Microstructure of paneer prepared by automated pressing technique

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Abstract

In this study, paneer was prepared by conventional method as well as by employing an automated pressing equipment and microstructure of paneer samples was studied by scanning electron microscopy. Further, pneumatic pressing of the hot coagulum was carried out at different pressures (2.5, 3.0, and 3.5 kg cm⁻²) and time durations (10, 12.5, and 15 min) using a semi-automated microprocessor based pneumatic pressing unit. It was found that the microstructure of paneer comprised mainly of continuous medium of casein matrix in which micro-pores were distributed. However, differences were observed in the way the curd particles were fused during the pressing process of paneer manufacture. Paneer obtained from the automated press was found to possess more uniformity than the one prepared by conventional method. This was attributed to more uniform pressure applied by the automated technique. Further, porosity values were correlated with the openness observed in the microstructure of paneer. It was found that at an optimized pressure and time combination, paneer had a more uniform structure along with porosity than conventionally made one. The dependence of the paneer properties on the pressure and pressing time was well fitted to polynomial quadratic model which showed influence of pressure applied is greater than the time of pressing.

Practical Applications

The SEM imaging of paneer prepared by automated pneumatic pressing based technique could be very useful in analyzing the microstructure which influences the texture and amount of water retention. Small to medium scale paneer manufacturers could very well utilize the automated pneumatic pressing technique to produce paneer of optimum texture and adequate amount of moisture. Paneer manufacturers of small to medium scale could very well utilize this pressing technique to increase their income at farm level.

1 INTRODUCTION

Paneer, as a very popular heat-acid coagulated product of milk in India and in many other parts of the world, is a highly rich source of nutrition for good quality protein, fat, vitamins and minerals like calcium and phosphorus (Dey et al., 2020; Kumar, Mishra, Sutariya, Chaudhary, & Rao, 2019). It is used in various culinary dishes and snack items throughout the Indian subcontinent. The paneer, generally having an appearance of white in color, has a sweetish-acidic nutty flavor, spongy kind of body and closely knit type of texture. It is extensively used for preparing variety of culinary dishes and snacks. Consumers give importance to body and texture of paneer as much as
they do to flavor. Further, paneer acts a substitute of meat to vegetarians in terms of nutrients and as well as providing “chewing” satiety. It has been reported that paneer available in market has a wide range of chewiness (Wangdare, Rao, Mishra, & Datir, 2017) because of several factors. It is difficult to produce paneer of same textural attributes batch to batch, because of several factors. It is difficult to produce paneer of same textural attributes batch to batch, 24 × 7. However, if we understand the factors affecting the texture and the mechanisms thereof, then it is possible to narrow down the textural variations caused during batch to batch manufacture, to a minimum and this achievement will in turn help producing uniform quality paneer, 24 × 7. In this regard, microstructural studies definitively contribute to our understanding of the reasons behind variations in texture of paneer as a cumulative result of various processing factors. In order to produce uniform quality paneer (Chitranayak et al., 2017; Chitranayak et al., 2017) developed an automated equipment for facilitating programmed pressing of paneer, as this pressing is an important unit operation of paneer manufacture. They reported an optimum pressure—time combination of 3.45 kg/cm² for 13.15 min which produced best quality paneer. They claimed that the paneer produced had a good uniform body and texture evaluated subjectively. However, these results need to be supported by objective methods like microstructure studies.

Some studies have been carried out earlier to elaborate microstructure of paneer. However, since paneer, too, is a heat-acid coagulum, commonly referred to as Indian cottage cheese, it is no surprise that its structure has resemblance to that of some varieties of cheeses. Glaser, Carroad, and Dunkley (1979) reported that the cow milk paneer resembled American cottage cheese in microstructure as studied by Scanning Electron Microscope (SEM), in terms of having uniformly distributed protein particles of small size. On the other hand, paneer, prepared from the milk of buffalo, contains higher density of fused protein particles. Fat globule membranes (FGMs) have fat globules with casein particles as attachments, very frequently observed in SEM images of raw paneer derived from buffalo.

Harwakar and Kalab (1980) observed a unique pattern in acid-coagulated milk gels, for example, GDL induced gels, and described it as core and lining structure. They also reported that similar structure was observed in other gels such as citric acid, hydrochloric and oxalic acid induced milk gels. They also showed significant change in core and lining structures by varying the coagulating temperature for milk and final value of pH for coagulation. The coagulation starting at 70°C indicated, only tentatively, the initiation of making these structures. The lining structure and core were fully developed at 90°C, apparently because of the complex interaction between k-casein and lactoglobulin.

In furtherance to above studies, SEM for comparing the microstructure of raw, cooked and fried paneer was employed by Kalab, Gupta, Desai, and Patil (1988). They reported that raw paneer contains the varying sizes aggregated protein particles and evenly distributed fat globules in its protein network. They compared the trend of microstructural development in paneer prepared from the milk of cow, buffalo, or mixed milks, at different conditions such as, raw, cooked or fried by SEM analysis. The granular structure of protein found to exist along with the formation of core and lining shape constitution in bigger size particles in raw paneer was reported while deep fried paneer at 175°C/4–5 min led to compaction of paneer structure due to compaction of individual particles. Microstructure of chhana, a product similar to paneer, was reported to be intermingled higher amount of mass of casein-whey protein complexes, which are interlinked to the thick bridging material interrupted by closely interspersed small voids. Moreover, the channa prepared from the buffalo milk had almost similar structure to cottage cheese and raw paneer and also found that they had denser and coarser matrix than that of the chhana prepared form cow milk (Adhikari, 1992). Bryant, Ustunol, and Steff (1995) reviewed that the Rheology, texture and other similar properties of channa, paneer and many similar coagulated dairy products could be very well characterized on the basis of their structure. Microstructure studies also support our understanding of mechanism of physicochemical changes taking place in food products, for example, protein–protein interactions in heat treated milk and milk products. The ultimate aim of the microstructure is to understand changes taking place during manufacture and how the structure of product develops. This will also help to understand the differences in quality of paneer prepared by altered processing parameters such as heat treatment of milk, coagulation agents, pressing conditions, etc.

