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# Isolation, characterization of wheat gluten and its regeneration properties

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Abstract In order to assess the effectiveness of different drying methods on physicochemical and reconstitution properties of wheat gluten, four wheat cultivars were selected and milled. Gluten was extracted and its wet and dry gluten content and water holding capacity were estimated. The washed starch and other flour constituents were dried. Isolated gluten was dried using three treatments viz. oven drying, vacuum drying and freeze drying. Dried gluten of four wheat cultivars were characterized for its water and oil absorption properties and thermal properties. The dried gluten and washed and dried flour constituents were then reconstituted and this flour was checked for flour quality (SDS volume. texture analysis and falling number). Only reconstituted flour using freeze dried gluten showed no significant difference to control flour in SDS volume and dough strength. In Falling number all reconstituted flour samples showed significant difference to control flour.

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Department of Food Technology and Nutrition, Lovely Professional University, Phagwara 144411, Punjab, India e-mail: nkft87@yahoo.com Keywords Gluten  $\cdot$  Water and oil absorption properties  $\cdot$  DSC  $\cdot$  SDS volume

## Abbreviations

- (F) Freeze dried gluten
- (O) Oven dried gluten
- (V) Vacuum dried gluten

## Introduction

Wheat contains a complex mixture of proteins that have unique property of being able to form viscoelastic dough when flour is mixed with water (Delcour et al. 2010). Gluten, the protein component of flour which gives the dough elasticity and strength, can be defined as the rubbery mass that remains when wheat dough is washed to remove starch granules and water soluble constituents (Wieser 2007). The baking industry requires flour to have defined quality characteristics including protein content, wet and dry gluten and rheological properties (Miralbes 2004).

Gluten may be defined as the 'cohesive, visco-elastic proteinaceous material prepared as a by-product obtained by isolation of starch from wheat flour. A biological definition might include the origins of the gluten–protein complex as being derived from the 'storage proteins of the wheat grain' (Shewry and Halford 2002). The final step of gluten extraction is the drying of the gluten extract. Drying of the gluten extract has received considerable attention by the researchers because high temperature drying often has negative effects on the gluten functionality (Czuchajowska and Paszczynska 1996). For commercial gluten production, the gluten is dried with high temperature air, whereas in the laboratory, freezing and vacuum drying have been reported to produce gluten with better functionality for bread making (Esteller et al. 2005). The gluten content is directly correlated to the grain protein, which is strongly influenced by the pedoclimatic conditions. However, the wheat genotype is considered the most important factor influencing the qualitative characteristics of gluten (Simic et al. 2006). Increase in total protein content of the flour positively correlates to the gluten content (Perten et al. 1992). Ratio between wet gluten (WG) content and grain protein (P) content (WG/P) is considered as an indicator of wet gluten production per protein unit. Simic et al. (2006) reported that Croatian wheat with WG/P ratios ranging between 2.7 and 3.0 have gluten with optimal baking characteristics, while cultivars with strong gluten characteristics showed the WG/P ratio closer to 2.3.

Bread making potential of flour is mainly based on the protein quantity and quality (Hruskova and Famera 2003). Gluten plays a key role in determining the unique baking quality of wheat by conferring water absorption capacity, cohesiveness, viscosity and elasticity on dough (Wieser 2007). The oil and water absorption capacity, emulsifying properties and foaming are commonly used to characterize food protein (Transmo et al. 2002). Singh and Singh (2006) reported that protein structure and the presence of other minor components influence water and oil absorption capacity of gluten.

The sedimentation value indicating correlation with gluten content, gluten quality and loaf volume depends on the protein composition and is mostly correlated to the protein content. Therefore, it is useful to measure sedimentation value (Hruskova and Famera 2003). Thermal analysis is a valuable tool for studying the effect of thermal processing on vegetable proteins (Leon and Rosell 2003). Differential scanning calorimetry (DSC) is a method well suited for the study of thermal denaturation processes (Hegg et al. 1978). When proteins are heated they usually undergo thermal denaturation; the proteins unfold and become less ordered forming a random coil. The denaturation of proteins has been extensively studied by differential scanning calorimetry (DSC) where denaturation is observed as an endothermic peak on the thermogram (Leon and Rosell 2003).

Therefore, the present study was designed to determine the effect of different drying methods on the water and oil absorption capacity and thermal properties of gluten isolated from four different Indian wheat cultivars. The regeneration potential of dried gluten was also determined.

