average force required for a probe to penetrate the extrudate. The range of hardness of extrudates was 11.66 N to 19.81 N. The control extrudates showed significantly (p < 0.05) lower hardness, if compared with protein enriched extrudates. Low in protein content and higher starch content in maize allowed higher enriched extrudates.

Addition of WPC-70 to the maize flour at 8% level of protein with 14% feed moisture showed significantly (p < 0.05) lower crispiness values when compared with the sample prepared with 4% WPC-70 and 12% feed moisture. It has been reported that the increase in moisture content of the feed mix might cause reduction of expansion due to reduced formation of air bubbles and number of internal cells in the extrudates [27]. They attributed it to the decrease of crispness in extrudates. Among the RC incorporated milk protein – maize composite based extrudates the sample containing 8% added RC protein with 14% moisture showed significantly (p < 0.05) higher hardness than those containing lower RC and lower feed moisture. Similar trend was recently reported by Seth et al., [25] who observed a significant increase in hardness with increase in feed moisture content increased hardness significantly, which attributed to reduction in expansion of the extruded product and lower rate of starch degradation at higher moisture values. Protein rich extrudates produce less expandable products and more rigid network resulting in higher resistance to shear and lower expansion [26].

3.2.5 Crispiness

Crispness test was performed to give an idea of the resistance, which the extrudate may offer, on first bite to the consumer. In this test number of peaks were observed that the maximum number of peaks give the better crisp product. The crispness of extrudates ranged between 25.66 to 53.33. Addition of WPC-70 to the maize flour at 8% level of protein with 14% feed moisture showed significantly (p < 0.05) lower crispiness values than the sample prepared with 4% WPC-70 and 12% feed moisture. It has been reported that the increase in moisture content of the feed mix might cause reduction of expansion due to reduced formation of air bubbles and number of internal cells in the extrudates [27]. They attributed it to the decrease of crispness in extrudates. Among the RC incorporated milk protein – maize composite based extrudates the sample containing 8% added RC protein with 14% moisture showed significantly (p < 0.05) lower crispiness than other formulations. Singh et al., [28] also reported that crispness increased with increase in temperature and decreased by enhancing the feed moisture.

Table 3. Physical and textural properties of milk protein- maize composite based extrudates

<table>
<thead>
<tr>
<th>Samples</th>
<th>Bulk density (g/cm(^3))</th>
<th>Expansion ratio</th>
<th>WAI (g g(^{-1}))</th>
<th>WSI (%)</th>
<th>Hardness (N)</th>
<th>Crispness (	extsuperscript{a})</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>0.08±0.004\textsuperscript{b}</td>
<td>2.62±0.07\textsuperscript{a}</td>
<td>5.81±0.18\textsuperscript{a}</td>
<td>32.81±0.10\textsuperscript{a}</td>
<td>11.66±0.52\textsuperscript{a}</td>
<td>25.66±1.15\textsuperscript{a}</td>
</tr>
<tr>
<td>C0812</td>
<td>0.06±0.003\textsuperscript{b}</td>
<td>2.50±0.1\textsuperscript{a}</td>
<td>5.51±0.14\textsuperscript{a}</td>
<td>31.67±0.10\textsuperscript{b}</td>
<td>14.55±0.29\textsuperscript{b}</td>
<td>49±0.06\textsuperscript{b}</td>
</tr>
<tr>
<td>C0612</td>
<td>0.07±0.004\textsuperscript{b}</td>
<td>2.45±0.06\textsuperscript{b}</td>
<td>5.24±0.20\textsuperscript{b}</td>
<td>31.76±0.19\textsuperscript{b}</td>
<td>13.10±0.70\textsuperscript{b}</td>
<td>40.66±0.57\textsuperscript{b}</td>
</tr>
<tr>
<td>W0814</td>
<td>0.07±0.007\textsuperscript{a}</td>
<td>2.25±0.06\textsuperscript{a}</td>
<td>5.71±0.07\textsuperscript{a}</td>
<td>31.47±0.06\textsuperscript{a}</td>
<td>19.81±0.69\textsuperscript{a}</td>
<td>33±2.64\textsuperscript{a}</td>
</tr>
<tr>
<td>W0412</td>
<td>0.09±0.004\textsuperscript{b}</td>
<td>2.24±0.05\textsuperscript{b}</td>
<td>5.70±0.08\textsuperscript{b}</td>
<td>31.62±0.26\textsuperscript{b}</td>
<td>16.58±0.49\textsuperscript{b}</td>
<td>53.33±1.52\textsuperscript{b}</td>
</tr>
<tr>
<td>C0814</td>
<td>0.07±0.004\textsuperscript{a}</td>
<td>2.44±0.05\textsuperscript{a}</td>
<td>5.71±0.10\textsuperscript{a}</td>
<td>31.37±0.16\textsuperscript{a}</td>
<td>16.62±0.10\textsuperscript{a}</td>
<td>40.33±1.52\textsuperscript{a}</td>
</tr>
</tbody>
</table>

