Influence of Addition of Wheat Gluten Fractions on Spectroscopic and Microstructural Assessment of Instant Noodles

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Abstract

Gluten protein fractions play a vital role in determining the quality of instant noodles. Thus, the effect of gluten fractions on microstructure and secondary structure of instant noodles prepared from two diverse wheat varieties DBW88 and HI1500 was studied by incorporation of gluten fractions at 3% and 5%. The highest percentage of intermolecular β-sheets (85.63%), intramolecular β-sheets (89.17%), β-turns (93.98) and anti-parallel β-sheets (92.85%) was found in variety DBW88 incorporated with 5% of HMW (High Molecular Weight) glutenins. Enhancement in the percentage of secondary structure component presented amide I band on the increasing proportion of glutenins, HMW and LMW (Low Molecular Weight) glutenin fraction displayed its polymeric character by higher disulphide bond formation in developed network of fortified instant noodles. The micrograph showed compact dense structure with long polymer strands on addition of glutenins and HMW glutenin fractions, while shortening of network was noticed on gliadin addition. A matrix with short strands and small continuous sheets enfolding the starch-protein network was revealed on LMW glutenin fractions addition. Thus, variation in microstructure and secondary structure of instant noodles was evident with the incorporation of gluten fractions derived from different varieties predicting its varietal variation.

Keywords

Fourier transform infrared spectroscopy, Secondary structure, Scanning electron microscope, Instant noodles microstructure, High molecular weight, Low molecular weight, Glutenin fractions

Introduction

Noodles have been serving as comfort food for the population of East and South east countries by virtue of its convenience, variety of flavors, palatability and affordable price [1]. The major ingredient used for the manufacturing of instant noodles is wheat flour. The prime components of wheat flour are protein and starch playing a major role in the quality of instant noodles. Protein quality parameters of wheat flour, as well as protein content, showed significant relationship with instant noodles quality parameters [2]. Wheat protein majorly comprised of gluten proteins formed by the combination of gliadins and glutenins where glutenins are responsible for elasticity and gliadins exhibit viscosity. Glutenins are also known as polymeric proteins composed of high molecular weight glutenin subunits (HMW-GS) and low molecular weight glutenin subunits (LMW-GS) linked by disulphide bonds [3]. Quality and quantity of gluten and the nature of its further subfractions plays a pivotal role in the dough rheology and, thereby, end-product quality. Evaluation of their specific role in the degree of gluten network formation and their individual role at microstructure level has not been explored to a great extent yet.
Fourier transform infrared (FTIR) spectroscopy is an advanced instrumental technique that takes less time to detect the proportion of the functional bond present in the sample. It has been exploited to uncover the secondary structure of the complex proteins including wheat proteins [4]. Secondary structure of wheat prolamins in the form of β-sheets, β-turns, α-helical and random structure has been elucidated along with the effects of hydration condition, freezing, sheeting, freezing [5-8] and other unit operations performed to prepare different wheat based products. Influence of gluten and its further subfractions on secondary structure of instant noodles has not been experimented yet. Thus, isolated gluten fractions were fortified in the flour to prepare instant noodles and explore the secondary structural effect of individual fraction.

Understanding of food microstructure has become vital in commercial food production, particularly in the development of new products, acknowledging the variations during processing, influence of ingredients and their interaction [9]. The microstructure of the three-dimensional network may be disclosed by examining hydrated gluten utilizing numerous microscopic techniques. Structural configuration formed by the combination of various proteins has mechanical properties differentiated from its fractions, which may be demonstrated in the material’s microstructure. Identification and modeling of microstructural characteristics of the gluten network along with their fractions interaction could be a substantial step toward improving flour industrial efficiency and establishing the knowledge of individual fractions on dough rheology and end product quality [10].

Thus, this study investigated the secondary structural and microstructural changes due to the incorporation of gluten and glutenin fractions in instant noodles which is very important to comprehend their role in its end-quality. To determine the proportional changes in the secondary structures, FTIR spectroscopy was employed, while to understand microstructural changes, scanning electron microscopy (SEM) was used.

