J Food Sci Technol (January–February 2012) 49(1):50–57
DOI 10.1007/s13197-011-0258-7

ORIGINAL ARTICLE

Studies on effect of additives on protein profile, microstructure and quality characteristics of pasta

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Revised: 17 May 2010 / Accepted: 2 June 2010 / Published online: 6 February 2011

Abstract Wheat storage proteins play a vital role in pasta making quality. In the present study, SDS-PAGE, Gel filtration chromatography and Scanning electron microscopy techniques were employed to understand the changes in the wheat protein fractions and their interactions with additives namely Sodium Steroyl Lactate (SSL), Glycerol Monostearate (GMS) and Hydroxy Propyl Methyl Cellulose (HPMC) during processing of pasta. SDS-PAGE studies indicated changes in High Molecular Weight Glutenin (HMW) fractions during drying stages of pasta preparation and in cooked pasta samples. In uncooked pasta, gel filtration patterns showed four peaks corresponding to different storage proteins whereas in the case of cooked pasta, these peaks were merged into three peaks. Pasta quality characteristics studies indicated that pasta with HPMC was found to have minimum percentage of cooking loss (5.6%), increased cooked weight (82 g), firmness (2.97 N) and high overall quality score (27) than GMS, SSL and control. Microstructure studies confirm the beneficial effect of HPMC. The present study indicated that HPMC is better additive for pasta manufacture followed by GMS. This could be due to interaction of HPMC with starch and protein matrix is different from that of GMS and SSL.

Keywords Pasta · Emulsifiers · Wheat proteins · Scanning electron microscopy · Electrophoresis · Gel filtration

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Introduction

The wheat semolina protein content and composition play an important role in the production of good quality pastas (Feillet and Dexter 1996; Matsuo 1994; Matsuo and Irvine 1970; Malcolmson et al. 1993). These proteins are divided into four main classes, of which the albumins and globulins are minor fractions, compared to the monomeric gliadins and the polymeric glutenins. The very large polymeric glutenin proteins composed of high-molecular weight glutenin (HMW) and low- molecular weight glutenin (LMW) subunits are linked together by disulphide bridges (Schofield et al. 1983). Payne et al. (1984) demonstrated that an allelic variation in the composition of the LMW prolamins was strongly correlated with the pasta making-quality characteristics. Anjum et al. (2008) reported that HMW subunits of wheat grain are of immense importance in determining the quality and the end use properties of the bread dough. Veraverbeke and Delcour (2002) reviewed the wheat protein composition and properties of wheat glutenin in relation to bread making functionality. Some of the synthetic/non-synthetic additives that incorporated during processing of pasta products are acidifiers, antioxidants, emulsifiers, acidity correctors, preservatives and flavour enhancers.

Commonly used emulsifiers in bakery and pasta products are sodium and calcium steroyl 2-lactylate (SSL and CSL), lecithin, monoglyceride (MG), Sucrose Fatty Acid Ester (SFAE) and Diacetyl Tartaric Acid Ester of Monoglycerides (DATEM). Cooking properties and sensory characteristics of pasta were optimum at an emulsifier concentration of 0.6% (Kovacs 1992). Monoglyceride significantly decreased the surface stickiness of cooked spaghetti (Matsuo et al. 1986). Addition of MG reduced torque, mechanical energy and dough temperature of pasta

dough (Hu et al. 1993; Henry 1995). Most dough strengtheners are anionic emulsifiers such as SSL, CSL and DATEM (Stampfli and Nersten 1995). By extensograph DATEM increased the ratio between resistance to deformation and extensibility, whereas MG and lecithin did not (Stampfli et al. 1996). HPMC has been used in various baked and pasta products for textural improvements. Xanthan gum, HPMC and other hydorcolloids have been tested for their potential as bread improvers and anti-staling agents (Guarda et al. 2004). HPMC was described as bound to the external part of the starch granules, reflecting a displacement of the lipids bounded to starch (Collar et al. 1998). Anton and Artfield 2008 reviewed role of hydrocolloids such as HPMC, Xanthan gum in bread making. The influence of HPMC on shelf-life of par-baked bread during its storage at low temperature and also on the staling of full baked bread was studied by Barcenas and Rosell (2007). Deshmukh et al. (2007) reported a valid method for determination of methyl cellulose (MC) and HPMC in food and food products. Use of HPMC and other hydrocolloids in the formulation of gluten free food products was reported earlier (Gallagher et al. 2005; Anton and Artfield 2008). There were reports on the use of additives in pasta product development (Stampfli et al. 1996; Stampfli and Nersten 1995; Tsen and Weber 1981). However most of the reports addressed only to product quality and rheological behaviour of pasta dough. Hence, the present study aims to understand the influence of mostly used additives such as SSL, HPMC and GMS on the protein profiles of pasta made from Indian *T.durum* wheat using SDS-PAGE, Gel Filtration Chromatographic techniques, Scanning Electron Microscopy, and pasta product quality analysis.

