Chapter 3

MATERIALS AND METHODS

Formulations for the fibre rich extruded snack food composed of rice flour (prepared from rice brokens) procured from rice mill Longowal, District Sangrur; pulse powder (a by-product from the pulse mill) from the Local pulse mill of LongowalSangrur; carrot (variety: Pusakesar) cultivated and grown in Sangrur, Punjab, India and the seasoning was procured from the SymegaSavoury International Ernakulum, Kerala, India. Vitamin C and β carotene and other chemicals used in the study were of extrapure/AR (grade) from Merck specialty India Pvt. Ltd.

Experiments were carried out in three phases. In the first phase, optimized product was prepared as per the standard method of Kumar et al., (2010) and further frying as well as application of seasoning was carried out according to a standard method adopted by a leading company at District Sangrur, India and stored for six months. In the second phase, optimized product was extruded at four different temperatures and stored for six months for studying the thermal kinetics, while in the third phase, the optimized product was fried at different temperature time combinations to get the optimum frying conditions.

3.1 Dry carrot pomace powder preparation

The carrots were washed in running tap water number of times to remove extraneous material. Trashes were removed with a plane stainless steel knife followed by trimming. A juicer mixer grinder cum food processor (Make: Maharaja Whiteline, Asiatic Engineers Pvt. Ltd., 600W) was used to extract carrot juice. After the juice extraction, pomace was collected and dried by following the method reported by Kumar et al., (2011, 2012a).
A hot air oven (Osaw Industrial Products Pvt. Ltd., India) was used for drying carrot pomace, which could regulate drying air temperature up to 250°C ±2 °C accuracy. The dryer consisted of a preheating and heating chamber with thermostat based control unit, an electrical fan, and measurement sensors. The samples were spread over the trays and the temperature of the dryer was set to 60°C as reported by Kumar et al., (2012a). The drying procedure was continued till the moisture content of the sample was reduced to about 5 ±1% (wet basis). The grinding was performed using the same food processor with grinder attachment. The material was ground to pass through the sieve of 2 mm size. The pomace powder so obtained was stored in sealed polythene bag for further use.

3.2 Extrudates preparation

The extrudates were prepared under the optimum conditions based on the extrusion studies (Kumar et al., 2010a). Ingredients were mixed and formulations were made as per the optimized conditions as rice to pomace and pulse ratio was 83.5:16.5, pulse and pomace ratio was 1:1 and the moisture content 19.23%, screw speed 310 rpm and temperature 110 °C for the carrot pomace based extrudates. Moisture was adjusted by sprinkling distilled water in dry ingredients. All the ingredients were weighed and then mixed in the same food processor for 10 min. The mixture was then passed through a 2 mm sieve to reduce the lump formation due to addition of moisture. After mixing, samples were stored in bags at room temperature for 24h (Stojceskaet al. 2008). Moisture content of samples was determined by hot air oven method (Ranganna 1995) prior to extrusion experiments.
3.3 Extrusion of samples

Extrusion of samples was performed using a co-rotating twin-screw extruder (Basic Technology Pvt. Ltd., Kolkata, India (fig.3.1 and 3.2) consisting standard screw profile. The length to diameter (L/D) ratio of the extruder barrel was 8:1. The barrel was provided with two electric band heaters and two water cooling jackets. The main drive of extruder was provided with a 7.5 HP motor (400 V, 3 ph, 50 cycles). The twin screw extruder was kept on for duration of 30 minutes to stabilize the set temperatures and samples were then poured in to feed hopper and the feed rate was adjusted to 4 kg/h for easy and non-choking operation. The die diameter was selected at 4 mm as recommended by the manufacturer for such product. The product was collected as the die end and packed in already numbered zipped lock packs and kept in a proper storage until the extrusion experiment was over.