Studies on optimization of pressing the coagulum during paneer manufacture are scanty, especially during mechanical and automated pressing. Kulshreshtha, Agrawal, and Singh (1987) had studied a laboratory-scale paneer press and correlated the paneer quality with the conditions of pressing. Pressing at pressures of about 98.1 k Pa produced paneer of uniform quality. When higher pressures were applied, it yielded hard paneer and decreased the yield due to more whey expression, whereas lower pressures yielded softer paneer, but enhanced yield. Thus, it was shown that application of proper pressure was crucial for getting proper texture and yield by controlling the moisture content. Chitranayak, Manjunatha, Mahesh Kumar, et al. (2017) tried to narrow down the variations of pressing by developing a microprocessor based automated paneer hoop-cum press unit to automate the pressure application and reported a significant improvement in the textural parameters of the paneer using the developed unit as shown in Figures 1–4. The paneer samples prepared by applying varying pressure and time combinations and the moisture content, bulk density and porosity of the paneer prepared were evaluated by standard methods (Chitranayak, Manjunatha, Menon, & Rekha, et al., 2017). They recommended a pressure in the range of 3.0 to 5.0 kg cm⁻² for obtaining good quality paneer. Arvind et al. (2019) very recently demonstrated the effect of matting temperature on quality of paneer. Now a days, there are different sensory validation tools used by new products developers for the improvement in organoleptic properties (Torres, Silva, Cutrim, & Cortez, 2020). In this research work, optimization of the pressure and time for the production of quality paneer from automated paneer press was carried out and the changes in microstructure, textural parameters and physico-chemical properties of paneer were investigated.

2 | MATERIALS AND METHODS

2.1 Preparation of paneer

Raw paneer was prepared by (1) conventional method and (2) employing an automated press developed by Chitranayak, Manjunatha, Mahesh
Kumar, et al. (2017). The method of preparation by the first method (Aneja, Mathur, Chandan, & Banerjee, 2002) is described below: Fresh crossbred-cow milk was procured from Institute’s Livestock Research Centre, filtered and standardized to 3.5% fat and 9.2% SNF. Three liters of the standardized milk was taken in a small steam kettle and heated to 82°C for 5 min. The milk was cooled to 70°C and citric acid (commercial grade procured from Mayura Scientific, Bengaluru) solution of 1% strength maintained at 70°C was slowly added to the milk, stirring the milk continuously for uniform distribution of the acid being added. The addition of the acid solution was stopped on complete coagulation.

**Figure 1** SEM images of paneer prepared by automated pressing technique with a magnification factor of (A) 250, (B) 500 and (C) 2000; M- Mechanical hole & C- Casein matrix

**Figure 2** SEM images of conventionally prepared paneer sample with a magnification factor of (A) 500, (B) 1000 and (C) 3000; C- Casein matrix
of milk indicated by appearance of greenish, clear whey on the surface. The coagulated milk was allowed for 10 min for settling of the curd particles. The mixture was then filtered through muslin cloth and the whey was drained off. The hot coagulum collected in the muslin cloth was carefully placed in stainless steel hoops and pressed in screw press for about half an hour. After pressing, the coagulum block was transferred to a vessel containing chilled water (4°C) and cooled for about 1 hr. The coagulum was then taken out of chilled water, drained for about 5 min, cut into desired pieces and stored till further analysis. Preparation of paneer by the second method was same as the first one except pressing conditions. The hot coagulum collected in the muslin cloth was carefully placed in a microprocessor based automated paneer hoop-cum press unit (Chitrnanayak, Manjunatha, Mahesh Kumar, et al., 2017), whose developmental work was conducted in our lab and its working in brief is explained herewith.

### 2.2 Working of microprocessor based automated paneer press

The microprocessor based automated paneer press consists of dentist type compressor (oil free), FRL unit (filtration-regulation-lubrication), pressing unit(pneumatic cylinder, connecting shaft, pressing plate and perforated hoop), tray collecting unit, microprocessor based automated instrumentation system (PID controller for pressure and time; RTD for temperature measurement, alarm/buzzer, solenoid valve, pressure gauges) and support for all other components of the press designed and developed (Figures 1-3). Fabrication and development of components of microprocessor controlled automated press was done on this basis. The design layout showing the different components of the proposed microprocessor controlled automated press for paneer is shown in Figure 1. Microprocessor controlled automated press consists of the following main units:

- Paneer pressing unit
- Automatic process control unit
- FRL unit
- Air compressor
- Support stand/frame and other minor accessories/components

Control panel, pneumatic cylinder, paneer hoop, detachable plate, connecting shaft, frame structure, stand and other parts were made using stainless steel (SS-304) which does not readily corrode, rust or stain with water as ordinary steel does. There are different grades and
surface finishes of stainless steel to suit the environment the alloy must endure. Stainless steel is used where both the properties of steel and corrosion resistance are required. It differs from carbon steel by the amount of chromium and nickel present. Fabrication of automated press was carried out as per FDA c-GMP and 3A hygiene standards (AMI, 2014; Marconnett, 2004). The various manufacturing processes involved in fabrication are described as per the selection of material of construction, testing of stainless steel for different grades, development of geometry, SS sheet cutting, welding and DP testing, grinding and polishing, testing and assembling of different components. The components and electronic instrumentation items were identified to be suitable, properly operational and functional for the required pressure, time and temperature. The components were assembled and integrated the microprocessor based PID controller, temperature controller, timing control circuit and all the other parts to develop the microprocessor based automated paneer press. The dentist type of air compressor was used for supplying the compressed air to the pneumatic cylinder (air-operated cylinder) to operate the main pressing unit, which passes through the FRL (Filtering-Regulating-Lubricating) unit and the solenoid valve. The developed automatic paneer hoop-cum-press unit has pressure, temperature and timer based control system. The pressure transducer monitors and controls the pressure applied over the hoop by means of pressure plates. The microprocessor based programmable digital timer indicates and controls the duration of application of the pressure. RTD, a temperature transducer employed to sense and indicate temperature. Internal connections of the pipelines were done in the pressing machine for the application and controlling the air pressure. One pipe-line was coming towards inside the valve for receiving the compressed air supplied by the air compressor and two pipe-lines were coming out from the solenoid valve for directing the flow of the compressed air supplied by the air compressor and other from bottom pipe-line was for removing the pressure for its upward movement after the set value of time. One pipe-line has been provided for pressure gauge to operate the pressure gauge and showing the pressure value on the control panel. As from the name of FRL unit, it can be understood that it is the combination of three important individual components (Filter, Regulator and Lubricator) which are necessary for a pneumatic circuit as shown in Figure 2. This unit consists of filter for filtering compressed air, pressure regulator for air pressure regulation and lubricator for supplying mist lubricant to the pneumatic cylinder for smooth operation and long life. The working of these three components explained below.