Materials and methods

Materials

Commercial sample of semolina from *T.durum* wheat was analysed for moisture, ash, gluten and protein content according to the AACC (2000) methods. Granulation test of the semolina was carried out with 200 g semolina sample in Plansifter (Buhler, Type MC 41 KS, Switzerland) using 670, 480, 340 and 193 micron sieves. The over-tailings of each sieve were weighed after running the sifter for 10 min.

Additives

Additives were selected based on the literature survey and preliminary laboratory trials. Glycerol monostearate (GMS) was procured from Biocon India Ltd., Bangalore, India. Sodium Steroyl Lactate (SSL) was procured from Organics, Mumbai, India and Hydroxy Propyl Methyl Cellulose (HPMC) was from Dow Chemicals, Mumbai, India

Sample preparation

Flow diagram of pasta sample preparation was shown in Fig. 1. Pasta dough was prepared using 1000 g of sample. The water used for control, SSL, GMS, and HPMC was 300, 295, 305 and 310 ml respectively. The amount of additives used was 0.5 g/100 g of semolina. The ingredients were mixed for 5 min in a Hobart mixer (Model N- 50, Ontario, Canada) at 59 rpm until coffee bean size dough pieces were formed. The dough was extruded using an extruder (La Monferrina, Italy) fitted with dies having perforation of 0.7 mm diameter. The strands of pasta were discarded initially. The pasta was dried at 75°C for 3 h in a Hot air drier (SAKAV, India).

Cooked pasta analysis

Cooking loss To evaluate the cooking quality, 25 g of pasta was added to 250 ml of boiling water. The samples were cooked for 10 min. At the end of 10 min cooking time, the material was drained for 5 min and volume of collected gruel was measured. The gruel was stirred well and 20 ml of the gruel was pipetted into a petri plate and evaporated into dryness over a water bath. Then the petri plate was transferred to a hot air oven maintained at 105±2°C and dried to constant mass (ISI 1993). Each analysis was repeated for four times.

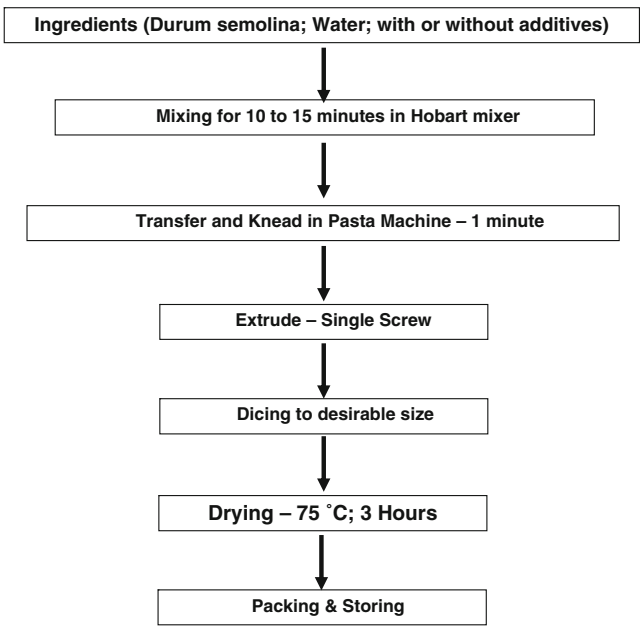

Fig. 1 Flow diagram for preparation of pasta samples

Cooked weight The pasta samples were cooked for 10 min and drained for 5 min. Cooked weight was determined by weighing the drained pasta and reported in grams (ISI 1993). Each sample was repeated for four times.

Pasta firmness The firmness was measured as described by Walsh et al. (1970). The firmness of pasta samples was measured by using a Texture Analyser model Tahdi (Stable Microsystems, U.K.). Three cooked pasta strands were sheared at a 90° angle. The shear was performed at a crosshead speed of 50-mm/min and load cell of 5 kg. The force required to shear the pasta was measured. The results reported are average of four readings.

Sensory evaluation A panel of trained judges carried out sensory evaluation of pasta by assigning scores for appearance (10), strand quality (10) and mouth feel (10). The overall quality (30) was taken as the combined score of all the three previous attributes mentioned above.

