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Scanning electron microscopic and electrophoretic studies of the baking process of south Indian *parotta*—an unleavened flat bread

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Abstract

The changes that take place in starch and protein molecules during processing of *parotta*, starting from wheat flour to baked *parotta*, were followed by scanning electron microscopy (SEM) and electrophoresis. The microstructure of wheat flour showed that the protein matrix was broken up into aggregates and starch granules were embedded in it. At the processing stage of *parotta* dough into a very thin sheet, the surface of dough appeared as a fine matrix of proteins and soluble solids with dispersed starch granules. The surface of baked *parotta* showed a still finer distribution of all the components. The microstructure of outer and middle layers of *parotta* showed clear differences. In the outer layer, starch granules were distorted and embedded in the protein matrix owing to the differential heating process whereas, in the middle layer, the extent of starch granule distortion was less than in the outer layer. Electrophoretic patterns of wheat flour, *parotta* dough and baked *parotta* revealed that less intense bands were observed in HMW regions of baked *parotta*. SEM and electrophoresis were found to be valuable tools for understanding molecular interactions between starch and protein molecules during the baking process of *parotta*.

Keywords: Wheat flour; Dough; Parotta; Scanning electron microscopy; Electrophoresis

1. Introduction

Wheat-based traditional products are consumed by millions of people in the Indian subcontinent. In India, 75–80% of the total wheat produced is used for making traditional products. Chapathi, puri, phulka, roti, *parotta*, bathura and nan are the main traditional products which form the staple items in the diet of the majority of the population. Basically *parotta* is prepared using wheat flour, salt, water and oil while sugar and egg form the optional ingredients. The dough prepared with salt and water is rested and later spread into a thin sheet with liberal application of oil. The sheet is folded into multiple layers and then coiled. After resting it is sheeted again into a circular disc of 15–17 cm diameter and to a thickness of 0.5 cm. It is baked on a hot plate maintained at about 230 °C for 4 min while turning every 30 s and applying about 2.5 ml of oil on each side during baking. The baked *parotta* is creamish white in colour and possesses several distinct layers. It is a soft textured product, with chewy characteristics, and is generally consumed along with vegetarian or non-vegetarian side dishes (Indrani, 1998).

There is not much information available on *parotta*, quite unlike the case of bread, which has been studied exhaustively. However, the information generated quite recently on *parotta* includes a description of *parotta* (Qarooni, 1996), optimization of ingredients for the preparation of *parotta* (Indrani & Venkateswara Rao, 2001), changes in the quality characteristics of *parotta* during storage (Indrani, Jyotsna Rao, Udayasankar, & Venkateswara Rao, 2000), and effect of quality of flour on the quality of *parotta* (Indrani & Venkateswara Rao, 2000). However, there is no report on the microstructure of *parotta* to facilitate a better understanding of the baking process of parotta. Different approaches to studying the microstructure of bread dough have been carried out using light microscopy (Amend & Belitz, 1991; Freeman & Shelton, 1991; Betchel, Pomeranz, & de Francisco, 1978), transmission electron microscopy (TEM) (Amend & Belitz, 1991; Betchel et al., 1978; Khoo, Christianson, & Inglett, 1969), freeze-fracture (Fretzdorff & Pomeranz, 1982) and scanning

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electron microscopy (SEM) (Aranyi & Hawrylewicz, 1968; Aranyi & Hawrylewicz, 1969; Gen, Angold, Williams, Ellis, Vauguan, & Galliard, 1990). SEM provides an appropriate means for characterising the physical properties and textural attributes of food ingredients in a formulated product (Belsie, Rasco, Siffring, & Bruinsma, 1993). His-Mei Lai (2001) reported the microstructure of rice pasta using SEM. The advantages that make the scanning electron microscope an extremely useful investigative tool for examination of the flour-dough transition include its very large depth of focus and the possibility of obtaining three-dimensional images of sample surfaces at relatively low magnifications with minimal preparation (Aranyi & Hawrylewicz, 1969). Electrophoretic methods have been reported elsewhere for assessing the baking process of bread (Lukow, Payne, & Tkauchuk, 1989; Payne, Nightingale, Krattiger, & Holt, 1987). Considerable work has also been reported on the influence of quality and quantity of protein fractions on chapati characteristics (Prabhasankar, 2002). However, there is no report on the electrophoretic study of the baking process of *parotta*. Hence, the aim of the present work was to study changes in the structures of the main components of wheat flour during processing of wheat dough to *parotta* by means of electrophoresis and SEM analysis.