Materials and Methods

Grain procurement and flour preparation

Grains of two different wheat varieties, namely DBW88 and HI1500 procured from Chaudhary Charan Singh Haryana Agriculture University (CCS HAU), Hisar (Haryana, India), and IARI Regional Centre, Indore (Madhya Pradesh, India), respectively. The varieties DBW88 and HI1500 had Glu-1 score of 10 and 4, respectively. The grains of the two varieties were ground in a pestle mortar, the freeze-dried gliadin and glutenin fractions were pulverized by using a Lark lyophilizer. 50 g of powdered gluten was extracted in 1 L of 70% (v/v) ethanol and mixed for 3 hrs at room temperature (22 °C) on a magnetic stirrer. The mixture was then centrifuged at 1000g for 30 min at 4 °C in a cooling centrifuge. The extraction was carried out three times. The alcohol insoluble were collected as glutenins, and the alcohol-soluble gliadins were recovered from the supernatant by evaporating the ethanol with a rotary evaporator at 30 °C. In a pestle mortar, the freeze-dried gliadin and glutenin fractions were pulverized.

Fractionation of HMW and LMW glutenin fractions

Defatted flour (24 g) was extracted (30 min, 60 °C) three times with 50% (v/v) propan-2-ol (1200, 1200, and 600 ml respectively) for the isolation of HMW-GS and LMW-GS as per Melas et al. [13]. The sample mixture was centrifuged for 30 min at 12,000×g, 20 °C to separate gliadins, albumins, and globulins. Glutenin subunits were extracted (30 min, 60 °C) from the final residue with 0.08 M Tris–HCl (pH 8.0) in 50% (v/v) propan-2-ol containing 1% (v/v) β-mercaptoethanol (120 ml). The sample mixture was centrifuged for 30 min at 12,000×g, 20 °C. The supernatant obtained was subjected to 40% (v/v) acetone precipitation for HMW-GS and then centrifuged for 30 min at 12,000×g, 20 °C. To precipitate LMW-GS, the supernatant acetone concentration was increased up to 80% (v/v) and centrifuged for 30 min at 12,000×g, 20 °C. HMW-GS and LMW-GS precipitates were washed with distilled water to obtain enriched protein fractions and then subjected to dialysis against 1% acetic acid solution for 72 hrs to maintain its functionality [14]. The enriched precipitates were freeze-dried and ground in IKA grinder (2 min) to pass through a 250 μm screen.

Preparation of instant noodles

Instant noodles were developed utilizing optimized processing conditions and formula by Gulia and Khatkar [15]. The dough was made by combining flour, guar gum, and water with dissolved salts (sodium chloride and kansui) for four minutes in a mixer. To stop moisture loss, the prepared dough was sheeted to a thickness of 3.2 mm and then allowed to rest in a zip-lock bag for 10 min. Ultimately, the dough sheet was made to 1.2 mm thick and let to rest in a zip-lock bag for 30 min. The sheets were divided into strands, then the steamer tray was set up for 6.4 min at 100 °C. The steamed noodles were cooked for two minutes at 142 °C in soybean oil, then allowed to cool for fifteen minutes. Samples of prepared instant noodles were swabbed to draw any extra oil and then placed in zip-lock bags for further investigation. The control samples were prepared from the respective flour i.e., DBW88 and HI1500. Further, flour samples blended with 3% and 5% of gliadins, glutenins, HMW and LMW glutenins which were used for preparation of instant noodles.

FTIR spectroscopy analysis

The secondary structure of gliadins, glutenins, HMW and LMW glutenin fractions incorporated instant noodles samples prepared from two wheat varieties i.e., DBW88 and HI1500 were studied by FTIR spectroscopy. IR-spectral studies were performed on Shimadzu IR affinity-I 8000 FT-IR
spectrometer under dry air at room temperature using KBr pellets. Sample (1 mg) was mixed with 300 mg of KBr supplied with FTIR unit. The samples were pressed directly on to attenuated reflectance KBr crystal into the sampling unit. Spectra were scanned between 1500 and 1700 cm\(^{-1}\), acquired at 4 cm\(^{-1}\) and signal averaged over 32 scans. According to Kaur et al. and Wang et al. \(^{16, 17}\), secondary structures were assigned as intermolecular β-sheets (1613-1620 cm\(^{-1}\)), intramolecular β-sheets (1627-1635 cm\(^{-1}\)), α-helices (1650-1660 cm\(^{-1}\)), β-turns (1670-1680 cm\(^{-1}\)), anti-parallel β-sheets (1680-1695 cm\(^{-1}\)). The percentage of mentioned structures was calculated using Origin software considering amid-I band (1580-1720 cm\(^{-1}\)) as 100%.