i. Filter: It was used in the FRL unit of the pressing unit to remove or filter out the impurities such dust particles from the compressed air supplied by the air compressor before it is fed to the pneumatic cylinder/components.

ii. Pressure regulator: It was used to stabilize the pressure applied to press the coagulum and regulate the operation of pneumatic cylinder.

iii. Lubricator: To provide lubrication for pneumatic components

The bourdon-tube based pressure gauge was connected through the pipe-line coming out from solenoid valve to know the exact value of
the air pressure supplied by the air compressor to the pneumatic cylinder for pressing the coagulum. The APC unit consists of pressure sensor, pressure regulator, temperature sensor (RTD type), microprocessor based PID controllers, microprocessor based digital process timing control circuit, solenoid valve, solid state hooper and digital indicators for pressure, temperature and time. The suitable pressure sensor of bourdon tube gauge type, operating in the pressure range of 0–10 kg/cm², FRL-unit for pressure regulator, also in the working range of 0–10 kg/cm², 5-way solenoid valve as controlling device, 7-segment digital indicators for pressure, temperature and time, LEDs and other small accessories selected and assembled. The bourdon tube based pressure gauge is shown in the control panel of Figures 2 and 3; a) which was to measure and indicate “Pressure” of the paneer pressing process. It has two scales: lower is 0–10 kg/cm² and upper is 0–150 psi to show the process pressure.

Support stand/frame was fabricated using SS 304 (Stainless Steel of grade 304) for supporting all the components of microprocessor controlled automated press. SS 304 had been used for the fabrication of all the body structure of the press, since it is corrosion less and carry very good strength. Electrical wiring and other minor accessories/components were made and used for assembling/connecting all the components. The assembled paneer press was tested and run under dead load continuously as well as at different load using paneer coagulum having weight 250 g to 8 kg (Figures 2 and 3). It was operated continuously without failure. Then the paneer press was used for conducting the experimental trials. In this machine, the coagulum was subjected to an optimized pressure of 3.45 kg/cm² for 13 min. After pressing, the coagulum was treated as described above.

2.3 | Experimental design

Response surface methodology (RSM) is a widely used mathematical and statistical method for modeling a process in which the response of interest is influenced by various variables. Different experimental designs are available for RSM. In this study, the experimental trials were carried out in a Face-Centred Central Composite Design which is a commonly used design for response surface analysis for optimization purposes. As per the design, trials of paneer preparation were conducted by 13 different combinations of pressure–time, as listed in Table 1. The levels of the pressure and time were so maintained as to get equidistant from each other with a difference of –1 or +1 between them. For example: difference between pressures was 0.5 kg cm⁻² and between time it was 2.5 min. Thus, by employing different combinations specified by the FCCC design, 13 trials were carried out. Each trial was conducted in triplicate and average of them was chosen for the statistical analysis by the Design Expert software.

2.4 | Selection of optimum pressure–time combinations

The ranges of independent and dependent variables were provided to the software. The following ranges of independent variables (treatments) provided to the software were: pressure: 2.5–3.5 kg cm⁻² and pressing time: 10–15 min. The ranges of various dependent variables provided were based on the results obtained by the experimental trials conducted in FCC design. Based on the inputs of dependent and independent variable ranges provided, the software gave a list of most optimum combinations which were again ranked based on the desirability. A treatment combination getting a desirability value of 1.0 (maximum) or most near to 1.0, occupies first rank followed by next and so on. The Design Expert program was run to get the optimum conditions and solutions. The optimized solutions given by the software having maximum desirability value was chosen as the most desirable treatment combination.

2.5 | Statistical analysis

The data of dependent (average values) and independent responses were fed into Design Expert software which fitted the data to second order polynomial model:

\[ Y = b_0 + b_1 \cdot X_1 + b_2 \cdot X_2 + b_3 \cdot X_1 \cdot X_2 + b_4 \cdot X_1^2 + b_5 \cdot X_2^2 \]

\[ Y = \text{dependent variable response} \]

\[ X_1 = \text{pressure}; X_2 = \text{time}; b_0 - b_5 = \text{regression coefficients}. \]

The goodness of fit was evaluated by regression coefficient \(R^2\). An \(R^2\) value as low as 0.80 and above was considered as good fit in view of the complex nature of the product. The significance of the treatment effects at \(p < .05\) was determined by ANOVA. The coefficient of variation less than 10% was considered as good fit. The influence of a parameter in linear term, interactive and quadratic terms was discussed based on the magnitude of the regression coefficients.