## Material and method

## Materials

Four wheat cultivars viz. C-306, Raj 3765, PBW-343 and KW-11 were procured form wheat breading farm, Haryana Agriculture University, Hisar, India. Airtight plastic containers were used for grain storage and flour storage. Parad

tablets (Himalya, India) were put into grains container enclosed in cloth for protection of wheat grains during storage.

#### Milling of wheat cultivars

The wheat grains were yeast treated using method as described by Siegel (1987). Wheat grains were steeped in water having yeast (57 g/kg grain), sugar (28.5 g/kg grain) and vinegar (10 ml/kg) at 35 °C to 40 °C for 2 h. Grains were then washed three times with tap water and dried under sunlight. Wheat grains were then tempered and moisture content was maintained to 16 %. The above pretreated wheat cultivars were milled using roller-mill (Chopin Laboratory CD-1 mill, France) and the flour obtained was stored in airtight plastic containers under ambient conditions for further analysis. In order to ensure the purity of the roller-milled flour samples from each lot, mechanical and manual cleaning of the roller-mill, including air blasting, were applied between milling of each of the wheat samples.

## Gluten extraction

The gluten extraction was carried out adopting the procedure as described in AACC (2005). Dough was prepared using 2 % sodium chloride solution at the rate of 60 % of the weight of flour. Prepared dough was kept immersed in water for 40 min. The dough was washed under stream of running water until most of the starch was washed out and the wash water was clear. The viscoelastic mass obtained was wet gluten. Added salt tighten up the gluten, decrease the binding of lipid during the separation process and wash out salt soluble proteins. The minimum gluten content of wheat flour should be about 24 % (wet) and 8 % (dry) (Singh and Singh 2006).

Wet gluten yield and gluten index

The wet gluten yield of wheat cultivars was determined. The dough was washed and gluten retained was collected and weighed for the determination of wet gluten yield. The wet gluten yield was calculated by the formula given below

Wet gluten yield = weight of wet gluten obtained  $\times$  100 weight of flour

Gluten index was determined according to AACC (2005). Wet gluten was centrifuged and the resultant mass was passed through a specially constructed sieve under standardized conditions. The percentage of wet gluten remaining on the sieve after centrifugation is defined as the Gluten Index. The gluten index was calculated by the formula given below

Gluten index = weight of wet gluten remained  $\times$  100 weight of wet gluten

# Dry gluten yield

The dry gluten yield was determined by drying wet gluten in freeze dryer for 24 h and dry yield was calculated.

Dry gluten yield = weight of dry gluten obtained  $\times$  100 weight of flour

# Moisture content of gluten

The Water absorption capacity of wet gluten was determined by method as described by AACC (2005). The moisture content of wet gluten was determined by drying of wet gluten in oven at 120 °C for 4 h.

# Gluten drying

Extracted gluten was dried using three methods viz. freezedrying, vacuum drying and oven drying. The gluten was freeze dried at -89 °C for 24 h, vacuum dried at 60 °C for 4 h, oven dried at 80 °C for 4 h. Dried gluten samples were powdered in a falling number milk (model 3100, Sweden) to pass through a 100 mesh sieve and packed in polyethylene bags and stored in air tight containers at refrigeration temperature (4 to 7 °C) till further analysis.

# Water absorption capacity

Water absorption capacity of dried wheat gluten samples was determined using method as described by Sosulsk (1962). The gluten sample (500 mg) was dispersed in 10.0 ml of water, mixed thoroughly and agitated for 1 h and centrifuged at 2000 rpm for 30 min. The supernatant was discarded and sediment was weighed.

Water absorption capacity = weight of sediment  $\times 100$  weight of sample

# Oil absorption capacity

The oil absorption capacity of dried wheat gluten samples was determined using the method of Lin et al. (1974). The gluten sample (500 mg) was added to 10.0 ml of refined soybean oil (Fortune brand), mixed thoroughly and agitated for 1 h. Then the sample was centrifuged at 2000 rpm for 30 min. The supernatant was discarded and sediment was weighed.