2. Materials and methods

2.1. Basic ingredients

Wheat flour obtained from variety HD 2329 (National Seeds Corporation LTD., New Delhi, India) was used for the studies.

The characteristics of the flour, such as moisture, ash, SDS-sedimentation value, protein, dry gluten, farinograph and extensograph characteristics, were determined using AACC methods (2000).

2.2. Formulation

Parotta was prepared according to the following formula: flour 100 g, salt 0.5 g, water, 62 ml (Farinograph water absorption). The *parotta* dough was rolled into a thin sheet with the application of commercially available refined groundnut oil (Postman brand, Ahmed mills, Mumbai).

2.3. Processing of parotta

Parotta was prepared according to the method of Indrani et al. (2000) as presented in Fig. 1.

2.4. Preparation for SEM

Preparation of samples for SEM was carried out according to a standard method with slight modifications (Sidhu, Siebel, & Meyer, 1990). *Parotta* dough and baked *parotta* sample pieces (size 20×20 mm) were defatted with hexane, followed by freeze drying using a Heto freeze dryer Model DW3 (Denmark). The freeze-dried samples were kept in a desiccator until further use.

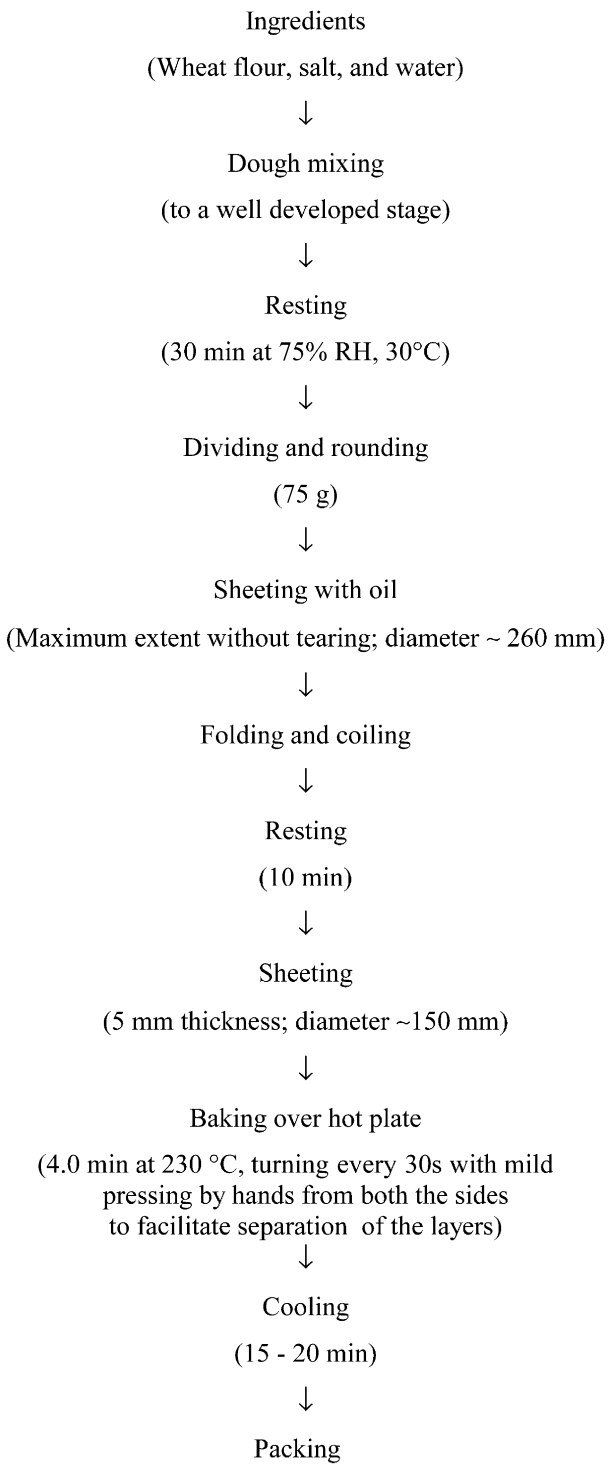

Fig. 1. Processing conditions for the preparation of *parotta*.