Fig. 3.1: Extruder
3.4 Stabilization of moisture

All the samples were kept at 60°C for 12 h for the stabilization of moisture or to remove extra moisture from the product in an incubator (Temperature precision ± 0.1°C, Orbital Incubator, Macro Scientific Works, New Delhi) as shown in Fig. 3.3.
3.4 Frying

Deep fat frying of the extrudates was carried out at 190 °C for 30 seconds using rice bran oil (IV-101.55 ± 0.05, SV-184.96 ± 0.04, refractive index- 1.469 ± 0.05 and specific gravity-0.9144 ± 0.03). The frying operation was carried out in deep fryer (Sew-Euro Drive India Pvt. Ltd., Vadodhara, India) of 5L capacity (Fig. 3.4).
3.5 Application of seasoning

*Achari* (Pickled) mango-flavoured seasoning was procured from SymegaSavoury Technology (Ernakulam, Kerala, India), which consisted of ingredients like spices and condiments, salt, mango powder, corn starch, acidity regulator, onion powder, asafoetida, permitted anti-caking agents, hydrolyzed vegetable protein, yeast extract and flavour enhancers and salt. Seasoning was added to the extruded snack food by mixing the seasoning with rice bran oil (A P Solvex, Dhuri, India) (85:15 by weight) and then adding 23.95 g of the prepared slurry to 100 g of the extruded snack.

3.6 Packaging and storage

Packaging of the extrudates was carried out in metallized polypropylene at the leading Snacks Company at Sangrur, Punjab (India). The packets of extruded snack food samples were stored in corrugated fibre box at 30°C and relative humidity of 50% in the research lab.
of Department of Food Engineering and Technology, SantLongowal Institute of Engineering and Technology, Longowal, Sangrur, Punjab (India) for six months. The extrudates were analysed for textural parameters, colour values and changes in the structure after every month for six months.

3.7 Evaluation of responses

3.7.1 Scanning electron microscopy

Scanning Electron microscopy (SEM) of the fibre rich snack food was conducted every month up to the 6 months. SEM (Jeol, Japan, Model-JSM 65100-LV) was used to illustrate the effect of storage on the microstructure of all the three samples (unfried, fried and fried with seasoning) stored using metalized polypropylene as packaging material.

Each extrudate was prepared for SEM examination by first drying it at 40 °C in vacuum oven and further the extrudates were cut by razor blade to obtain an intact cross section. Samples were mounted on aluminium stubs using glue adhesive (Fewikwik, Pidilite, India) and sputter coated with a thick gold palladium layer with a sputter coater (Jeol, Japan, Model- JS31100) (Fig. 3.5). Photomicrographs of each extrudate sample up to six months of storage were taken at 700 and 1500 magnifications. The magnifications were varied to get the more accurate and desirable results *(Harris et al 1988).*
Fig. 3.5 Scanning Electron Microscope

Fig. 3.6 Sputter coating of the extrudate samples
3.7.2 Texture analysis

Textural properties of the extrudates were determined by crushing method using a TA–XT2 texture analyzer (Stable Micro Systems Ltd., Goldming, UK) equipped with a 500 kg load cell (Fig. 3.6). An extrudate about 40 mm long, was compressed with a probe (SMS – P/75 – 75mm diameter) at a crosshead speed 5 mm/s to 3 mm of 90% of diameter of the extrudate. The highest first peak value was recorded as hardness, as this value indicated the first rupture of snack at one point and this value of force was taken as a measurement for hardness and the total number of peaks was taken as a measurement of crispiness (Stojceska et al. 2008).

Fig 3.7 Texture analyser
3.7.3 Colour analysis

The colour of extrudates in terms of L, a, b values was determined using colorimeter (Model NP-3000, Nippon, Japan). Colour space is based on the concept that colours can be considered as combinations of colour plotted in a cubical form. The maximum and minimum for L-values are 100 and zero, indicates perfect reflecting diffuser and black respectively. The redness/greenness and yellowness/blueness are denoted by a-values and b-values, respectively (Hunter Lab, 2008). Before measuring, the colorimeter was standardized with black and white calibration tiles provided with the instrument. Extrudate samples were cut longitudinally and the colour was measured in three places of each sample. Average values were recorded for the study (Stojceska et al. 2008). Colour analysis of the extrudates was conducted every month up to 6 months of storage.

Fig 3.8 Color analyser

The CIE Lab colour space is based on the concept that colours can be considered as combinations of red and yellow, red and blue, green and yellow, and green and blue (Fig. 3.8 and Fig. 3.9).
To determine the exact combination of colours of a product, coordinates of a three dimensional colour space are assigned. The L*, a* and b* values were recorded in the software provided in an attached PC.