# Thermal properties of wheat gluten

The thermal properties of wheat gluten were determined adopting the procedure described by Leon and Rosell (2003). Powdered dried gluten samples were analyzed to check the thermal properties by differential scanning calorimetry (DSC-822, Mettler Toledo, Greifensee, Switzerland) equipped with a thermal analysis data station. The dried gluten samples (8.0 mg) were loaded into a 100  $\mu$ l capacity aluminium pan. Samples were hermetically sealed. The DSC analyzer was calibrated using an empty aluminium pan as reference. Sample pans were heated at a rate of 10 °C from 40 to 130 °C. Onset temperature (To); Peak temperature (Tp); conclusion temperature (Tc) and Enthalpy of gelatinization ( $\Delta$ H gel) were calculated automatically. The Peak height index (PHI) was calculated by the ratio  $\Delta$ Hgel/(Tp- To).

# Regeneration of gluten

During gluten extraction, the washed flour constituents were collected and dried in petriplates at 80 °C in oven for 4 h. The blend of dried flour constituent's powder was mixed with dried gluten in 9:1 ratio (as wheat contain 8-12 % gluten protein) and evaluated for flour quality attributes to check the regeneration potential of gluten.

# SDS-sedimentation volume test

Sodium dodecyl sulfate (SDS Solution) sedimentation volume of flour samples was estimated according to the method as described by Axford et al. (1978).

# Falling number test

The falling number was determined by approved method as described by AACC (2005) and the results were expressed in time as seconds.

# Dough tensile strength and percent elongation at break

Dough tensile strength and percent elongation at break of dough samples was determined using a Texture Analyzer TA.XT2i (Stable Micro System, Surrey, UK), operated according to the ASTM Standard Method. Three specimens (40 mm wide) of each film were measured and cut using cutter. The peak loads and extension at break were recorded for testing film specimens. The tensile strength [force (g)] and % elongation at break were calculated according to the ASTM method. Each test piece was placed centrally on the sample platform of Kieffer with the extension hook previously positioned beneath. Percent Elongation at Break was determined by the following equation

% elongation = distance Sample Stretched  $\times 100$ original length of sample

## Statistical analysis

Means, standard error mean (SEM), linear regression analysis with 95 % confidence intervals were calculated using Microsoft Excel 2007 (Microsoft Corp., Redmond, WA). Data was subjected to a single way analysis of variance (ANOVA).

#### **Results and discussion**

#### Gluten yield

The data regarding wet gluten yield, gluten index and dry gluten yield and water absorption capacity of gluten are presented in Table 1. Gluten is an important constituent of wheat because it provides strength and texture to baked wheat products and make it capable to form lots of bakery products from it. More the gluten content better will be the quality of wheat flour for bread.

## Wet gluten yield

The wet gluten yield of wheat cultivars flour ranged between 30.28 and 36.54 %, for KW-11 and C-306, respectively. No significant difference was observed between KW-11; PBW-343 and C-306; Raj-3765 (Table 1). Our observations are in accordance with Singh and Singh (2006) and Kumar et al. (2013). The wet gluten yield indicates the quality of protein and baking quality of flour. Autran et al. (1997) observed that pentosans and hemicelluloses in flours have a strong effect on

Table 1	Physico-chemical	analysis of gluten
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Wheat flour samples	Physico-Chemical properties of gluten				
	Wet gluten (%)	Gluten index	Dry gluten (%)	Water absorption capacity (%)	
KW-11	$30.60 {\pm} 0.26^{a}$	66.25±1.71 <sup>a</sup>	$8.65{\pm}0.12^{a}$	$353.81{\pm}1.88^{a}$	
PBW-343	$30.28{\pm}0.10^a$	$69.48{\pm}1.22^{ab}$	$8.75{\pm}0.15^a$	$346.21{\pm}3.94^{a}$	
C-306	$36.54{\pm}0.35^{c}$	$75.96{\pm}0.83^{b}$	$10.35{\pm}0.10^{c}$	$353.09{\pm}1.52^{a}$	
Raj-3765	$33.79{\pm}0.30^b$	$73.42{\pm}1.91^{b}$	$9.77{\pm}0.12^{b}$	$345.88{\pm}1.57^{a}$	

Data are presented as mean  $\pm$  SEM (n=3)

<sup>a-b</sup> Means with same superscript in column do not vary significantly (p < 0.05) from each other

gluten yield and that flour processing properties are strongly determined by the way flour milling fractions are blended. In a response surface study on gluten extraction from low-grade flour and durum flour, it was found that the protein concentration in protein fraction increased as the water content in the dough increased from 400 to 710 g/Kg (Dik et al. 2002).