2.5. Scanning electron microscopy

A Leo Scanning Electron Microscope Model 435VP (UK) was used. The surface of sheeted *parotta* dough and baked *parotta* samples was separately placed on the sample holder with the help of double-sided scotch tape and sputter-coated with gold (2 min, 2 mbar).

Finally, each sample was transferred to the microscope where it was observed at 15 kV and a vacuum of 9.75×10^{-5} torr.

2.6. Electrophoretic characterization

The effect of the baking process of parotta on the quality of protein fractions was studied using SDS-PAGE (sodium dodecyl sulphate-polyacrylamide gel electrophoresis). The samples were raw material (wheat flour), parotta dough and baked parotta suspended in a solution containing sodium dodecyl sulphate (4%), β-mercaptoethanol (5%), bromophenol blue (0.001%), glycerol (20%) and 0.06 M Tris–HCl (pH 6.8) to give a concentration of 30 μg flour μl $^{-1}$. The samples were heated in a boiling water bath for 5 min, followed by centrifuging at 10,000×*g* for 5 min and 20 μl of supernatant were loaded onto gels. SDS-PAGE analysis was carried out according to Prabhasankar and Haridas Rao (2001) in 10% acrylamide gel. Gels were stained overnight with 0.1% (w/w) Comassie Brilliant Blue R-250 in methanol/acetic acid/water (25:10:65) followed by destaining.

3. Results and discussion

3.1. Quality characteristics of wheat flour

The flour used contained 0.45% ash, 10.0% gluten, 10.8% protein and had 63 ml SDS-sedimentation value, 62% farinograph water absorption. The dough showed 8 min stability, 460 BU extensograph resistance to extension, 160 mm extensibility and 2.9 ratio figure (Table 1). This indicated that the flour was of medium strength and was suitable for *parotta* making (Indrani & Venkateswara Rao, 2000).

3.2. Microstructure of wheat flour

The images obtained in the SEM of flour and its physical appearance are shown in Fig. 2. The physical appearance of flour was similar to any other refined wheat flour. The SEM observation of wheat flour was similar to that reported in the classical studies of Aranyi and Hawrylewicz (1968, 1969) and Rojas, Rosell, de Barber, Perez-Munera, and Lluch (2000). Wheat flour preserved some characteristics of the intact endosperm tissue but the original highly compact packed structure of the kernel appeared to be broken up into aggregates of protein matrix embedding groups of cellular components, mainly starch granules of two different sizes—the larger lentil shaped granules and the smaller round shaped granules as reported by Hoseney (1994) can be observed. Some starch granules appeared damaged as a consequence of milling.

3.3. Microstructure of parotta dough

The microstructure characteristics of wheat flour dough and gluten are highly dependent on the preparation procedures and the specific technique applied. In fact, dough and gluten have been described as veil-like structures that seem to be stretched over and around the starch granules (Aranyi & Hawrylewicz, 1968; Khoo et al., 1969), thin creep-like sheets (Evans, Volpe, & Zabik, 1977), films of proteins that form layers and three-dimensional sponge-like structure (Freeman & Shelton, 1991; Freeman, Shelton, Bjerke, & Skierkowski, 1991). Berglund, Shelton, and Freeman (1990, 1991), working with low-temperature SEM (LSEM), found that dough may exhibit a reticular pattern, depending on the sample preparation procedure. Based on their results of environmental scanning electron microscopy (ESEM), Bache and Donald (1998) suggested that the gluten network at a microscopic level is an artifact of severe dehydration, and that the true network exists at a molecular level. Khoo et al. (1969) stated that when fully hydrated in mixed dough, the protein forms a veil-like film over the external surface of the starch granules which can be observed in a protein matrix. Hoseney (1994) also observed that SEM of freeze-dried optimally mixed dough showed no intact flour particles, but instead an apparently random mixture of protein fibrils with adhering starch granules (Hoseney, 1985). Lee, Ng, Whallon, and Steffe (2001), made a similar observation in their study in which developed dough made from cracker flour was subjected to a Laser scanning confocal microscopy (LSCM) study. They reported that non developed dough had the least protein matrix, the developed dough had the most protein matrix and dough strength was related directly to the amount of protein matrix present. They confirmed that extensional deformation creates more protein matrix than does shear deformation. Schluentz, Steffe, and Ng (2000) examined the ultra-structure of developed, partially developed and non-developed doughs by using SEM and also found that developed dough had the most protein matrix formation and non-developed dough had the least.