**SEM analysis**

For microstructural analysis of fortified and control freeze-dried instant noodles, fractured samples were fixed on carbon tape pasted on the sample stub. The exposed fractured area of the sample was coated with gold NP using Sppurtex coater (DIT-290305 CIR). The coating was done for 0.5 sec with a thickness of 2-3 nm to make the entire sample conductive. These gold-coated samples were analyzed on a scanning electron microscope (JSM-7610F Plus JEOL, JAPAN) with EDAX-APEX software. The micrographs of freeze-dried instant noodles were viewed at magnification of 100X.

**Results and Discussion**

Secondary structure analysis of control and supplemented instant noodles with gliadins, glutenins, HMW and LMW glutenin fractions

The amide I band is fractionated into 5 components representing the different secondary structure of control and fortified instant noodles with 3% and 5% of gliadins, glutenins, HMW and LMW glutenin fractions prepared from wheat varieties DBW88 and HI1500 as shown in table 1. The FTIR spectra of control and fortified instant noodles with 3% and 5% of gliadins, glutenins, HMW and LMW glutenin fractions prepared from wheat varieties DBW88 and HI1500 as shown in figure 1. On comparing the secondary structure of the control instant noodles prepared from varieties DBW88 and HI1500, the content of the intermolecular β-sheets (23.50%), β-turns (31.77%) and anti-parallel β-sheets (33.14%) were comparatively higher in variety DBW88 with lower content of intramolecular β-sheets (21.77%), and α-helical and random structure (25.61%). This indicates that the percentage of polymeric proteins responsible for forming inter-and intra-disulphide bonds are present higher in proportion in variety DBW88.

However, the higher content of α-helical and random structure is present in the variety HI1500 indicating the higher proportion of monomeric proteins i.e. gliadins responsible for fluidity of the network. Similar observation was revealed in a recent work where gliadins lead to higher percentage of α-helical when observed in dough fortified with gliadins fraction \(^{18}\). As the proportion of α-helical structure is negatively correlated with the β-sheets content, where the later was found to be positively associated with dough strength, on the contrary former had negative impact on dough properties and then ultimately on end product quality \(^{5}\).

On gliadins inclusion in instant noodles, the proportion of intermolecular β-sheets increased from 25.55% at 3% to 27.98% at 5% fortification level in variety DBW88 on comparing with its control (23.50). The proportion of intermolecular β-sheets in instant noodles of variety HI1500 decreased from 18.23% at 3% to 16.90% at 5% supplementation of gli-
The classification of LMW-GS on the basis of amount of glutenins in variety Hi1500 than variety DBW88. This might be due to the variation in the subunits involved in more bonding due to the structural variation.

Higher percentage of β-sheets and β-turns structure signifies the dough strength of variety DBW88 due to the higher content of glutenins of strong variety as revealed by Dhaka and Khatkar [21, 22]. Dhaka and Khatkar [21] noticed the presence of higher proportion of inter and intra molecular β-sheets and β-turns in good bread making variety Hi977, whereas lower intensity of β-turns and spiral structure in case of poor bread making variety C306. Similar results were observed here where lower content of β-turns and anti-parallel β-sheets were found in variety Hi1500 than variety DBW88. Glutenin fractions are responsible for the formation of higher proportion of β-turns forming spiral structure due to the presence of highly polar glutamine amino acid [23, 24].

LMW glutenins addition in variety Hi1500 exhibited stronger influence on enhancement in the secondary structure than in variety DBW88. The rise in content of secondary structure components was noticed in intermolecular β-sheets from 61.31% at 3% to 76.88% at 5%, intramolecular β-sheets from 52.59% at 3% to 74.69% at 5% and anti-parallel β-sheets from 60.10% at 3% to 79.13% at 5% addition in variety Hi1500. In the secondary structure of LMW-GS, subunits with N-terminal proposed to exhibit irregularly dispersed β-turns, while C-terminal forms α-helical structure [25]. The classification of LMW-GS on the basis of amount of cysteine residues are chain extenders, chain branchers and chain terminators, where three or more cysteine residues forming intermolecular disulphide bonding are “chain branchers”, whereas “chain extenders” have two cysteine residues and “chain terminators” are just with one cysteine only [26].