2.6 | SEM (scanning electron microscopy) for microstructure analysis of paneer

The standard methodology of paneer sample preparation for SEM imaging was followed (Syed & Winlow, 1989). Initially the paneer samples prepared by using the automated pressing machine were cut to the size of cube (1 mm × 1 mm × 1 mm) size. The primary and secondary chemical fixation of paneer sample was carried out with the suitable chemicals. The further steps followed in the preparation of paneer sample and the chemicals for SEM analysis are described as follows:

Chemical fixation: The primary fixation of paneer was done by fully immersing the cube of paneer sample of size 1 mm × 1 mm × 1 mm in 3% buffered solution of glutaraldehyde with phosphate buffer of 0.1 M at normal room temperature and leaving the sample in buffer solution for incubation for 2 hr (2–4 hr, max. 24–48 hr).

Washing: The sample was rinsed by phosphate buffer solution of 0.1 M whose pH value was 7.2 (3 × 10 min).

Post-fixation: The paneer sample was immersed in 1–2% buffered solution of Osmium Tetra-Oxide (i.e., OsO₄, Mol. Wt. 254 g.) in 0.1 M
Phosphate buffer having pH value of 7.2, for 2 to 4 hr at ambient room temperature, in an adiabatic container of light weight. For this purpose, 1% buffered solution of Osmium Tetra-Oxide (i.e., OsO₄) was prepared by mixing 0.25 g of Osmium Tetra-Oxide (OsO₄) with 25 mL of 0.1 M Phosphate buffer, whereas this Phosphate buffer was prepared by mixing 12.5 mL of 0.2 M phosphate solution with 12.5 mL of distilled water. Similarly, the 2% buffered solution of Osmium Tetra-Oxide (i.e., OsO₄) was prepared by mixing 0.25 g of osmium tetra-oxide (OsO₄) with 12.5 mL of 0.1 M phosphate buffer, whereas the phosphate buffer was prepared by mixing 6.25 mL of 0.2 M phosphate solution with 6.25 mL of distilled water.

The paneer sample was, thereafter, washed thrice in 0.1 M phosphate buffer having pH value of 7.2 for 10 min during each washing (i.e., 3 times × 10 min.).

The above-washed paneer sample was dehydrated in ethanol solutions (in water) of varying strengths in following graded series for 15 min during each dehydration:

1. 30% ethanol
2. 50% ethanol
3. 70% ethanol
4. 80% ethanol
5. 90% ethanol
6. 96% ethanol
7. 100% ethanol × 2 times

Critical point drying (CPD): This is a standard way to dehydrate the biological tissue before placing the sample for examination in the Scanning Electron Microscope. In the step of critical point drying, an appropriate inert fluid having just higher critical temperature than the ambient for a viable pressure, replaces water in biological tissue.

Sample mounting: The samples of paneer pieces were mounted on the holders or stubs using double-sided conductive tapes.

Metal coating: For the overall improvement in the SEM image, the coating of paneer samples was done in the process of sample preparation. In the step of metal coating, conductive layer of metal is formed on the sample which inhibits charging, limits thermal damage and enhances the secondary electron signal needed for the surface configuration investigation in SEM.

- Loading the coated sample in the SEM
- Focusing/viewing specimens in the SEM

### 2.7 Physicochemical characteristics of paneer

Moisture content (% on wet basis) of paneer was evaluated by standard gravimetric method (BIS, 1981). To evaluate the porosity in percentage the following standard method was applied as: The paneer samples prepared from the automated pressing technique were cut into cubes of size 1.5 × 1.5 × 1.5 cm ($V_0 = 3.375$ cm$^3$) and the initial weights ($M_0$) of the cubes taken and noted down. These weighed samples of paneer cubes were made to soak in deionized-cum-distilled water (having 1:20 ratio by volume) for about 10 to 12 hours overnight in the refrigerator. After taking out these paneer cube samples from distilled water and wiping their surface water, the weight of the samples was taken ($M_1$). The porosity value in percentage was evaluated as,

$$\text{Porosity} = \left[ \frac{(M_1 - M_0)}{V_0 \rho} \right] \times 100\%$$

where $\rho$ is the density expressed in g cc$^{-1}$ and the density, $\rho$ of water = 1 g cc$^{-1}$ (at 4°C, the temperature of its maximum density).
TABLE 2 Physico-chemical characteristics of paneer

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Conventionally prepared (14.13 kg /30 min)</th>
<th>Automated press (3.45 kg/cm²/13.15 min)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Moisture content</td>
<td>50.56</td>
<td>50.74</td>
</tr>
<tr>
<td>Porosity</td>
<td>15.45</td>
<td>15.82</td>
</tr>
<tr>
<td>Bulk density</td>
<td>1.46</td>
<td>1.14</td>
</tr>
<tr>
<td>Hardness</td>
<td>43.73</td>
<td>34.22</td>
</tr>
<tr>
<td>Chewiness</td>
<td>19.6</td>
<td>18.75</td>
</tr>
</tbody>
</table>

The standard methodology was used for evaluating the bulk density of the prepared paneer samples and the equation used was,

\[ \text{bulk density} = \left( \frac{M_0}{V_0} \right) \text{ gram per cc.} \]

Hardness and chewiness of paneer samples, as shown in Tables 2 and 3, were determined by texture profile analysis with the help of Texture Analyser (Stable Micro Systems, UK) (Chitranayak, Manjunatha, Menon Rekha, et al., 2017). The evaluation of paneer samples for sensory characteristics was undertaken by a panel of semi-trained judges on a 9-point hedonic scale (Land & Shepherd, 1988) and shown in Table 4.