3.8 Effect of extrusion temperature on the microstructure, textural and functional attributes of carrot pomace based extrudates

The extrudates were prepared under the optimum conditions based on the extrusion studies (Kumar et al. 2010a). Ingredients were mixed, and formulations were made as per the optimized conditions as reported by Kumar et al. (2010a) as discussed earlier in 3.2. To study the effect of temperature on microstructure, textural and functional attributes, the temperature was varied between 110°C and 140°C for the preparation of carrot pomace-based extrudates.
3.8.1 Scanning electron microscopy

Scanning electron microscopy (SEM) of the fiber-rich extruded snack food was conducted for all designed extrusion temperatures and of the optimized product at the selected magnification level every month up to the 6 months using method as discussed earlier in section 3.7.

3.8.2 Texture analysis

Textural properties of the extrudates were determined for all the designed extrusion temperatures at one month interval up to the 6 months by the method, discussed earlier in section 3.7.

3.8.3 Colour analysis

The colour of extrudates in terms of L, a and b-values was determined for all designed extrusion temperatures at one month interval up to the 6 months by the method, discussed earlier in section 3.7.3.

3.8.4 Estimation of β-carotene.

β-Carotene was estimated by spectrophotometric method based on the chromatographic separation using an adsorbent having varying affinities as per the standard method (Sharma et al., 2009; Srivastava and Kumar 2003).

3.8.5 Estimation of Vitamin C by High-Performance Liquid Chromatography Method

3.8.5.1 Standard Solution.

Vitamin C 50 g was weighed into 50 ml volumetric flask, and dissolved and diluted to make up volume with water. One millilitre from 50-ml solution was pipetted into 100 ml volumetric flask, and the volume was made up with water. The vitamin C was then identified and quantified as per the retention time and the peak area/height of the standard and sample as per the standard method (Fontannaz et al., 2006).
3.8.5.2 Sample Preparation and High-Performance Liquid Chromatography Separation.

1 gram sample was weighed into 100 ml volumetric flask. 75 millilitre water was added and sonicated for 5 min to dissolve the sample. Adjustment of volume was done with water followed by filtration through a 0.45 mm syringe. High-performance liquid chromatography with ultraviolet detector (Model 10 AVP, Make Shimadzu Corporation, Tokyo, Japan) along with c-18 column (25 cm length 4.6 mm diameter) was used, and the conditions maintained are described in Table 1.

**TABLE 1. HPLC CONDITIONS FOR VITAMIN C**

<table>
<thead>
<tr>
<th>Parameters</th>
<th>HPLC Conditions</th>
</tr>
</thead>
<tbody>
<tr>
<td>Mobile phases</td>
<td>(a) mobile phase A (buffer solution) pH of water was adjusted to 2.1 with H₂SO₄</td>
</tr>
<tr>
<td></td>
<td>(b) mobile phase B acetonitrile</td>
</tr>
<tr>
<td>Flow rate</td>
<td>1.0 mL/min</td>
</tr>
<tr>
<td>Detection</td>
<td>220 nm</td>
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</tbody>
</table>

HPLC, high performance liquid chromatography

3.9 Effect of frying time and temperature on the different properties of carrot pomace, pulse powder and rice flour based extrudates

The extrudates were prepared under the optimum conditions based on the extrusion studies (Kumar *et al.* 2010a) as discussed earlier in section 3.2. The frying temperature was varied between 170 to 200 °C to study the effect of frying time and temperature on microstructure, textural and functional attributes.
3.9.1 Frying of extrudates

Deep fat frying of the extrudates (100g) was carried out at 170, 180, 190 and 200°C for 5, 10 and 15 seconds using rice bran oil (2 litres). The frying operation was carried out in electronic deep fryer (Euro-Pro Operating LLC, Canada) of 4L capacity.