In this study, the microstructure of *parotta* was analysed for the first time by SEM. Freeman et al. (1991) and Rojas et al. (2000) demonstrated the convenience of using SEM and cryo SEM for the analysis of bread dough microstructure.

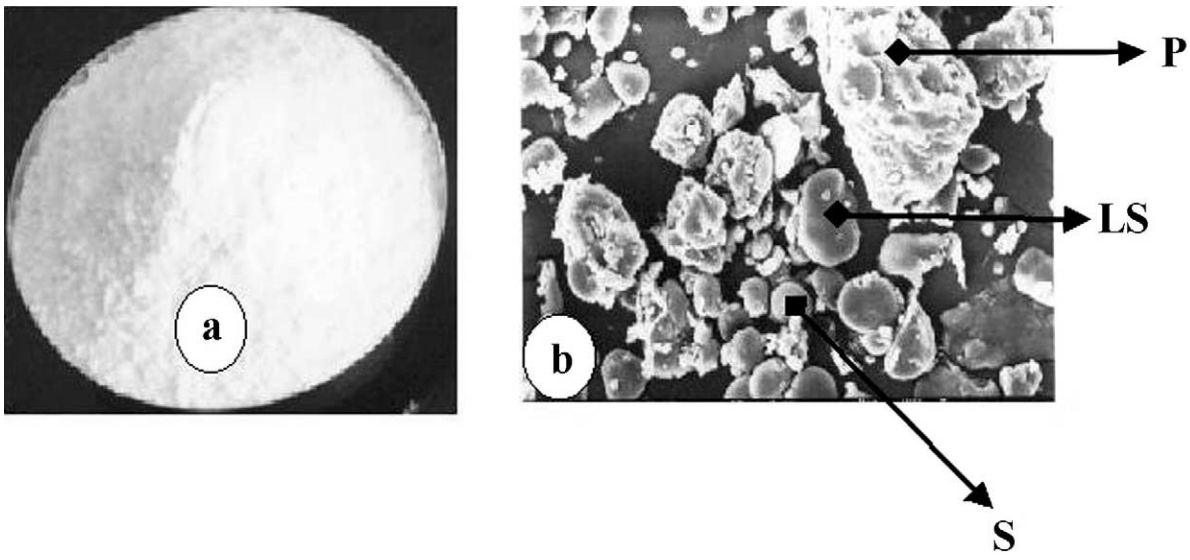

Fig. 2. Physical and microstructure of wheat flour. (a) Physical appearance of flour. (b) Microstructure of wheat flour using SEM with magnification of 500×. P, Protein fibrils with starch granules; LS, Large starch granules; S, Small starch granules.

Preparation of *parotta* includes several steps (Fig. 1), but the most important step that governs the quality of *parotta* is sheeting of dough with application of oil into a very thin sheet. During this process the dough should not tear (Indrani & Venkateswara Rao, 2000). Hence this particular processing step was selected and subjected to SEM analysis. The physical appearance of sheeted *parotta* dough and its microstructure are shown in Fig. 3. The physical appearance of sheeted *parotta* dough seems to be a very thin and transparent sheet of dough. The proteins in wheat are solubilised as a result of the effect of salt in the formulation and they constitute the amorphous matrix with embedded starch granules. Even in this dough system, some broken starch granules were found, owing to a certain amount of starch damage during the milling process.