3 | RESULTS AND DISCUSSION

3.1 | Scanning electron microscopy (SEM) for microstructure analysis of paneer

Figure 5 shows microstructure of conventionally made paneer at magnifications of 500, 1,000 and 3,000 ×. In 500 magnification (Figure 5a), small holes (shown by smaller arrows) may be noticed embedded in casein matrix (C). Larger spaces (bigger arrows) can also be noticed dispersed in the casein matrix. The small holes represent spaces occupied by fat globules which have been removed during alcohol/acetone treatment of paneer samples. Whereas, larger spaces were the voids left by moisture or whey dispersions. These are more evident at 1,000 × magnification (Figure 5b). The structural changes that take place during paneer manufacture explain the moisture retention in paneer. The paneer structure is formed by fusing of curd particles under hot conditions (Desai, Gupta, Patel, & Patel, 1991). These observations were found to be in accordance with those described by Kalab et al. (1988) for raw paneer, who also reported, through SEM, distribution of fat globule and moisture voids in the casein matrix of paneer at the same magnification used in the present study. However, Kalab et al. (1988) reported core and lining structure of paneer matrix using transmission electron microscopy (TEM). In this study, we did not employ TEM, and so no core and lining structure was observed; scanning electron microscopy (SEM) employed portrays only surface structure. At 3,000 × magnification, large moisture voids (arrows) may be observed (Figure 5c). At the same, “smoothness” of the matrix may also be observed. The phenomenon of fusing of curd particles under hot condition could be understood very well because β-lactoglobulins in milk undergo heat denaturation and interact with casein micelles imparting them some sort of surface stickiness which binds the curd particles together during pressing (Haque & Kinsella, 1988; Webb & Johnson, 1965). The casein as a result gets hydrated and attains plastic texture (Guinee, 2003). Ray (2008) also employed SEM to study moisture retention in raw paneer. He also reported similar observations of fat globule voids in paneer matrix, larger ones in buffalo milk paneer. He also reported fat globule agglomerates in the microstructure. These observations of fat globules and moisture voids are also reported by Ahmed and Bajwa (2019) and Rao and Mathur (1990) in paneer, and Adhikari, Mathur, and Patil (1992) in chhana.

These observations may be contrasted with the microstructure of paneer obtained by automated technique (Figure 6). At 250 ×, several fat globule (small arrows) and moisture voids (bigger arrows) were noticed more uniformly distributed in the casein matrix (C) (Figure 6a). This is also evident at 500 × magnification, indicating that the automated press used in the study resulted in more uniform distribution of fat globules and moisture in paneer matrix (Figure 6b). Further magnification of 3,000 × indicates uniform and smoother nature of casein matrix (C) than conventionally prepared paneer (Figure 6c). This may be attributed to the fact that in conventional paneer, pressure may not be uniformly applied throughout the body of coagulum, and the pressure applied might have got concentrated only at central portions of the coagulum. However, in automated press which was based on compressed air technique, the pressure has been applied uniformly on the coagulum. These are supported by the results of porosity, bulk density and chewiness. The porosity of the experimental paneer was slightly higher resulting in slightly lower bulk density. Because of uniform distribution of moisture, the hardness and chewiness values were also less than those of conventionally made paneer (Table 3). The significant microstructural difference between the two samples was also revealed by uneven network of casein matrix. This could be valid reason for difference in moisture and whey protein retention between the two samples and hence yield as well as shelf life. Moisture, fat and whey protein dispersion pattern into casein network was better in automatic pressed paneer as compared to control as indicated by less scattering of light due to fine and well entrapment of whey which resulted less shining of SEM image of automatic pressed paneer sample as compared to control. Paneer consists of water (major portion), followed by protein, fat and other minor components like lactose and ash. During preparation of paneer, the components of paneer underwent physico-chemical changes resulting in microstructural changes. Casein-whey protein and complex particles aggregated and ultimately fused to form a continuous network as visible in the images shown. Fat globules remain embedded in the network. The water or whey got entrapped in capillary spaces as well as in the void spaces, which were observed in the SEM images. SEM image of paneer prepared using microprocessor based automatic press indicated finer and evenly distributed casein matrix. Ahmed and Bajwa (2019) reported different microstructures of paneer as a result of different coagulating agents employed like fruit juices. Lemon and amla juices resulted in coarser structures than citric acid. Lemon juice as a highly
Table 3: Moisture content, porosity and bulk density of paneer pressed at different pressure and time of pressing combination in the developed automated controlled paneer press

<table>
<thead>
<tr>
<th>Property</th>
<th>T₁</th>
<th>T₂</th>
<th>T₃</th>
</tr>
</thead>
<tbody>
<tr>
<td>MC</td>
<td>54.73</td>
<td>51.60</td>
<td>51.34</td>
</tr>
<tr>
<td>P</td>
<td>21.55</td>
<td>15.75</td>
<td>19.30</td>
</tr>
<tr>
<td>BD</td>
<td>1.03</td>
<td>1.05</td>
<td>1.07</td>
</tr>
</tbody>
</table>

Note: P₁ = 2.5 kg/cm², P₂ = 3.0 kg/cm², P₃ = 3.5 kg/cm², T₁ = 10 min, T₂ = 12.5 min, T₃ = 15 min. Abbreviations: BD, bulk density; MC, moisture content; P, porosity.

Table 4: Change in sensory attributes of paneer pressed manually at different weight-time of pressing combination

<table>
<thead>
<tr>
<th>Sensory attributes</th>
<th>P₁</th>
<th>P₂</th>
<th>P₃</th>
</tr>
</thead>
<tbody>
<tr>
<td>Color and appearance</td>
<td>7.50</td>
<td>8.00</td>
<td>7.00</td>
</tr>
<tr>
<td>Body and texture</td>
<td>7.86</td>
<td>7.28</td>
<td>6.00</td>
</tr>
<tr>
<td>Flavor</td>
<td>8.21</td>
<td>7.57</td>
<td>7.50</td>
</tr>
<tr>
<td>Overall acceptability</td>
<td>7.93</td>
<td>7.35</td>
<td>7.00</td>
</tr>
</tbody>
</table>

Note: P₁ = 2.5 kg/cm², P₂ = 3.0 kg/cm², P₃ = 3.5 kg/cm², T₁ = 10 min, T₂ = 12.5 min, T₃ = 15 min.