Table 1: Amide band I represent different proportion of secondary structure of control and gluten fractions incorporated instant noodles samples of varieties DBW88 and Hi1500.

<table>
<thead>
<tr>
<th>Instant noodle’s samples prepared from blends*</th>
<th>FTIR Wave number of amide band I representation</th>
</tr>
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<tbody>
<tr>
<td></td>
<td>Intermolecular β-sheets (%)</td>
</tr>
<tr>
<td></td>
<td>1613-1620 cm⁻¹</td>
</tr>
<tr>
<td>Control sample (DBW88)</td>
<td>23.5</td>
</tr>
<tr>
<td>DBW88 + 3% gliadins</td>
<td>25.55</td>
</tr>
<tr>
<td>DBW88 + 5% gliadins</td>
<td>27.98</td>
</tr>
<tr>
<td>DBW88 + 3% glutenins</td>
<td>49.75</td>
</tr>
<tr>
<td>DBW88 + 5% glutenins</td>
<td>69.96</td>
</tr>
<tr>
<td>DBW88 + 3% LMW glutenins</td>
<td>61.22</td>
</tr>
<tr>
<td>DBW88 + 5% LMW glutenins</td>
<td>64.16</td>
</tr>
<tr>
<td>DBW88 + 3% HMW glutenins</td>
<td>65.03</td>
</tr>
<tr>
<td>DBW88 + 5% HMW glutenins</td>
<td>85.63</td>
</tr>
<tr>
<td>Control sample (HI1500)</td>
<td>20.45</td>
</tr>
<tr>
<td>HI1500 + 3% gliadins</td>
<td>18.23</td>
</tr>
<tr>
<td>HI1500 + 5% gliadins</td>
<td>16.9</td>
</tr>
<tr>
<td>HI1500 + 3% glutenins</td>
<td>41.64</td>
</tr>
<tr>
<td>HI1500 + 5% glutenins</td>
<td>52</td>
</tr>
<tr>
<td>HI1500 + 3% LMW glutenins</td>
<td>61.31</td>
</tr>
<tr>
<td>HI1500 + 5% LMW glutenins</td>
<td>76.88</td>
</tr>
<tr>
<td>HI1500 + 3% HMW glutenins</td>
<td>60.81</td>
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<tr>
<td>HI1500 + 5% HMW glutenins</td>
<td>57.98</td>
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</tbody>
</table>

*DBW88 and Hi1500 are the wheat variety samples, LMW-Low molecular weight and HMW- High molecular weight.

Adins. In terms of β-turns and anti-parallel β-sheets, the rise of percentage was higher in variety DBW88 than in variety Hi1500 when gliadins fractions were incorporated in the instant noodles. On the contrary, the proportion of α-helical and random structure and intramolecular β-sheets was higher in instant noodles of variety Hi1500 than of variety DBW88 on the addition of gliadins. Gliadins samples for secondary structural analysis exhibited more content of α-helical and random structure, however, glutenins samples disclosed the majority of highly aggregated structure in the form of β-sheets [19]. The tendency for the formation of greater β-sheets was by the virtue of enhanced protein aggregates, intermolecular disulphide bonds, disulphide cross links, other non-covalent and irreversible bonds [20].

On glutenins addition in instant noodles, both varieties exhibited great variation in terms of its secondary structure. The percentage of intermolecular β-sheets got raised from 49.75% at 3% to 69.96% at 5% incorporation level of gliadins in variety DBW88, meanwhile the other components like β-turns and anti-parallel β-sheets enhanced from 55.61% at 3% to 77.89% at 5% and 56% at 3% to 79.96% at 5% addition, respectively. In variety Hi1500, increment in inter-and intra-molecular β-sheets, α-helical structure, β-turns and anti-parallel β-sheets was observed when gliadins were incorporated at 3% and 5% in instant noodles, but rise was comparatively higher in variety DBW88. This might be due to the variation in the subunits involved in more bonding due to the structural variation.

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Influence of Addition of Wheat Gluten Fractions on Spectroscopic and Microstructural Assessment of Instant Noodles

Dewan and Khatkar.