3.9.2 Determination of functional properties

3.9.2.1 Colour analysis

The colour of extrudates in terms of L, a and b values was determined using color spectrophotometer (Model color i5, Gretag Macbeth Regensdorf Switzerland). Colour space is based on the concept that colours can be considered as combinations of colour plotted in a cubical form. The maximum and minimum for L values are 100 and zero, indicates perfect reflecting diffuser and black respectively. The redness/greenness and yellowness/blueness are denoted by ‘a’ and ‘b’ values, respectively. Before measuring, the colorimeter was standardized with black and white calibration tiles provided with the instrument. Extrudate samples were cut longitudinally and the colour was measured at three different places of each sample. Average values were recorded for the study.

3.9.2.2 Oil absorption

Oil absorption capacity of ground carrot pomace based extrudates was determined gravimetrically by Soxhlet extraction with petroleum ether (AOAC, 1995).

3.9.2.3 Sensory evaluation

Sensory analysis was conducted in terms of overall acceptability for all the samples. A panel of semi-trained panellists of 5 members were asked to assess the extrudates and mark them on a hedonic rating test (1 - dislike extremely, 5 - neither like nor dislike, and 9 - like extremely) in accordance with their opinion for taste, texture, colour and overall acceptability.
(Ranganna, 2003). About 25 g servings of extrudates were kept in plates. All tests were performed in partitioned booths under uniform lighting conditions, and the subjects were not informed about the background of the study. The samples scoring an overall quality of 5 or above were considered acceptable and those scoring below 5 were considered unacceptable.

3.9.2.4 Frying kinetics

For the design process, kinetic modelling is necessary to derive basic kinetic information for a system in order to describe the reaction rate as a function of experimental variables and, hence, to predict changes in a particular food during processing (Van Boekel 1996). Foods mainly follow the zero and first order reaction kinetics (Kumar et al. 2012b; Singh 2000); therefore zero-order (Eq. 1) and first-order (Eq. 2) degradation reaction kinetics was adopted for describing the rate of reactions.

\[
C = C_0 \pm k_1 \times x \pm k_2 \times y \\
C = C_0 \times \exp (\pm k_1 \times x \pm k_2 \times y)
\]

where \(C\), \(C_0\), \(x\), \(y\), (+) and (−) indicate measured value of response, initial value of the corresponding response, frying temperature, frying time, formation and degradation of any quality parameter, respectively; \(k_1\) and \(k_2\) are reaction rate constants.

3.9.2.5 Free fatty acid (Acid value) and peroxide value

Acid value is the number of milligrams of KOH required to neutralize the free fatty acids present in one gram of the oil or fat (Ranganna, 2003). Peroxide value of an oil or fat is used as a measurement of the extent to which rancidity reactions have occurred during storage. Free fatty acid and peroxide values were determined using the standard method recommended by AOCS (2004).
3.9.2.6 Texture analysis

Textural properties of the extrudates were determined by crushing method using a TA–XT2 texture analyzer (Stable Micro Systems Ltd., Goldming, UK) equipped with a 500 kg load cell. An extrudate about 40 mm long, was compressed with a probe (SMS – P/75 – 75mm diameter) at a crosshead speed 5 mm/s to 90% of diameter of the extrudate. The highest first peak value was recorded as this value indicated the first rupture of snack at one point and this value of force was taken as a measurement for hardness (Stojceska et al., 2008). Crispness is represented by number of fractures performed during complete rupture of the extrudates. Same was measured by total number of peaks generated during the period.

3.9.2.7 Estimation of β-carotene

β-Carotene was estimated by spectrophotometric method based on the chromatographic separation by using an adsorbent having varying affinities as per the standard method (Sharma et al. 2009; Srivastava & Kumar 2003).

3.9.2.8 Scanning Electron microscopy

Scanning electron microscopy (SEM) of the extruded snack food fried at optimized frying temperature was conducted immediately after frying and after six months of storage at the selected magnifications as per the method discussed earlier in 3.7.1.

3.10 Statistical analysis

The results were analysed by statistical software Statistca 7. The analysis of variance (ANOVA) for significance was conducted at a confidence level of 95% (p≤0.05). The significance of difference between mean values was assessed with Duncan’s multiple range test. The results were analyzed by statistical software Statistica 7 and Microsoft Excel (data analysis), 2007. The significance of difference between mean values was assessed with Duncan’s multiple range test.