## Gluten index

Gluten quality is defined by the degree of elasticity and extensibility. The gluten index ranged between 66.25 and 75.96 % for KW-11 and C-306, respectively (Table 1). The gluten index of KW-11 was significantly lower than C-306 and Raj-3765, where as PBW-343 had non significant difference between KW-11, C-306 and Raj-3765. Magdic et al. (2006) determined gluten index and their values were ranged from 62 to 99 %. There was a strong correlation between the extensographic parameters and Gluten Index indicates that it can be accepted as a fast and reliable tool for describing gluten strength of Croatian wheat flour (Horvat 2002).

## Dry gluten yield

The dry gluten yield of wheat cultivars flour ranged between 8.65 and 10.35 % for KW-11 and C-306, respectively. The dry gluten content of KW-11; PBW-343 was significantly lower than Raj-3765 and C-306. Raj-3765 had significantly lower dry gluten content than C-306 (Table 1). It gives the dry matter of gluten protein present in flour and water absorption capacity of gluten present in flour. However results obtained by Singh and Singh (2006) were ranging between 5.9 and 10.1 % of dry gluten yield. Supekar et al. (2005) determined the dry gluten content in the range of 9.4 to 12.7 %. Similar results were reported by Pharande et al. (1988).

## Water absorption capacity of gluten

The Water absorption capacity indicates the water holding capacity of dough during processing of different bakery products. The Water absorption capacity of wet gluten was ranging between 346.21 and 353.81 % for PBW-343 and KW-11, respectively (Table 1). No significant difference was observed between water content of gluten of all wheat cultivars. Esteller et al. (2005) reported that gluten is of two types, 'non-vital' and 'vital'. Non-vital wheat gluten is the gluten that has undergone irreversible denaturation and cannot be revitalized. It absorbs water in an amount related to its particle size and distribution. In contrast, vital dry gluten in contact with water re-hydrates rapidly and regains its intrinsic functionality. The functionality of gluten is very important for improving baking performance and may be affected by several factors at different stages of gluten extraction. The vitality of gluten has been

Table 2 Water and oil absorption capacity of dried gluten

Dried wheat gluten	WAC (%)	OAC (%)
KW-11 (F)	298.83±4.99 <sup>c</sup>	$260.15 \pm 8.71^{a}$
(V)	$249.94{\pm}6.42^{a}$	$256.09{\pm}6.79^{a}$
(0)	$291.79 \pm 4.22^{\circ}$	251.96±11.33 <sup>a</sup>
PBW343 (F)	$309.95{\pm}5.05^{d}$	$268.20{\pm}9.25^{a}$
(V)	$258.65 \pm 3.33^{ab}$	$248.05{\pm}5.05^{\mathrm{a}}$
(0)	$309.21 \pm 7.82^{d}$	$246.19{\pm}2.28^{a}$
C-306 - (F)	$354.22 \pm 7.57^{\rm f}$	$356.00 \pm 5.64^{e}$
(V)	$291.05 \pm 3.70^{\circ}$	$342.04{\pm}6.20^{de}$
(0)	$342.22 \pm 3.92^{e}$	$330.25 {\pm} 5.09^{d}$
Raj3765 (F)	$331.83 \pm 3.04^{e}$	$319.89 \pm 5.42^{cd}$
(V)	$311.71 \pm 1.31^{d}$	301.09±13.53 <sup>c</sup>
(0)	$272.25 {\pm} 5.86^{b}$	$271.28 {\pm} 6.76^{b}$

Data are presented as mean  $\pm$  SEM (n=3)

(F) Freeze dried gluten, (O) Oven dried gluten, (V) vacuum dried gluten <sup>a-b</sup> Means with same superscript in column do not vary significantly (p<0.05) from each other

related to the rate of water absorption and the degree of viscoelasticity (Frederix et al. 2004)

#### Characterization of dried wheat gluten

The water and oil absorption capacity and thermal properties of gluten dried by three methods viz. freeze drying, vacuum drying and oven drying were determined and data regarding the observations is presented in Tables 2

 Table 3
 Characterization of dried wheat gluten

and 3. The gluten was freeze dried (-89 °C/24 h), vacuum dried (60 °C/4 h), oven dried (80 °C/4 h) and powdered. The powdered dried gluten samples were characterized to check the retention of vitality of gluten. Drying of the gluten extract has received considerable attention by the researchers because high temperature drying often has deleterious effects on the gluten functionality (Czuchajowska and Paszczynska 1996). For commercial gluten production, the gluten is dried with high temperature air, whereas in the laboratory, vacuum and freeze drying have been reported to produce gluten with better functionality for bread making (Esteller et al. 2005).