3.4. Microstructure of outer and middle parotta layers

Baked *parotta*, unlike other flat breads, possesses several distinct and transparent layers. The physical appearance and microstructure of outer and middle *parotta* layers are shown in Fig. 4. Rojas et al. (2000) observed breadcrumb structure using cryo-SEM. The bread crumb showed the characteristic structure of an open sponge with interconnected cavities inside large gas cells. Khoo et al. (1969) also reported that the larger gas cells distinguish bread crumb from pre-baked dough, as seen under the SEM. But no such gas cells could be observed in the microstructure of baked *parotta,* as there is no fermentation and no gas formation in the process of preparation of *parotta*.

The physical appearance of the *parotta* shows several layers. A slight difference between the microstructures

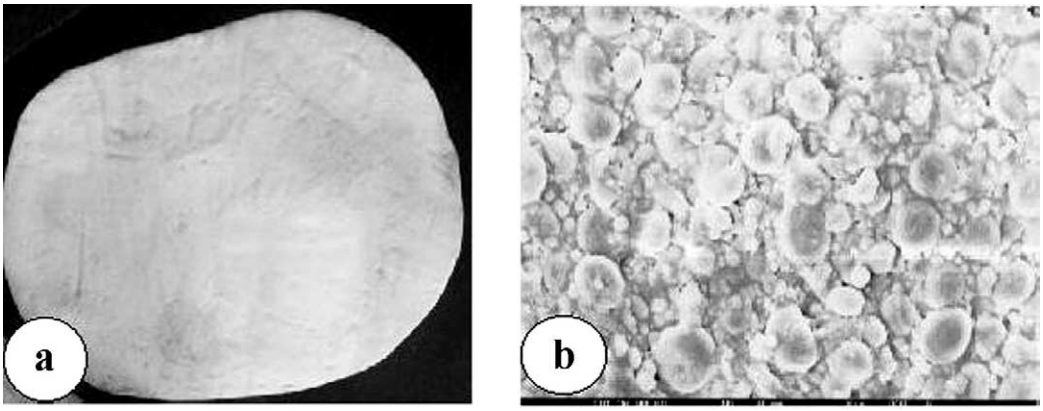

Fig. 3. Physical and microstructure of sheeted parotta dough. (a) Physical appearance of sheeted parotta dough. (b) Microstructure of sheeted parotta dough using SEM with magnification of 500×.