\textsuperscript{a}Means with the different letter within column are significantly different (p<0.05)

\textsuperscript{b}Mean ± S.E (from 4 determinations).

\textsuperscript{c}No of positive peaks in force deformation curve of texture analysis.
3.2.6 Color

Since color is an important quality factor which is directly related to the acceptability of food products, Color like other food products is an important physical property for extrudates products as well [29].

$L^*$ value varied between 78.06 and 81.37 within the combination of variables studied. The lightness of extrudate ($L^*$) and the redness ($a^*$) were mostly dependent on barrel temperature, feed moisture, and feed rate. The yellowness parameter ($b^*$) was only slightly dependent on extrusion variable. From the data presented in Table 4, it is evident that $L^*$ and $b^*$ value of samples containing RC exhibited significantly ($p<0.05$) higher when compared with WPC-70 added samples. Extrusion parameter, feed moisture of 12% has shown positive significant ($p<0.05$) effect on colour value of milk protein – maize composite extrudates irrespective of level of protein. Increasing protein concentrations in the formulation increased the yellowness (higher $b^*$ values) of extrudates [23]. Alteration in colour values during the present investigation was influenced by the type and level of proteins and feed moisture. All WPC-70 containing extrudates were, darker, lower yellow and marginally lower $a^*$ (redness) values. Since, WPC-70 had lactose content higher than that of RC, which might have readily participated in complexation reaction of maillard browning with available nitrogenous compounds.

3.2.7 Resistant starch

The resistant starch content of extrudates are presented in Table 5. The control extrude has higher resistant starch content than protein incorporated samples. The resistant starch content of control sample was 0.17 g/kg of sample however it varied in the range of 0.03-0.11 g/kg in milk protein added extrudates. The added milk proteins must have denatured during extrusion and participated in complex formation with maize flour constituents particularly with starch. Extrusion usually lowers RS content of extrudates [30], although the process can increase RS content if the specific extrusion conditions lead to the formation of amylose- lipid complexes [31]. Similar trend of results were observed by Mahasukhonthachat et al., [32], Wolf [33] and they also reported a decrease in RS content (or increased digestibility) after extrusion processing.

3.2.7.1 Changes during storage

Increase in resistant starch content in extrudates during storage may be attributed to the formation of amylose-lipid complex, starch-protein interaction and lower moisture content in extrudates, which enhances the period of starch digestibility during enzymatic hydrolysis. Retrogradation of starch molecules is affected by number of factors including moisture content, formulation variables (presence of other carbohydrates, proteins and lipids), additives, storage temperature and salts [34]. The changes in the resistant starch content of extrudates during storage at 25°C and 37°C are illustrated in Table 6. The resistant starch content of extrudates stored at 25°C increased from initial value of 45 to 128 mg/100 g of sample, while for extrudates stored at 37°C, it increased from 45 to 132 mg/100 g of sample at the end of storage period. It is evident that there was a significant increase in resistant starch content after 2 week of storage. This result suggests that RS content in the extrudates may be due to retrogradation of starch during storage after extrusion [30].

3.3 TBA and FFA value

The extent of lipid oxidation in the extrudates during storage was measured in terms of TBA value. The TBA value is an index of lipid oxidation measuring malonaldehyde (MDA) content which is formed through hydroperoxides and it is the initial reaction product of lipid oxidation [35]. The changes in TBA value of extrudates during storage at 25°C and 37°C are illustrated in Table 6. The TBA value of extrudates increased from initial value of 0.154 to 0.173 and 0.174 for the sample stored at 25°C and 37°C, respectively at the end of storage period. There was no significant difference ($p<0.05$) in TBA value for the samples stored at 25°C and 37°C. However, the increase in TBA value at both storage temperatures was significant. The increase in TBA value of extrudates could be due to increase in oxidation of lipids particularly maize lipids which are rich in PUFA. Both heating and presence of oxygen might have promoted the oxidation of lipids resulting in formation of oxidative products.