Statistical analysis

Statistical analysis of data was done by using Duncan's New Multiple Range Test as described by Steel and Torrie (1960).

Extraction of protein from pasta samples for SDS-PAGE and gel filtration studies

The pasta dough, dried pasta and cooked pastas samples were freeze-dried and powdered with mortar and pestle. Powdered samples (1 g) were suspended in 10 ml of phosphate-SDS buffer (pH 6.9), vortexed for 15 min and centrifuged at 10,000 rpm for 20 min in a high-speed centrifuge. The clear supernatant was used for SDS-PAGE and Gel filtration studies.

Biochemical characterization (SDS-PAGE) of protein fractions

SDS-PAGE (SDS-Polyacrylamide gel electrophoresis) analysis was carried out on horizontal electrophoresis system (Broviga, India) using 12.5% gel according to the method of Prabhasankar (2002). The pasta dough samples were freeze-dried and powdered with mortar and pestle. To the powdered pasta samples (10 mg), 300 µl of sample buffer (0.5M Tris HCl containing 25% of glycerin; 0.1% of bromophenol blue; 14.4 milligram percentage of 2-mercaptoethanol) was added and vertexed thoroughly, boiled for 5 min, cooled and spinned. 75 µl of the sample was loaded into each well of 12% poly acrylamide gel. Electrophoresis was run at constant voltage (50 V) for overnight until the tracking bromophenol blue dye reaches the end of the gel. The protein bands in gel after the electrophoresis was fixed for 15 min in solution of methanol: acetic acid: water (4.5:4.5:1.0). Then the gel was stained using coomassie brilliant blue (0.05% w/v in acetic acid: methanol: water (10:25:65) for overnight and destained repeatedly in the same solution without the dye.

Gel filtration chromatography of protein fractions of pasta

Size exclusion or Gel filtration chromatography was performed on a column of Sepharose 6B (Pharmacia, Sweden). The pre-swollen gel was equilibrated with elution buffer (50 mM Phosphate buffer pH 6.9 containing 0.5 g/100 ml SDS) and packed on a glass column (100× 0.9 cm). The extracted proteins were chromatographed using the same buffer at a flow rate of 14 ml/h and the fractionated proteins were monitored at 280 nm in a UV-visible spectrophotometer (Spectronic Genesus 5, Milton Roy).

Scanning electron microscopy

Freeze-dried uncooked and cooked pasta were taken and cut transversally with a sharp blade without damaging the structure. A Leo scanning electron microscope (Model 435 VP, Leo Electronic Systems, Cambridge, U.K) was used to scan the images. Freeze-dried samples from the surface and cross section were mounted on the specimen holder 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.

Results and discussion

Quality characteristics of raw material

The Durum semolina used for the present study had 9.2% moisture, 0.82% ash, 10.2% dry gluten and 11.8% protein on dry weight basis. The particle size distribution test showed that 6.8% of semolina retained on 32 W (670 µm) sieve, 26% on 45 W (480 µm) and 6.5% on 60 W (340 µm) and meets the required specifications for pasta making (Prabhasankar et al. 2007).

Changes in the wheat protein fractions during pasta making

The influence of processing steps on wheat protein fractions of pasta dough and semolina (on flour basis) was monitored by SDS-PAGE and shown in Fig. 2. The studies indicated that total band intensity of lane 5 (dried pasta) is observed lightly compared to the other lanes. This could be due to lower extractability of proteins from the

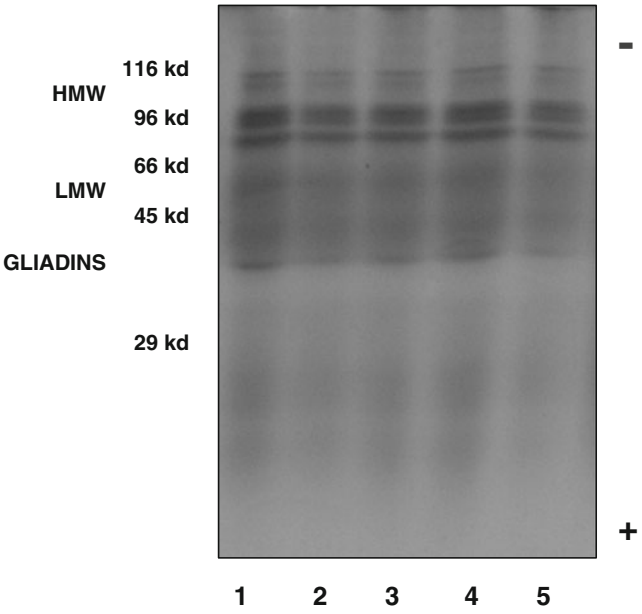

Fig. 2 SDS-PAGE pattern of semolina, pasta at different drying conditions and cooked pasta. *Lanes: 1. Semolina; 2. Pasta dough (0 h); 3. Pasta dough (1 h) 4. Pasta dough (2 h); 5. Dried pasta (3 h); 6. Cooked pasta*