Figure 5: Developed microprocessor based automated paneer press showing all components

Acidic coagulating agent led to bigger clumps of Paneer samples with irregularly shaped interstitial void spaces (Figure 5b), whereas amla curd having less acidity appeared to be yielding quite fine Paneer samples found to be compact with less interstitial spaces and consequently less moisture within these interstitial spaces. Change in sensory attributes of paneer pressed manually at different weight-time of pressing combination is given in Table 4. It was observed that the validity of the consumer data in the characterization of the sensory properties of the manually pressed paneer had familiarity with the automated machine pressed paneer, hence, inferred the acceptance of the same. Nascimento et al. (2020) studied the acceptance and sensory profile of mixed juices and concluded that the organoleptic characteristics of the mixed juices were accepted by the consumers.

3.2 Effect of pressure applied and pressing time on physicochemical properties of paneer

3.2.1 Moisture content

The response surface generated by the polynomial model of second order for the moisture content of paneer is shown in Figure 7a. It can
be seen from the figure that as and when the pressure and pressing time was increased the paneer moisture content had been decreasing linearly. Absence of any distortion of the response surface indicated that there were no interaction effects. The moisture content of paneer sample varied from 46.88 to 54.25% (Table 1). The effect of pressure applied and time for which pressure was applied on moisture content of the product during pressing was significant ($p < .05$).

### 3.2.2 | Porosity

Porosity values measured as % are displayed in Figure 7b as influenced by pressure and time. It may be seen that as pressure increased the porosity decreased and then almost remained the same. Whereas, as time increased, the porosity decreased. However, there seemed to be no interaction or synergistic effects because the response surface was uniform in its dimensions. The porosity value of paneer ranged from 15.01 to 19.25 (Table 1). The porosity value decreased as pressing pressure and time increased ($p < .05$).

### 3.2.3 | Bulk density

The response surface generated by the polynomial model of second order for bulk density of paneer has been depicted in the Figure 7c. The response surface is almost a prefect square indicating absence of interaction effects. It also reveals that the influences of pressure and time were linear in nature. The bulk density of paneer ranged from 1.07 to 1.20 g cm$^{-3}$ (Table 1). The bulk density value increased as a function of the pressure and time ($p < .05$).

### 3.3 | Effect of pressure – Time on texture profile properties of paneer

#### 3.3.1 | Hardness

Hardness is the force or the maximal stress (force per bottom area of pressing cylinder) required during its first compression to compress the paneer cube. In the study, the hardness of the paneer ranged from 24.05 to 44.22 N as presented in Table 1. As the pressing time increased, the hardness also increased. Similarly, as the pressure applied increased, the hardness also increased, but up to about 3.0 kg pressure. In this case, the interaction effects of the treatments were not significant as indicated by the uniform shape of the response surface. The hardness increased as a function the applied pressure and time ($p < .05$). There was slight rise in the value of hardness when the pressure by the automated pressing technique was increased as shown in the curve in Figure 8a.

#### 3.3.2 | Chewiness

Chewiness is defined as the work done during the compressing and disintegrating the paneer cube into a swallowable state. During
texture profile analysis test, chewiness value is obtained by multiplying hardness, cohesiveness and springiness values. The chewiness value of paneer showed an increasing trend for higher value of duration of pressing applied by the automated pressing technique (Figure 8b). The response surface was almost a uniform square indicating linear influence of pressure and time with no interaction and quadratic effects. The effect of pressure and time on chewiness of the product during pressing was significant ($p < .05$), which ranged from 10.65 to 23.42 N (Table 1).

### 3.3.3 | Effect of pressure—time on overall acceptability of paneer

Overall acceptance score is based on the other sensory parameters viz. flavor, body and texture and color and appearance (Dwivedi et al., 2014). The score of overall acceptability graded on nine-point Hedonic scale should be as high as possible nearing to 9.0 (like extremely), but a score of above 7.2 (above “like moderately”) (80% of maximum of 9.0) may be considered well accepted. The paneer prepared with different levels of pressure and time had overall acceptability score ranged from 7.35 to 8.25 (Table 1). The effect of applied pressure and time on overall acceptance of the product was significant ($p < .05$) on linear terms, but interactive and quadratic effects were not significant as indicated by the uniform dimensions of the response surface. In Figure 8c, shown as the 3D depiction, the value of overall acceptability was on higher side at the central portions of the curve for paneer samples prepared for different combinations of time and pressure applied by automated press.

### 3.4 | Observations from regression analysis and model coefficients

Regression analysis is a useful statistical tool to quantify the dependence of one or more variables on independent variables. It gives us a well-defined model which describes mathematical relationship of independent variable as a function of dependent variables. Regression analysis and ANOVA were used in this study for fitting the second order quadratic model to the data and for examining statistical significance of the terms, respectively. Table 5 shows the ANOVA parameters revealing the statistical significance of independent variables in linear, interactive and quadratic terms. The estimated $R^2$ and CV values of the quadratic polynomial model for various responses such as overall acceptability, moisture content, porosity, bulk density, hardness and chewiness are tabulated in the Table 2. The $R^2$ values of all the models were 0.80 and above, indicating a good fit of the polynomial model, except chewiness model for which $R^2$ was 0.76. Coefficient of variation in the data ranged between 1.75 and 7.26 indicating that the variation in the data was within the acceptable limit of 10%. This shows that the responses obtained were mostly as a result of application of treatment rather than random variations.

The best fit polynomial models for various variables are as follows:
Using the above equations, the values of dependent variables can be calculated at any pressure and time combination within the range studied. The data thus calculated at various pressure-time combinations have been plotted as response surfaces diagrams by the software (Figures 7a–c and 8a–c). These diagrams vividly portray the combined effects of various combinations of the independent variables (pressure and time) on dependent parameters studied. We can instantly perceive the influence of various combinations of pressure and time on the dependent variables, for example linear influence on moisture content of paneer (Figure 5a) or no quadratic or interaction influences on overall acceptability score (Figure 8c).