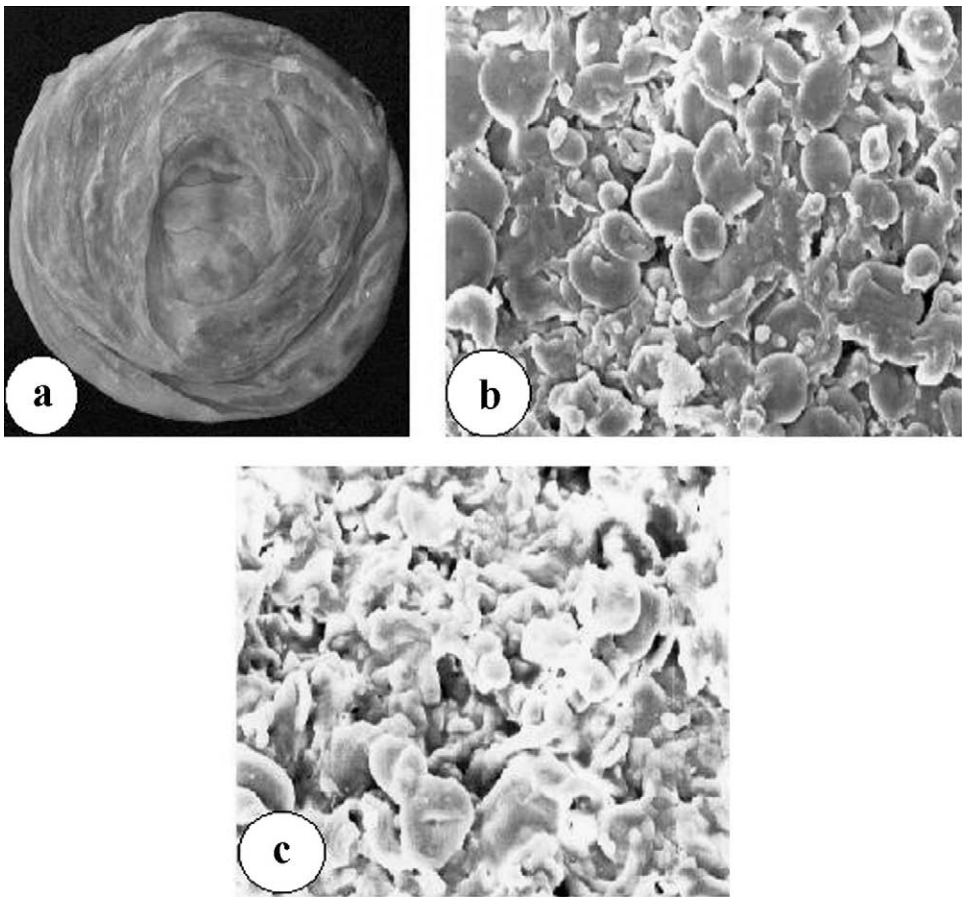

Fig. 4. Physical and microstructure of baked parotta. (a) Physical appearance of baked parotta (b) Microstructure of middle layer of the baked parotta using SEM with magnification of 500×. (c) Microstructure of outer layer of the baked parotta using SEM with magnification of 500×.

of outer and middle layers of *parotta* was observed. The microstructure of outer layers of *parotta* showed a change in shape of both types of starch granules (large and small) owing to gelatinisation. Protein and starch molecules were found to adhere closely. However, in the case of middle layers, the protein and starch molecules were much closer and deformation of protein and starch molecules was less. Possibly the presence of fat between layers of parotta might have maintained the identity of starch granules. Sidhu et al. (1990) studied the extent of starch gelatinisation in a few common Indian unleavened flat breads—*chapathi, parontha* and *poorie*. The starch in crumb areas of these flat breads is gelatinised to a greater extent than the crusts. Srivastava, Meyer, Haridas Rao, and Seibel Empar (2002) reported that scanning electron micrographs clearly indicated that the starch in the crumb of chapati granules formed a continuous matrix. Empar et al. (2001) reported a study of final-fried frozen battered squid ring—a fried product very popular in Spain prepared using a batter coating of wheat flour, corn flour, salt and leavening. The batter crust microstructure revealed gelatinised starch granules and gas cells. A similar observation was made in our study, wherein the microstructure of outer layers of *parotta* showed gelatinised starch granules. The gelatinised starch granules were immersed in the continuous matrix formed by the denatured protein. Both gelatinsation of starch granules and denaturation of protein matrix are a consequence of the heat to which these components are subjected during the baking process. The loss of water and merging of all the structural components which takes place when the *parotta* is baked, primarily affecting the protein matrix, and the quality of the protein matrix which is dependent on the quality of wheat used, may be responsible for the resultant chewiness and layer separation of *parotta* which is a typical quality attribute of the product.

3.5. Electrophoretic characterisation

Changes in protein composition during the baking process of parotta were determined by SDS-PAGE under reducing conditions. The SDS-PAGE patterns of wheat flour, parotta dough and baked parotta are shown in Fig. 5. The electrophoretic pattern indicated that major protein fractions in wheat flour and parotta dough were intact, but in the case of baked parotta, the intensities of protein bands in HMW regions were less