The chain extending effect of LMW glutenin fractions on dough and gluten has been recorded by Dewan et al. [18], Dewan and Khatkar [22], respectively. The possibility of presence of chain extending subunits of LMW glutenins existed with variety HI1500 predicting the strong ordered structure with higher proportion of inter-and intra-molecular β-sheets, β-turns and anti-parallel β-sheets due to the formation of disulphide bonds in higher proportion than on incorporation of LMW glutenins from variety DBW88.

When HMW glutenins were added in instant noodles prepared from DBW88, there was surge in the intermolecular β-sheets (from 65.03% to 85.63%) and intramolecular β-sheets (from 81.84% to 89.17%) along with β-turns (from 78.29% to 93.98%) and anti-parallel β-sheets (from 86.97% to 92.85%) when percentage increased from 3% to 5%. While in variety HI1500, a rise in intermolecular β-sheets from 20.45% (control) to 60.81% (3% HMW glutenins) but decline on 5% addition with 57.98%. However, on evaluating similar patterns incurred in other secondary structure like intramolecular β-sheets, β-turns and anti-parallel β-sheets. The native secondary structure of HMW-GS has been proposed to form high amount of intermolecular β-sheets by the virtue of more creation of hydrogen bonds due to N and C terminal [27]. Gluten secondary structure is influenced by the variation in the subunits of HMW glutenins. Higher percentage of β-sheets and β-turns are responsible for the dough strength and ultimately end product quality [28]. α-helical and β-sheets secondary structure have been correlated negatively to each other stating the rise in former one might lead to fall in the later indicating that α-helical are negatively associated with the dough and end product quality [5].

Microstructure of instant noodles supplemented with gliadins, glutenins, HMW and LMW glutenin fractions

The micrograph of freeze-dried instant noodles from control and supplemented with gliadins, glutenins, HMW and LMW glutenin fractions prepared from wheat varieties DBW88 and HI1500 presented in figure 2. In the micrograph of the control dough both varieties DBW88 and HI1500, the uniformity in the matrix with no cavities was revealed in the instant noodles prepared from variety DBW88 than variety HI1500 where number of hollow gaps were clearly visible predicting its weak structure and network. The weak structure of variety HI1500 might be due to the variation in its balance of gliadins and glutenins, where when the proportion of viscous proteins i.e., gliadins increased, it leads to weakening of the network. On the contrary, the higher proportion of glutenins is responsible for the strengthening and toughening of the network as well as dough, thereby end product. This was supported by the evidence derived from the secondary structure evaluation of the control instant noodles of both varieties DBW88 and HI1500.

Gliadin fortification in both the varieties displayed the creation of more gaps and cavities, but proportion of hollow caves was higher in varieties HI1500 which increased with percentage of gliadins inclusion from 3% to 5%. The prominent and excessive breakage along with discontinuity in the matrix was observed in the 5% gliadin addition in variety HI1500. However, on 3% incorporation in strong variety DBW88, the matrix was still more uniform network with one gap and few ridges in the instant noodles, but the percentage of gaps and cavities got increased on 5% supplementation of gliadins. This denoted the opening of gluten network due the monomeric nature of the gliadins leading to the shorting of the gluten network developed leading to the rise of cavities. Gliadins have been visualized as single molecules under SEM analysis [29].

Therefore, these monomeric proteins are responsible for opening of network, leading to more viscosity of dough and thereby reducing the elasticity by acting as plasticizers. As
Influence of Addition of Wheat Gluten Fractions on Spectroscopic and Microstructural Assessment of Instant Noodles

Dewan and Khatkar.

The 1Dx5 subunit contains an additional cysteine at the initial terminal of repetitive domain, while the 1Bx14 and 1Bx20 subunits comprise of two cysteine residues with one at N-terminal and other at C-terminal [35]. The former subunit leads to additional bond formation responsible for dough extension and strong network, while latter subunit might create short network sheets with lesser strength comparatively. This could be the possible reason for the variation in the microstructure of the HMW glutenins of both varieties.

Recently, glutenins addition in the dough have been revealed in the formation of strands due to its polymeric nature [18, 34] which was noticed here also in the micrographs. The variation in both varieties HI1500 and DBW88 might be due to its glutenin subfractions which have illustrated in the further micrographs of instant noodles incorporated with glutenin fractions.