dried pasta. However, the intensities of HMW bands (MW 97–116 Kd) were less than that of semolina. In the case of pasta dough, gluten formation may occur during mechanical mixing leading to lower extractability of the protein fractions. However, in the case of semolina the protein molecules are loosely bound and labile in native state and get extracted under normal and milder extraction methods. This could be due to denaturation of wheat protein fractions and subsequent re-aggregation during cooking of pasta. The present study indicated that wheat protein fractions play a vital role in pasta product development. Similar results were observed in different stages of *parotta* preparation (Prabhasankar et al. 2003). Singh (2005) studied the changes in wheat protein profile during bread baking using SE-HPLC. His studies indicated that decrease in protein solubility due to aggregation and/ of cross-linking was observed with time of baking. Results of the present study were also corroborated with earlier report on changes in wheat protein fraction during bread baking using SE-HPLC (Singh 2005).

Effect of incorporation of additives on the cooking quality characteristics of pasta

The data on the effect of additives on the quality characteristics of pasta is shown in Table 1. The quality characteristics study data such as, cooked weight, cooking loss, firmness and overall score of acceptability indicated that the use of HPMC improved the pasta making quality with improved cooked weight, minimum cooking loss and maximum overall score compared to control and other additives whereas SSL had an adverse effect on quality of pasta. Shiau (2004) studied the effect of SSL at different levels of incorporation on noodle texture quality. He found that the cutting forces of extruded noodles increased as the concentration of SSL increased from 0.5-1.5 g/100 g. However, present study compares incorporation of SSL, GMS and HPMC at 0.5% level on firmness (cutting force) of pasta. The results indicated that incorporation of 0.5% SSL resulted in less firm pastas than that of HPMC and GMS. Cooking loss is a commonly used predictor of overall spaghetti cooking performance by both consumers and industry. Shiau (2004) observed that SSL delayed the initial swelling temperatures of starch granules in the dough and raised the gelatinization temperature and resulted in increased cooking loss and more elastic pasta products. Similarly increased cooking losses were obtained in pasta containing SSL. This increase in cooking loss could be due to the disruption of the protein-starch matrix and the uneven distribution of water within the pasta matrix resulting from the competitive hydration tendency, thus preventing starch swelling due to limited water availability (Shiau 2004). The addition of glycerol monostearate (GMS) reduced the cooking loss for noodles incorporated with corn/ potato starch (Kaur et al. 2004; Defloor et al. 1991). Their studies also indicated that addition of GMS increased the consistency of bread dough. Similarly in the present study, addition of GMS to pasta product also decreased the cooking loss and improved the final quality characteristics of the product. This was corroborated with earlier report on the influence of additives in vermicelli made from *T. aestivum* wheat (Defloor et al. 1991). Pasta containing gums showed reduced cooking loss values (Shiau 2004). In the present study, HPMC showed reduced cooking loss.

Table 1 Effect of emulsifiers and gum on quality characteristics of pasta

	Cooked weight (g/25 g)	Cooking loss (%)	Appearance (10)	Strand quality (10)	Mouth feel (10)	Overall quality (30)	Texture (N)
Control	75±0.46 [a]	7.1±0.16[a]	7.5±0.16[a]	8.0±0.18[a]	7.0±0.16[a]	22.5±0.18[a]	2.5±0.01[a]
SSL	68±0.41[b]	9.1±0.18[b]	6.5±0.12[b]	7.0±0.16[b]	5.0±0.12[b]	18.5±0.16[b]	2.2±0.02[b]
GMS	77±0.52[a]	6.2±0.16[c]	8.5±0.16[c]	8.5±0.18[c]	8.0±0.18[c]	25.0±0.18[c]	2.5±0.01[c]
HPMC	82±0.64 [c]	5.6±0.12[d]	9.0±0.18[d]	9.5±0.21[d]	8.5±0.16[d]	27.0±0.21[d]	2.9±0.02[d]

[a, b, c, d] Column wise values with different superscripts are significantly different ($p<0.05$). Average of n = 4

Since HPMC is also a gum, the soluble nature and interaction that the subsequent hydrated polysaccharide network may have encapsulating in the starch-protein matrix contributing to the lower cooking loss. Gum forms a network around the starch granules, encapsulating them during cooking and restricting the excessive swelling and diffusion of the amylose content. However, present study doesn't confirm exact mechanism involved in this interaction /encapsulation. An in-depth study of molecular level interaction may be needed to prove the above hypothesis.