3.5 | Optimization of pressure and pressing time

There were three combinations of pressure-time recommended by the software with desirability of above 0.90: (1) 3.45 kg cm\(^{-2}\) for 13.15 min and (2) 3.49 kg cm\(^{-2}\) for 12.96 min and (3) 3.47 kg cm\(^{-2}\) for 12.61 min for which desirability values were 0.923, 0.912 and 0.910, respectively (Table 3). Among the two combinations, the first one was considered as most recommended because of highest desirability value (0.923). Using this combination, the predicted properties of paneer are: moisture content 50.74%, porosity 15.82, bulk density 1.14 g cm\(^{-2}\), hardness 34.22 N, chewiness 18.75 N.s and overall acceptability score 8.25.

3.6 | Validation

Experiments were conducted at derived optimum processing conditions to validate model and then the overall acceptability, moisture content, porosity, bulk density, hardness and chewiness of paneer were evaluated. The data and results based on experimentation (average value of three readings) and the values of various quality parameters based on prediction are showed in Table 6. For all the responses, t test was used for finding the significant difference between predicted and actual response values at 5% level of significance and it was found that there was no significant difference, so the response surface optimization model was adequate.

4 | DISCUSSION

The rheological properties of paneer are closely related to its structure. These are dependent on the manufacturing conditions. The
development of paneer structure starts from heat treatment stage of milk itself. In raw milk, native casein micelles are stabilized by net negative charges and steric repulsions (Walstra, 1990). During the heat treatment of milk, at the temperature of 70°C onwards, the whey proteins undergo denaturation, as the hydrophobic interactions and intermolecular di-sulfide bonds lead to association of these denatured whey proteins with casein micelles involving κ-casein (Haque & Kinsella, 1988). These denatured whey proteins because of their sticky nature coat on the casein micelles accomplishing casein-whey protein interactions (Webb & Johnson, 1965). Denaturation of proteins means opening up of their structural coil because of breakage of intact bonds by heat energy (Lehninger, 1982). This leads to the exposure of more number of hydrophilic groups which were embedded inside the structure of undenatured proteins, hence denatured whey proteins bind more strongly with casein micelles. The combination of caseins and denatured whey proteins has better water binding ability, so imparts typical chewy body to the product; consumer and gets smooth mouthfeel on eating the paneer. During acidification of milk to pH of about 5.1–5.2, during manufacture, most of the colloidal calcium phosphate is solubilized in the form of micelles, with the altering charges on individual micelles, leading to the increased ionic strength of the solution. Consequently, the intermolecular and interionic forces responsible for the integrity of these solubilized micelles (e.g., such casein particles as deplete in Ca and P) are considerably different from the intermolecular forces present in native micelles. The very balance between the opposite intermolecular forces of attraction and repulsion, so crucial to gelation properties, stands disturbed (Lucey & Singh, 2003). During the subsequent coagulation process, the casein curd particles because of charge neutralization on casein micelles group together as clumps as syneresis progresses, but still remain loosely bound with each other. They are made to be tightly bound to each other during subsequent pressing stage under hot conditions to form a unified curd block. The whey protein coating around the casein micelles is still fresh and sticky under hot condition. Hence, before the stickiness is lost, the curd has to be pressed in hot condition so that the curd particles fuse together to form a single block. This was also probably one of the factors responsible for the core and lining structure reported by Kalab et al. (1988). This was not observed

| Table 5 | ANOVA results for various responses using quadratic response surface model |
|---|---|---|---|---|---|---|---|
| Factors/variables | Df | OA | MC | PR | BD | Hardness, N | Chewiness, N |
| Model | 5 | 6.39* | 6.52* | 5.66* | 20* | 30.76* | 15.89* |
| X₁ | 1 | 5.73* | 11.09* | 14.85* | 32.48* | 2.22* | 0.40 |
| X₂ | 1 | 0.34 | 1.96 | 2.65 | 7.53* | 137.47* | 31.35* |
| X₁X₂ | 1 | 5.31 | – | 0.41 | – | 0.45 | – |
| X₁² | 1 | 0.13 | – | 8.58 | – | 13.64 | – |
| X₂² | 1 | 1.35 | – | 1.49 | – | 2.31 | – |
| Lack of fit | 3 | 2.34 | 0.38 | 4.18 | 2.45 | 3.16 | 0.76 |
| Residual | 7 | | | | | | |
| Total | 12 | | | | | | |
| R² of fitted polynomial model | – | 0.82 | 0.86 | 0.80 | 0.80 | 0.96 | 0.76 |
| CV% in the data | – | 2.11 | 3.58 | 6.75 | 1.75 | 6.30 | 7.26 |

Note: BD, bulk density; Df, degree of freedom; MC, moisture content; OA, overall acceptability; PR, porosity; X₁ = pressing pressure; X₂ = pressing time.
*Significant at p < .05.

| Table 6 | Comparison of experimental values with predicted values |
|---|---|---|---|---|---|---|
| Response | Predicted values | Experimentally verified values |
| Overall acceptability score | 8.25 | 8.24 | 8.25 | 8.50 |
| Moisture content, % | 50.74 | 50.63 | 48.89 | 51.16 |
| Porosity | 15.82 | 16.02 | 15.96 | 15.28 |
| Bulk density, g cm⁻³ | 1.14 | 1.15 | 1.15 | 1.09 |
| Hardness, N | 34.22 | 32.84 | 31.85 | 33.55 |
| Chewiness, N | 18.75 | 18.44 | 17.82 | 17.85 |

Software suggested optimum combinations
(1) 3.45 kg/13.15 min; desirability: 0.923
(2) 3.49 kg/12.96 min; desirability: 0.912
(3) 3.47 kg/12.61 min; desirability: 0.910
in the present study because of not employing TEM technique. So, it can be understood that the structure of paneer is mainly casein-whey protein complex in which other ingredients like fat globules, lactose and minerals are embedded. Whey or water may be expected to be entrapped in the capillaries of the proteinous matrix of paneer as reported for other proteinous products by Kinsella and Fox (1987).