The extent of lipolysis was estimated by using FFA Value and presented in Table 6. Free fatty acid (FFA) value of 16.43 µeq/ml in fresh extrudates increased slightly to 17.53 and 17.04 µeq/ml after 4 weeks storage. Statistically increase in FFA value was significant; however
significant variation was only noticed between the extrudates stored at 25 and 37°C at the later stages of storage. FFA content indicates the degree of hydrolytic rancidity and presence of lipases and fats in cereal grains often lead to increase in FFA content. Since extrusion processing inactivates enzymes therefore there was not much rise in FFA content in extrudates.

3.4 Changes in Sensory Attributes of Extrudates During Storage

Sensory evaluation plays a vital role in determining the shelf-life of the product. The samples stored at 25°C and 37°C were evaluated by a 9 semi-trained sensory panel members after one week storage interval and scores are presented in Table 7. Although sensory scores decreased for all the attributes, however the sensory scores for color and appearance, texture and overall acceptability did not have any significant variation (p>0.05) during the entire storage period. The flavour score got decreased significantly (p<0.05) from the initial sensory score 7.8 to 6.2 in the 4th week of storage period. Change in flavour attribute could be due to lipid oxidation and lipase mediated hydrolysis. Panelists also reported that during the later part of storage there was slight bitterness in the extrudates. Raw corn flavour is considered as desirable sensory attributes and the intensity of it, decreased at the later stages.

Table 4. Hunter color parameters of milk protein - maize composite based extrudates

<table>
<thead>
<tr>
<th>Samples</th>
<th>L*</th>
<th>a*</th>
<th>b*</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>80.59±0.02d</td>
<td>1.26±0.03c</td>
<td>29.3±0.01c</td>
</tr>
<tr>
<td>C0812</td>
<td>81.37±0.03a</td>
<td>1.42±0.06a</td>
<td>29.56±0.28a</td>
</tr>
<tr>
<td>C0612</td>
<td>81.33±0.05a</td>
<td>1.45±0.04a</td>
<td>29.20±0.02a</td>
</tr>
<tr>
<td>W0814</td>
<td>78.06±0.03a</td>
<td>0.98±0.02a</td>
<td>25.70±0.02a</td>
</tr>
<tr>
<td>W0412</td>
<td>78.45±0.02b</td>
<td>1.46±0.01c</td>
<td>27.42±0.02c</td>
</tr>
<tr>
<td>C0814</td>
<td>79.37±0.02c</td>
<td>1.18±0.01b</td>
<td>26.93±0.02b</td>
</tr>
</tbody>
</table>

*a,b,c,d* Means with the different letter within column are significantly different (p<0.05) Mean ± S.E (from 4 determinations)

Table 5. Resistant starch content of milk protein- maize based extrudates

<table>
<thead>
<tr>
<th>Samples</th>
<th>Resistant starch content (g/ 100 g sample)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>0.17±0.02b</td>
</tr>
<tr>
<td>C0812</td>
<td>0.06±0.01b</td>
</tr>
<tr>
<td>C0612</td>
<td>0.08±0.01b</td>
</tr>
<tr>
<td>W0814</td>
<td>0.03±0.01a</td>
</tr>
<tr>
<td>W0412</td>
<td>0.03±0.01a</td>
</tr>
<tr>
<td>C0814</td>
<td>0.11±0.02c</td>
</tr>
</tbody>
</table>

*a,b,c,d* Means with the different letter within column are significantly different (p<0.05) Mean ± S.E (from 3 determinations)

Table 6. Changes in resistant starch, TBA and FFA Value of extrudates during storage