Effect of emulsifiers and gums on protein fractions of pasta

To understand the role of emulsifiers and gum on wheat protein fractions of pasta dough, SDS-PAGE followed by gel filtration chromatographic studies were carried out. SDS-PAGE pattern (Fig. 3) indicated that wheat protein fractions were unaffected in the case of SSL and GMS incorporated pasta. However, in the case of HPMC, the intensity of all fractions is less, which could be due to stronger interaction of HPMC with the protein fractions. In the case of emulsifiers (SSL and GMS) incorporated cooked pastas, a high molecular weight band having approximate (≈72 kd) was seen (Fig. 3).

In pasta uncooked sample, gel filtration pattern showed distinct four peaks obtained (Fig. 4a). The first peak (eluting in void volume Vo) corresponds to the high molecular weight glutenins and second, third and the fourth peak correspond to the low molecular weight glutenins eluting in the order of decreasing molecular size. In uncooked pasta samples with emulsifiers, there is no denaturation of the protein fractions but mild aggregation due to mechanical mixing may take place. It can be seen that in uncooked pasta containing SSL (Fig. 4b), the aggregation is more compared to all the other emulsifiers. This may be due to SSL, which is an anionic emulsifier. It promotes aggregation in the dough by incorporating negative charges into the complex and thus bringing the overall net charge to zero. Aggregation of low molecular glutenins is much higher in HPMC (Fig. 4d) than in SSL. Aggregation may also be due to polymerization resulting from cross-linking between the denatured proteins. Similar observations were made by Singh (2005) for protein fraction changes during bread making.

In cooked pasta samples, there is denaturation of proteins due to cooking. Due to increase in temperature, the protein loses its native configuration and begins to unfold. This can be clearly seen in cooked control pasta (Fig. 5). Similarly in cooked pasta with emulsifiers, there is a rapid denaturation of the HMW glutenins and aggregation with the low molecular weight glutenins. In cooked samples all the three protein peaks are clearly seen. Similar observations were reported by Singh (2005) on changes in wheat proteins during bread making using SE-HPLC.

Scanning electron microscopic studies (SEM)

To understand the effect of emulsifiers and gum on the wheat protein matrix, SEM was carried out using freeze-dried pasta samples and the micrograph images are shown in Fig. 6. In wheat, generally the starch granules are bound to the protein matrix and form a network. In pasta control uncooked sample (Fig. 6) the scanning electron micrograph studies showed the intact starch granules embedded in the protein matrix. The gluten network forms a uniform layer over the starch matrix. In the presence of GMS (Fig. 6), the gluten network has been strengthened. However in the case of SSL, the starch granules are not intact and they show