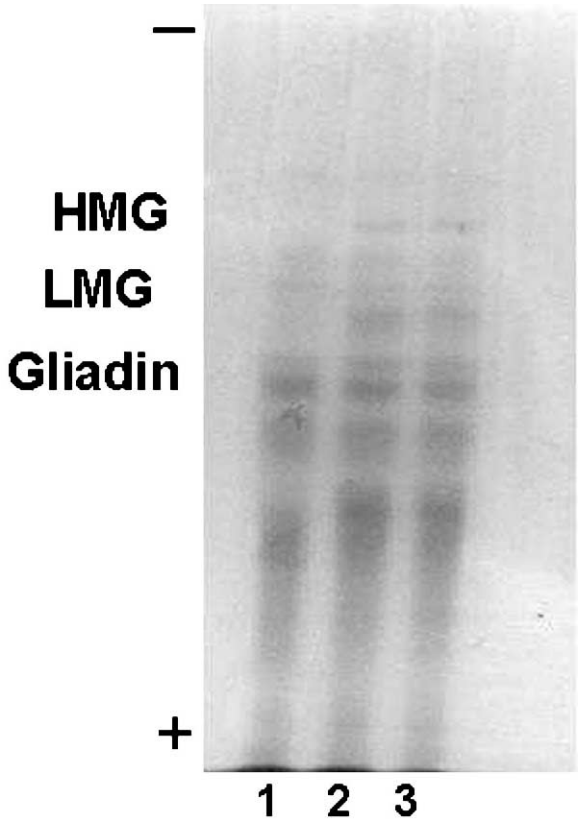

Fig. 5. SDS-PAGE of protein fractions from Lanes: 1. Baked parotta; 2. Parotta dough; 3. Wheat flour.

intense than in the wheat flour and parotta samples. The intensities of bands, particularly in the glutenin region, were reduced by baking.

The electrophoretic observations of protein bands are substantiated with the aid of micrographs. In the initial wheat flour, protein bands were intact and distinct. The major protein bands, such as gliadins, LMG and HMG, were clearly visible. In the micrograph of wheat flour (Fig. 2), starch granules and protein matrix aggregates were observed and possibly may have facilitated better extraction of major proteins. In the parotta dough, due to hydration of proteins and dough development owing to mixing, a continuous protein film in which starch granules were embedded was observed (Fig. 3). The major protein fractions did not show much change in the electrophoretic pattern of unheated parotta dough compared to the flour (Fig. 5). In the case of baked parotta, the protein bands in the HMW region appeared to be less intense. Rearrangement of disulphide bonds might have occurred on heating. As a reducing agent was used during preparation of samples for SDS-PAGE, a complete recovery of HMG subunits was theoretically possible. However, on heating, degradation and/or aggregation of proteins by covalent, non-disulphide linkages may have taken place, leading to an irreversible change in the sample composition and differences in

Table 1
Characteristics of the wheat flour used

Parameters	
Chemical	
Moisture (%)	11.5
Ash(%)	0.45
SDS-Sedimentation value (ml)	63
Protein (%)	10.8
Dry gluten (%)	10
Farinograph characteristics	
Water absorption (%)	62
Dough development time(min)	3.5
Dough stability (min)	8.0
Mixing tolerance index (BU)	20
Extensographic characteristics	
Resistance to extension, R (BU)	460
Extensibility, E (mm)	160
Ratio figure R/E	2.9
Area (cm^2)	118

electrophoretic patterns. Similar reports regarding the effects of high temperature on protein fractions, especially the glutenin fraction, have been noted by us earlier (Prabhasankar & Haridas Rao, 2001). Duodu, Nunes, Delgadillo, Parker, Mills, Belton, and Taylor (2002) reported that, in the electrophoresis of uncooked and cooked protein-body-enriched sorghum and maize, there were low molecular weight protein bands observed under reducing conditions in uncooked and cooked sorghum and maize. These bands were fainter for the cooked protein-body enriched samples than the uncooked. A similar trend was observed in our study when electrophoretic patterns of parotta dough were compared with baked parotta. Result of the present study were in line with an earlier report made on the storage study of frozen yeast raised dough (Kenny, Wehile, Dennehy, & Arendt, 1999). The micrograph of baked parotta showed a denatured protein network and the outlines and shapes of starch granules were also distorted indicating gelatinisation because of the baking process (Fig. 4). This could be a possible reason for the bands being less intense in the HMW region. Electrophoretic characterisation revealed that the baked parotta showed less intense protein bands in the HMW region as against the intense protein bands in flour and parotta dough (Fig. 5)

4. Conclusion

For the first time, the microstructure evolution and electrophoretic characterisation from flour to *parotta* is presented in this paper. SEM and electrophoresis have proved to be valuable tools for observing the molecular interactions of the major components of wheat flour during the baking process of parotta.

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