<table>
<thead>
<tr>
<th>Storage period (weeks)</th>
<th>Resistant Starch Content (g/100 g Of Sample)</th>
<th>TBA Value</th>
<th>FFA Value (µeq/ml)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>25°C</td>
<td>37°C</td>
<td>25°C</td>
</tr>
<tr>
<td>0</td>
<td>0.045±0.002a</td>
<td>0.045±0.002a</td>
<td>0.154±0.002a</td>
</tr>
<tr>
<td>1</td>
<td>0.045±0.003a</td>
<td>0.045±0.002a</td>
<td>0.162±0.004a</td>
</tr>
<tr>
<td>2</td>
<td>0.051±0.004a</td>
<td>0.045±0.002a</td>
<td>0.167±0.004a</td>
</tr>
<tr>
<td>3</td>
<td>0.126±0.001b</td>
<td>0.128±0.001b</td>
<td>0.169±0.004a</td>
</tr>
<tr>
<td>4</td>
<td>0.128±0.001b</td>
<td>0.132±0.001b</td>
<td>0.173±0.005a</td>
</tr>
</tbody>
</table>

*a,b,c,d* Means with the different letter within column are significantly different (p<0.05) Mean ± S.E (n=3)
Table 7. Changes in sensory attributes during storage

<table>
<thead>
<tr>
<th>Storage period (Weeks)</th>
<th>Color and appearance</th>
<th>Flavour</th>
<th>Texture</th>
<th>Overall acceptability</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>7.6±0.20&lt;sup&gt;a&lt;/sup&gt;</td>
<td>7.5±0.17&lt;sup&gt;a&lt;/sup&gt;</td>
<td>7.8±0.40&lt;sup&gt;a&lt;/sup&gt;</td>
<td>7.60±0.37&lt;sup&gt;a&lt;/sup&gt;</td>
</tr>
<tr>
<td>2</td>
<td>7.3±0.40&lt;sup&gt;a&lt;/sup&gt;</td>
<td>7.5±0.26&lt;sup&gt;b&lt;/sup&gt;</td>
<td>7.6±0.47&lt;sup&gt;a&lt;/sup&gt;</td>
<td>7.50±0.34&lt;sup&gt;a&lt;/sup&gt;</td>
</tr>
<tr>
<td>3</td>
<td>7.2±0.40&lt;sup&gt;a&lt;/sup&gt;</td>
<td>7.2±0.37&lt;sup&gt;a&lt;/sup&gt;</td>
<td>7.5±0.37&lt;sup&gt;a&lt;/sup&gt;</td>
<td>7.40±0.39&lt;sup&gt;a&lt;/sup&gt;</td>
</tr>
<tr>
<td>4</td>
<td>7.1±0.39&lt;sup&gt;a&lt;/sup&gt;</td>
<td>6.2±0.38&lt;sup&gt;a&lt;/sup&gt;</td>
<td>7.0±0.28&lt;sup&gt;a&lt;/sup&gt;</td>
<td>7.30±0.40&lt;sup&gt;a&lt;/sup&gt;</td>
</tr>
</tbody>
</table>

**Mean ±S.E (n=3)**

4. CONCLUSIONS

The study indicated that milk protein sources had significant effect on textural, nutritional and sensory attributes of milk protein-maize composite extrudates. The optimized product prepared from RC substitution had more protein content (17.03±0.49%), lower hardness of 13.91±0.99 N, higher L* Value 81.37±0.03. Storage stability of milk protein-maize based extrudates has resulted in non-significant difference in sensory attributes, TBA and FFA value and significant difference in resistant starch. Storing the samples at 25°C develops resistant starch. Hence, the product had potential market as innovative product for effective utilization of underutilized maize and milk protein for highly nutritious food. It could be an alternate source for “Nutrimix” as well as snack food for under-nourished population in developing countries.

DISCLAIMER

The products used for this research are commonly and predominantly use products in our area of research and country. There is absolutely no conflict of interest between the authors and producers of the products because we do not intend to use these products as an avenue for any litigation but for the advancement of knowledge. Also, the research was not funded by the producing company rather it was funded by personal efforts of the authors.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

REFERENCES

10. Hwang MP, Hayakawa K. Bulk densities of cookies undergoing commercial baking


31. Svihus B, Uhlen AK, Harstad OM. Effect of starch granule structure, associated components and processing on nutritive


© 2020 Ponbhagavathi et al.; This is an Open Access article distributed under the terms of the Creative Commons Attribution License (http://creativecommons.org/licenses/by/4.0), which permits unrestricted use, distribution, and reproduction in any medium, provided the original work is properly cited.