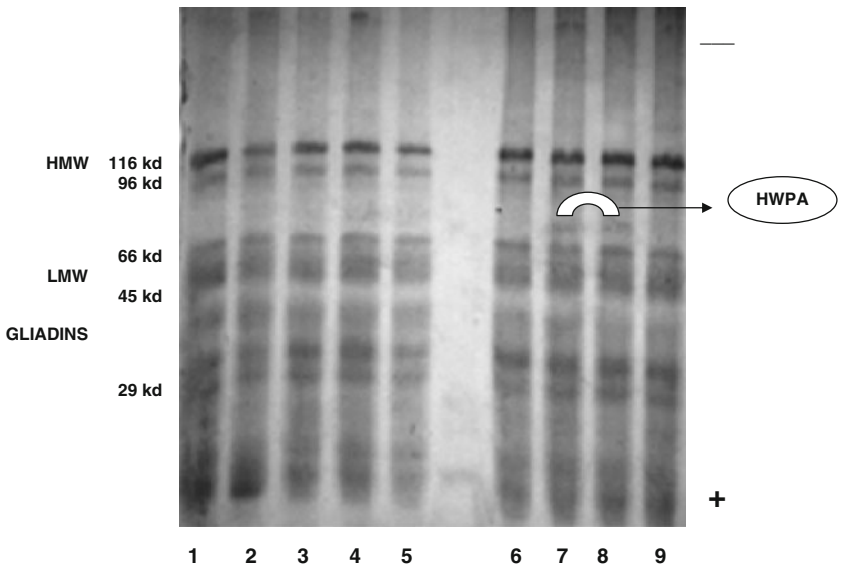

Fig. 3 SDS-PAGE pattern of uncooked and cooked pasta with three different additives. *Lanes 1. Semolina 2. Pasta uncooked control 3. Pasta uncooked containing Sodium Steroyl Lactate (SSL) 4. Pasta uncooked containing Glycerol Mono Stearate (GMS) 5. Pasta uncooked containing Hydroxy Propyl Methyl Cellulose (HPMC) 6. Pasta cooked control 7. Pasta cooked containing SSL 8. Pasta cooked containing GMS 9. Pasta cooked containing HPMC. HWPA: High molecular weight protein aggregate (≈72 kd)*

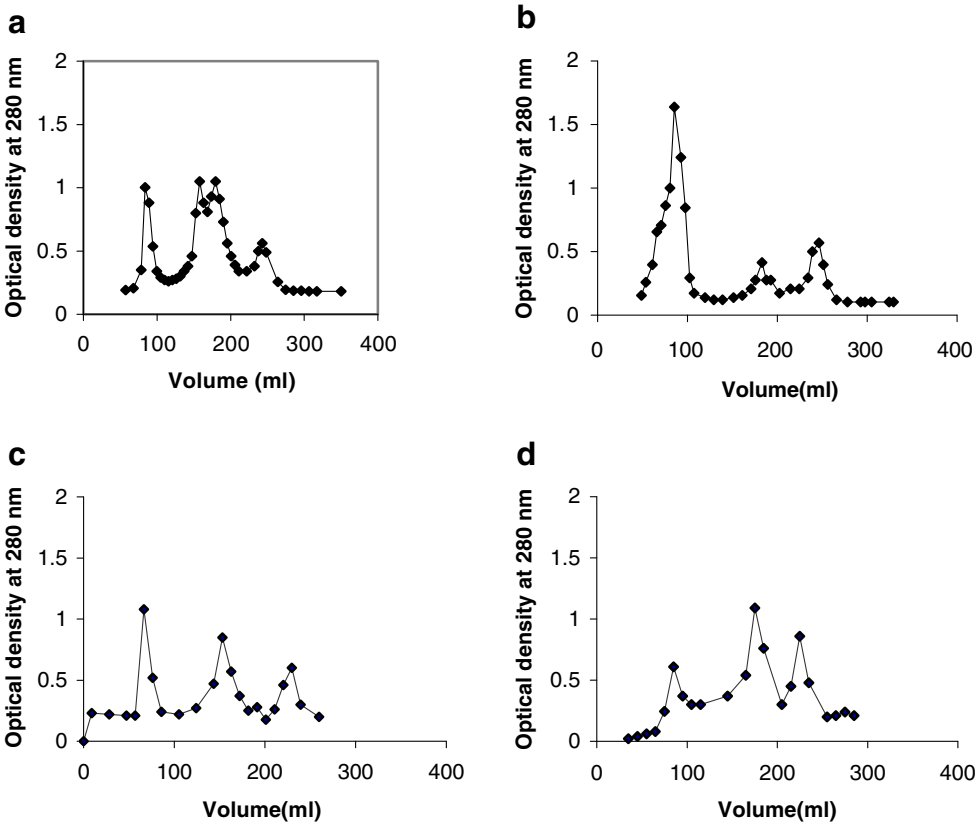

Fig. 4 Gel-filtration elution profiles of protein fractions in control and uncooked pasta with emulsifiers **a**. Control pasta; **b**. Pasta with SSL; **c**. Pasta with GMS; **d**. Pasta with HPMC

weak attachment to the protein matrix. Gluten network has not completely covered the starch granules. In the case of HPMC, there is a thin film of protein matrix, which covers the entire starch granules. There is a uniform gluten network envelope in which starch granules are gelatinized. Cooked pasta SEM (Fig. 6) indicated that HPMC and GMS has very uniform gluten network envelope in which starch granules are gelatinized, whereas in the case of SSL, starch granules uncovered by gluten network were observed. HPMC inclusion into pasta product show a different mechanism in which HPMC encapsulates the starch granules as a protective coat thus reducing the potential degradation of starch and overall leaching of starch upon cooking (Fig. 6). The SEM images of both cooked and uncooked pasta samples containing HPMC appeared to support this hypothesis. In the present study, the SEM images of pasta products studies were correlated with that of quality characteristics. Other microstructural studies of the bread crumbs also revealed possible interactions between HPMC and bread constituents suggesting that HPMC could involve all the bread constituents and block internal interactions (Barcenas and Rosell 2007). Rosell et al. (2007) also showed that HPMC in regular breads could interfere either in the starch-gluten interactions or in the formation of physical entanglements. Similar kind of observation was seen in SEM of cooked pasta with HPMC of present study. Jyotsna et al. (2004) in their studies, observed that in micrographs of vermicelli, honeycomb like gluten-starch network has been affected by addition of different additives.