On glutenins inclusion in the instant noodles in both varieties contributed to the denseness and compactness of the developed network. In variety DBW88, formation of ridges and polymeric strands in the matrix increased with the increase in concentration of glutenins from 3% to 5% in instant noodles. While short uniform sheets with few break points and cavities were noticed in HI1500 with 3% glutenin addition. On 5% supplementation of glutenins in variety HI1500, continuous viscous uniform sheets with few hollow gaps were observed on comparison with 3% glutenin addition. On comparison of instant noodles micrograph of both the varieties with glutenin addition, dense and uniform matrix with less cavities were noticed in variety DBW88 than in variety HI1500. Previous studies of glutenin microstructure have elaborated as sheets of arranged proteins in the form of spiral structure [32] and then with contradiction in the form of fibril morphology at the atomic level due to hydrogen bond formation [33].

On the addition of LMW glutenin fractions, the pattern of intense structure with hollow pockets were observed in instant noodles prepared from both the varieties DBW88 and HI1500. In variety DBW88, short strands of LMW glutenins forming network, where gaps in the form of pockets were observed on 3% fortification. On increasing the concentration to 5% of LMW glutenins in variety DBW88, surface compactness increased with decrease in hollow gaps revealing the strength giving property of LMW glutenins. While comparing the addition of LMW glutenins in variety DBW88 with variety HI1500, compact structure with fewer cavities was found in variety HI1500 than in variety DBW88 at 5% fortification level. However, on 3% addition of LMW glutenins in variety HI1500 displayed short, sheeted structure with few hollow gaps and short strands probably denoting the LMW-GS polymers. HMW-GS, being the backbone of gluten network is distributed in the continuous pattern, however, LMW-GS occurs as aggregates in a discrete manner [36].

LMW-GS chain terminating effect have been postulated by Dangi et al. [34] where 1% inclusion of purified fraction of LMW-GS exhibited short polymer connection in the dough network. Here, in variety DBW88, LMW glutenins exhibited the chain terminating effect in the form short sheets with more breakage while in variety HI1500, LMW glutenins re-
vealed more strengthening in the network on comparison by the virtue of subunits responsible for chain extension. This might be due to the presence of more cysteine residues present in the latter than the former. As on the basis of structural characteristics, out of B, C and D types, B-types subunit act as a chain extender unit by the virtue of their ability to form two intermolecular disulphide bonds [37]. This can be the possible reason for the formation of more uniform structure with fewer cavities in LMW glutenins supplemented instant noodle’s microstructure of variety HI1500.

Conclusions

The consequence of the research revealed that the secondary structure and microstructure of instant noodles changes significantly on the incorporation of gliadins, glutenins, HMW and LMW glutenin fractions at the level of 3% and 5% in the respective base flour of varieties DBW88 and HI1500. Varietal as well as proportional influence was observed with significant difference in both strong variety DBW88 and weak variety HI1500. Gliadins inclusion in variety HI1500 escalated the α-helical structure more due to its monomeric nature and presence of higher proportion of gliadins in native form. Glutenins of variety DBW88 created higher proportion of β-sheeted structure than the glutenins of variety HI1500 due to the allelic variation.

Microstructural differences of both the varieties were noticed due to the varietal and native composition, where more open and short sheeted structure was observed in variety HI1500, while glutenins of variety DBW88 predicting more bonding created dense and compact network. The contrasting behavior of HMW and LMW glutenin fractions of both varieties was associated with the allelic variation due to structural differences, number of cysteine residues and its location. LMW glutenins of variety HI1500 enhanced the β-sheets and β-turns in instant noodles due to the inclusion of chain extender subunits while increased inter and intramolecular β-sheets along with anti-parallel β-sheets and β-turns was noticed on HMW glutenins of variety DBW88 by the virtue of its subunits with more cysteine residues creating more disulphide bonds and spiral structure. This secondary structural revelation was supported by micrographs of HMW and LMW of both varieties. Dense and uniform sheets with long strands were formed on inclusion of HMW glutenins of variety DBW88 and LMW glutenins of variety HI1500.

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Conflict of Interest

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S249
Influence of Addition of Wheat Gluten Fractions on Spectroscopic and Microstructural Assessment of Instant Noodles

Dewan and Khatkar.