#### Water absorption capacity

The water absorption capacity of dried wheat gluten was ranging between 249.94 and 354.22 % for KW-11 (V) and C-306 (F), respectively (Table 2). Higher water absorption capacity of gluten indicates a high content of starch. The significant lower (p<0.05) water absorption capacities was observed in vacuum dried gluten samples in comparison to freeze dried and oven dried gluten samples of all four wheat cultivars which may be due to differeance in drying conditions. The water absorption capacity is considered as a critical function of protein in viscous food like soups, gravies, dough and baked products mainly breads and cakes. Singh and Singh (2006) determined water absorption capacity of freeze dried gluten was ranging from 266.6 to 412.2 %.

Dried gluten samples	D.S.C					
	TO (°C)	TC (°C)	T P (°C)	∆Hgel j/g	PHI	
KW-11 (F)	78.39±1.35 <sup>ab</sup>	111.88±0.45 <sup>ab</sup>	136.84±0.53 <sup>a</sup>	$38.28{\pm}0.44^{a}$	$0.66 {\pm} 0.016^{ab}$	
(V)	$77.99 {\pm} 1.67^{ab}$	$110.25 {\pm} 0.57^{ab}$	$136.68 {\pm} 0.87^{a}$	$35.76 {\pm} 0.41^{ab}$	$0.61 {\pm} 0.015^{ab}$	
(0)	$73.63 {\pm} 1.27^{a}$	$109.46 {\pm} 0.69^{a}$	136.72±0.69 <sup>a</sup>	$33.55 {\pm} 0.38^{ab}$	$0.53{\pm}0.012^{a}$	
PBW343 (F)	$84.51 \pm 1.46^{b}$	$115.15 {\pm} 0.48^{\rm b}$	$136.81 \pm 0.65^{a}$	$42.84 \pm 0.49^{bc}$	$0.82{\pm}0.023^{b}$	
(V)	$81.89 \pm 1.41^{ab}$	$115.03 \!\pm\! 0.53^{b}$	$136.92{\pm}0.89^{a}$	$39.77 {\pm} 0.45^{a}$	$0.72 {\pm} 0.019^{ab}$	
(0)	$74.09 \pm 1.28^{a}$	$109.69 {\pm} 0.89^{a}$	$136.71 {\pm} 0.68^{a}$	$32.44{\pm}0.37^{a}$	$0.52{\pm}0.011^{a}$	
C-306 (F)	$91.26 \pm 1.58^{b}$	$117.83 \!\pm\! 0.84^{b}$	$136.67 {\pm} 0.73^{a}$	$50.23 \pm 0.58^{\circ}$	$1.12 \pm 0.036^{\circ}$	
(V)	$81.64{\pm}1.41^{ab}$	$117.86 {\pm} 0.65^{\rm b}$	136.76±1.06 <sup>a</sup>	$47.94 {\pm} 0.55^{bc}$	$0.87 {\pm} 0.022^{bc}$	
(0)	$78.19 {\pm} 1.37^{ab}$	$115.49 {\pm} 0.42^{\rm b}$	$136.77 {\pm} 0.68^{a}$	$38.41 {\pm} 0.44^{ab}$	$0.66 {\pm} 0.016^{ab}$	
Raj3765 (F)	$88.86 \pm 1.53^{b}$	$117.08 {\pm} 0.58^{\mathrm{b}}$	$136.77 {\pm} 0.77^{a}$	$41.68 {\pm} 0.48^{b}$	$0.87 {\pm} 0.027^{bc}$	
(V)	$79.35{\pm}1.37^{ab}$	$116.33 {\pm} 0.73^{\rm b}$	$136.85{\pm}0.84^{a}$	$37.80{\pm}0.44^{ab}$	$0.66 {\pm} 0.016^{ab}$	
(0)	$76.54{\pm}1.42^{ab}$	$114.93 \!\pm\! 0.84^{b}$	$136.74{\pm}0.72^{a}$	$36.47{\pm}0.42^{ab}$	$0.61 {\pm} 0.014^{ab}$	

Data are presented as mean  $\pm$  SEM (n=3)

(F) Freeze dried gluten, (O) Oven dried gluten, (V) vacuum dried gluten

<sup>a-b</sup> Means with same superscript in column do not vary significantly (p < 0.05) from each other