Relationship between protein profiles, microstructure and quality characteristics of pasta

The present study deals the role of storage protein profiles (gliadin, LMW and HMW) in two different aspects of pasta preparation. The protein profile changes happening during processing of pasta were demonstrated with the help of electrophoresis, gel filtration and microstructure. The results indicated that incorporation of additives affects the protein profiles of pasta dough, and in turn the quality of pasta such as texture, sensory and cooking quality. Among additives used, HPMC was found to be more suitable than GMS and SSL. The present study indicated that HPMC resulted in the good quality pasta than that of GMS and SSL in terms of cooking quality, texture, and sensory attributes. In the case of HPMC incorporated pasta dough microstructures, the gluten network was strength, which in turn resulted in the good quality of pasta. Similarly, HPMC incorporated pastas had good cooking quality, which was demonstrated by the low cooking loss and high cooked weight. HPMC had different kind interaction with storage proteins than GMS and SSL.

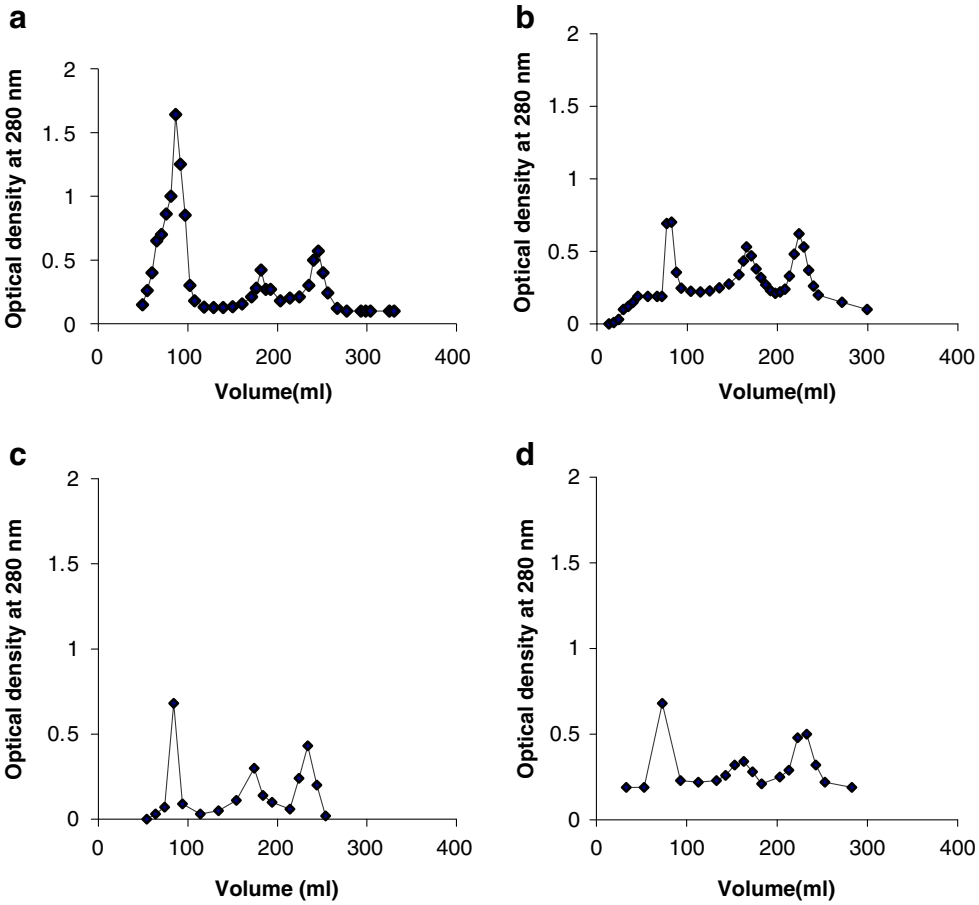

Fig. 5 Gel-filtration elution profiles of protein fractions in control and cooked pasta with emulsifiers. **a**. Control pasta; **b**. Pasta with SSL; **c**. Pasta with GMS; **d**. Pasta with HPMC