When casein-whey protein complex aggregates are larger, more void spaces are formed in the network imparting sponginess to the product and acting as entrapments for moisture/whey. In paneer, the casein micelles are several times larger than the casein micelles present in either fresh milk or heat-treated milk. The dimension of casein micelle swells from 100 nm in fresh milk to 300 nm upon the heat treatment of milk. One may frequently observe the intact fat globule membranes in raw paneer. The texture and microstructure of paneer are determined by its manufacturing process as well as its milk components like casein micelles, whey proteins, fat globules, lactose and minerals including additives, if any. Microstructure simultaneously influences the physical properties of the Paneer product sample like its firmness, elasticity, susceptibility to syneresis and mouth-feel. The paneer prepared by the automated press possessed uniform body and texture and more uniformly distributed porosity corroborating the earlier subjective studies reported in literature. It is concluded that the more uniform microstructure observed in the experimental paneer strengthened the observation that the paneer prepared by automated press had better textural acceptability than the conventionally prepared paneer.

The minimum moisture content observed, that is, 46.88% is on lower side because generally the moisture content of paneer is not less than 50%. This shows that the pressure applied and time duration are on higher side. The moisture content of paneer sold in the market varies widely, because it depends on several factors like coagulation temperature, duration between coagulation and starting of pressing, uniformity of pressing, etc. Hence, it is difficult to produce paneer with same moisture content batch to batch, so batch to batch variations are bound to be there. That is why, FSSAI has set only upper limit for the moisture content as 65% FSSAI, (2018) which is actually scaled down value from 70% limit of earlier regulations. Moisture content of paneer is very important because it affects shelf-life, body and texture and handling and packaging. As per the available information in the literature, it was found that the consistent values of porosity level for paneer have not been reported so far. However, it is reported that the buffalo milk paneer is more porous and spongy than cow milk paneer for various reasons like more calcium content of buffalo milk (Sachdeva, Singh, & Kanawjia, 1985; Vishweshwaraih & Anantakrishnan, 1985). Thus, it may be understood that application of higher pressures leads to compaction and less porosity. It was observed that, the highest value of bulk density (1.28 g cc\(^{-1}\)) was observed for 3.5 kg cm\(^{-2}\) pressure and 15 min pressing duration while the lowest value (1.03 g cc\(^{-1}\)) was observed for 2.5 kg cm\(^{-2}\) pressure and 10 min pressing duration.

It could be found that the value of hardness increased as the value of pressing duration increased from 10 min to 12.5 min. The hardness value reached to the maximum when the duration of pressing was further increased to 15 min. The duration of pressing significantly affected the hardness value of paneer. The hardness of paneer cubes is imparted by pressing process aided by interaction between k-casein and \(\beta\)-lactoglobulin during heat treatment of milk (Haque & Kinsella, 1988) helping to form a typical paneer structure. If the temperature goes below this range during pressing its texture as well as the chewiness was found to be of poor quality and if the temperature remained higher during the press then also its quality was adversely affected resulting in hard and dry paneer. Denaturation of proteins means opening up of their structural coil because of breakage of intact bonds by heat energy (Lehninger, 1982). This leads to the exposure of more number of hydrophilic groups which were embedded inside the structure of undenatured proteins, hence denatured whey proteins bind more strongly with casein micelles. The combination of caseins and denatured whey proteins has better water binding ability, so imparts typical chewy body to the product; also, consumer gets smooth mouth feel on eating the paneer. The developed paneer press can be used for pressing paneer ranging from 250 g to 8 kg with better quality of paneer in reduced process time and cost. It will be very useful for small to medium scale paneer manufacturers. The developed automated paneer press improves the status of mechanization for paneer manufacturing, reduces drudgery, process time and cost, allows precise control of process parameters, enhances the yield, improves quality, shelf-life by maintaining hygiene and improves sensory attributes than the paneer pressed by manual method.

### 5 CONCLUSION

Paneer samples possessed protein continuous matrix in which fat globules and moisture droplets were distributed. The distribution was more uniform in automated press paneer than on conventionally prepared paneer. This resulted in slightly better porosity in the experimental sample and lower hardness and chewiness values. This underlines the importance of automated pressing technique in getting uniform body and texture of paneer than conventionally made paneer, since the paneer prepared by the automated press possessed uniform body and texture and have more uniformly distributed porosity. Further, these results had indicated that, pressure, time and pressure-time interactions significantly affected the bulk density of paneer, hence improving the chewiness. As and when the load of pressure along with the duration of the pressure applied increased by the automated pressing technique, the reduction in inter-particle spaces in paneer had taken place which resulted in varied texture of the paneer. Even though the values of bulk density is not a parameter considered commercially important for paneer, the observation that it is dependent on the intensity of pressure applied would help in understanding the texture and many other properties of paneer. It could have an economic significance for the manufacturers especially in terms of yield and bulk appearance. The bulk density is also scarcely reported in literature. The results of present investigation would be useful information for the stakeholders of paneer industry. The dependence of the paneer properties on the pressure and pressing time was well.
fitted to polynomial quadratic model which showed influence of pressure applied is greater than the time of pressing. It was concluded that small to medium scale paneer manufacturers could very well utilize the semi-automated pneumatic pressing technique to produce good quality paneer.

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AUTHOR CONTRIBUTIONS
Chitranayak Sinha: Conceptualization; data curation; formal analysis; investigation; methodology; validation; writing-original draft; writing-review & editing. Manjunatha M: Formal analysis; investigation; project administration; supervision; writing-original draft; writing-review & editing. Jayaraj Rao: Data curation; formal analysis; investigation; methodology; project administration; supervision; writing-review & editing. Pushpanayak Sinha: Methodology; supervision; writing-review & editing. Khushbu Kumari: Data curation; methodology; validation.

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