Table 4 SDS volume of flour and reconstituted flour

Flour samples	SDS volume (ml)				
	Flour	Freeze dried gluten reconstituted flour	Vacuum dried gluten reconstituted flour	Oven dried gluten reconstituted flour	
KW-11	36.56+0.59 <sup>a</sup>	35.79+0.35 <sup>a</sup>	33.53+0.30 <sup>b</sup>	28.68+0.46 <sup>c</sup>	
PBW-343	$38.51 \pm 0.34^{a}$	38.23+0.59 <sup>a</sup>	$34.52 \pm 0.40^{b}$	30.18+0.09 <sup>c</sup>	
C-306	$48.50 \pm 0.39^{a}$	$47.47 \pm 0.78^{a}$	$44.32 \pm 0.44^{b}$	38.49+0.55 <sup>c</sup>	
Raj-3765	36.32.0.64 <sup>a</sup>	35.68+0.26 <sup>a</sup>	$32.58 \pm 0.32^{b}$	29.51+0.61 <sup>c</sup>	

Data are presented as mean  $\pm$  SEM (n=3)

 $^{\rm a-b}$  Means with same superscript in row do not vary significantly (p<0.05) from each other

#### Oil absorption capacity

The oil absorption capacity of gluten obtained from different wheat cultivars and dried by three methods (freeze drying, vacuum drying and oven drying) was ranged between 246.19 and 356.00 % for PBW-343 (O) and C-306 (F), respectively (Table 2). The oil absorption capacity of oven dried samples was lower than freeze and vacuum dried gluten samples. Freeze dried gluten samples shows higher oil absorption capacity than vacuum and oven dried gluten samples. The oil absorption is an important parameter during frying and cooking of noodles. Somewhat similar oil and water absorption capacities were reported in wheat gluten by Singh, and Singh (2006) and Kaushik et al. (2013).

#### Thermal properties

The thermal properties viz.  $T_O$ ; Onset temperature,  $T_P$ ; Peak temperature,  $T_C$ ; conclusion temperature, ( $\Delta$ Hgel); Enthalpy of gelatinization and PHI of gluten is presented in Table 3. The  $T_O$  (Onset temperature) was ranged between 73.63 and 91.26 °C for KW-11 (O) and C-306 (F), respectively. The results revealed that oven dried gluten denaturation started

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Table 6 Tensile strength of flour and reconstituted flour

Flour	Tensile strength [force (g)]				
samples	Flour	Freeze dried gluten reconstituted flour	dried	Oven dried gluten reconstituted flour	
KW-11	$23.79{\pm}0.78^a$	22.16+0.87 <sup>a</sup>	17.32+0.98 <sup>b</sup>	17.21+1.09 <sup>b</sup>	
PBW-343	$29.78{\pm}1.41^{a}$	28.43+1.09 <sup>a</sup>	$23.10 + 1.16^{b}$	23.34+0.87 <sup>b</sup>	
C-306	$47.80{\pm}2.81^{a}$	$46.23 \pm 1.27^{a}$	$40.21 \pm 0.59^{b}$	$40.03 \pm 0.78^{b}$	
Raj-3765	$32.97{\pm}1.56^{a}$	$31.42 \pm 0.92^{a}$	26.46+1.89 <sup>b</sup>	26.54+1.05 <sup>b</sup>	

Data are presented as mean  $\pm$  SEM (n=3)

 $^{\rm a-b}$  Means with same superscript in row do not vary significantly ( $p{<}0.05)$  from each other

earlier than vacuum and freeze dried gluten. The  $T_P$  (peak temperature) was ranged between 109 and 117.86 °C for KW-11 (O) and C-306 (F), respectively. Results revealed that oven dried gluten denatured earlier than vacuum and freeze dried gluten. The transition temperature Tc (conclusion temperature) was ranging between 136.67 and 136.92 °C, which revealed that gluten totally denatured at 136 °C. Non significant difference between all gluten samples revealed that there was non significant effect of different wheat cultivars and different drying temperature on gluten denaturation temperature. The AHgel values for glutens isolated from different wheat cultivars in dry form ranged from 32.44 to 50.23 for PBW-343 (O) and C-306 (F), respectively. The lower values of transition temperatures of wheat gluten are because of the low level of starch. The transition temperatures of gluten samples are moderate so the percentage of starch is low in these samples. Fujio and Lim (1989) reported that transition of wheat gluten could be detected by monitoring the change in color when it was being heated under pressure. They summarized that wheat gluten may be considered to be an amorphous biopolymer, which explains the occurrence of the glass transition and the absence of a denaturation peak when DSC examines it. The Peak height index (PHI) values for glutens