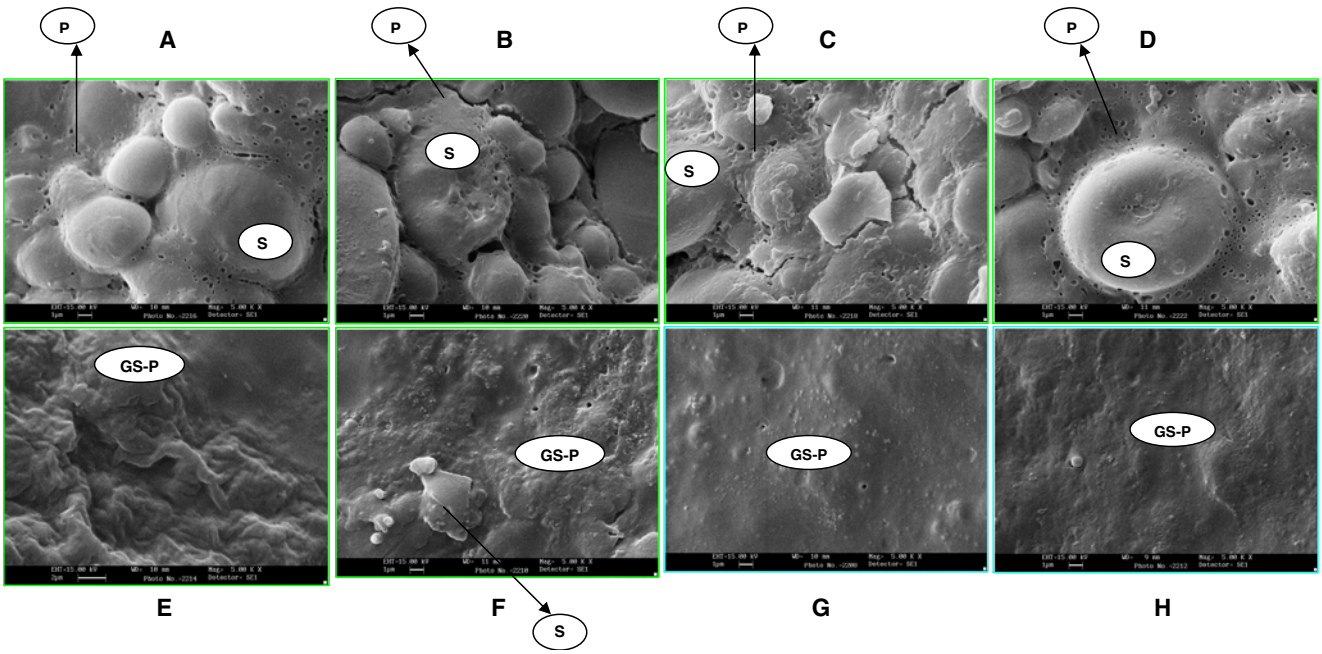

Fig. 6 Scanning Electron Micrographs of transverse sections of uncooked and cooked pasta (5000 X). Uncooked: **a**. Control; **b**. SSL; **c**. GMS; **d**. HPMC. Cooked: **e**. Control; **f**. SSL; **g**. GMS; **h**. HPMC. *S- Starch granules; P- Protein Matrix; GS-P: Gelatinized Starch- Protein network*

Conclusion

The present study indicated that wheat storage protein fractions play a major role in determining final quality of pasta products as evidenced by SDS-PAGE and gel filtration studies. Among the two emulsifiers [Sodium Steroyl Lactate (SSL), Glycerol Monostearate (GMS)] and one gum [Hydroxy Propyl Methyl Cellulose (HPMC)] chosen, pasta with HPMC was found to be most beneficial. This is because, the cooking loss for pasta containing HPMC is very less followed by GMS and SSL, and is evident from the scanning electron micrographs of cooked and uncooked HPMC samples. Thus, it is concluded that pasta with HPMC is having better quality characteristics than GMS and SSL as evidenced by SDS-PAGE, Gel filtration and SEM studies.

Acknowledgment The authors are greatly indebted to Dr.G. Venkateswara Rao, Head, Flour Milling, Baking and Confectionery Technology Department for his valuable suggestions and encouragement. The authors are also indebted to Mr.K.Anbalagan Central Instrumentation Facility Services, Central Food Technological Research Institute (CFTRI), Mysore, for technical help in carrying out scanning electron microscopy. Authors thank the Director, CFTRI for encouragement and permission to publish the work.

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