 Table 5
 Falling number of flour and reconstituted flour

Flour samples	Falling number (s)						
	Flour	Freeze dried gluten reconstituted flour	Vacuum dried gluten reconstituted flour	Oven dried gluten reconstituted flour			
KW-11	447.12±12.15 <sup>a</sup>	325.67+11.32 <sup>b</sup>	323.25+10.91 <sup>b</sup>	321.56+8.56 <sup>b</sup>			
PBW-343	$392.46{\pm}5.89^{a}$	315.36+13.46 <sup>b</sup>	314.69+9.43 <sup>b</sup>	314.25+9.34 <sup>b</sup>			
C-306	$483.01{\pm}8.09^{a}$	357.82+16.23 <sup>b</sup>	355.34+13.57 <sup>b</sup>	356.34+10.45 <sup>b</sup>			
Raj-3765	$458.20{\pm}13.27^a$	336.94+9.12 <sup>b</sup>	336.23+12.45 <sup>b</sup>	334.74+12.78 <sup>b</sup>			

Data are presented as mean  $\pm$  SEM (n=3)

<sup>a-b</sup> Means with same superscript in row do not vary significantly (p < 0.05) from each other

ranged from 0.53 to 1.12 for KW-11 (O) and C-306 (F), respectively. From above results, it may be concluded that freeze dried gluten had less conformational and structural changes after drying in comparison to oven and vacuum dried gluten. This implies that added freeze dried gluten improves baking characteristics and retains vitality of bakery products.

# Regeneration properties of gluten

The regeneration properties of dried gluten were determined. The flour components which were washed during gluten separation were collected and dried. The dried flour components and dried gluten powder were mixed in 9:1 ratio and their SDS volume, falling number and tensile strength was determined and compared with their respective control flour samples.

## SDS (Sedimentation volume)

The SDS sedimentation volume is a good indicator of wheat flour quality. The SDS sedimentation volume of wheat flour ranged between 36.56 and 48.50 ml for KW-11 and C-306, respectively (Table 4). Freeze dried gluten reconstituted flours showed non significant difference with their respective control flours, whereas, vacuum and oven dried flour was significantly different from control flour. Present SDS values are in agreement with the values obtained by Supekar et al. (2005). The SDS sedimentation value of wheat flours is based on the fact that the gluten protein absorbs water and swells considerably when treated with lactic acid.

#### Falling number

The falling number instrument analyzes viscosity by measuring the resistance of a flour and water paste to a falling plunger. The falling number of wheat flour samples ranged between 392.46 and 483.01 s for PBW-343 and C-306, respectively (Table 5). The falling number of all reconstituted samples was significantly different from their respective control flours. Falling number indicates flour quality. More the falling number low will be the amylase activity and viceversa. Different products need different level of  $\alpha$ -amylase for best quality product. The bread formed from more amylase flour loose the texture. If the amylase activity is low, the bread becomes hard and grainy in texture. According to AACC (2005) high falling number (above 300 s) indicates minimal enzyme activity and sound quality of wheat flour. A low falling number (below 250 s) indicates substantial enzyme activity and sprout-damaged wheat or flour. Even after reconstitution, all flour samples falling number were above 300, thus indicated minimal enzyme activity and sound quality of wheat flour.

#### Tensile strength

The texture analyzer TXT2I was used for detecting the textural quality of food products. The tensile strength ranged between 23.79 and 47.80 g for KW-11 and C-306, respectively (Table 6). Freeze dried gluten reconstituted flour showed a non significant difference in dough strength with their respective control flour, whereas, vacuum and oven dried flour had significantly different from control flour. More the tensile strength; better would be the quality of flour for bread manufacturing and vice-versa. Tensile strength indicates the quality and quantity of gluten protein. The tensile strength indicates the flour quality, elasticity and strength of gluten protein.

#### Conclusion

The gluten dried by freeze drying had best water and oil absorption properties. The thermal properties remained similar for gluten dried using three drying methods. The reconstitution properties were also best for freeze dried gluten, therefore gluten should be dried using a freeze drying method for use in baking purposes.

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