

## **Improvement in Functional and Rheological Properties of Gluten by Enzyme Treatment**

Vishal R. Parate<sup>1</sup>, Sumit S. Pathak<sup>1</sup> and M. I. Talib<sup>1</sup>

<sup>1</sup>(Department of Food Technology, University Institute of Chemical Technology, North Maharashtra University, Jalgaon, India)

---

**Abstract:** Enzyme treatment to the wheat flour enhances its functional properties as enzyme (diastase) is going to react between the bonding of the starch and the protein to improve the visco-elastic properties of the dough. The present study was carried out with the aim to improve the functional and rheological properties of the gluten from Durum 206A Wheat variety by the diastase enzyme treatment. In the present study the flour of the Durum 206A Wheat flour was kneaded to dough by adding the enzyme solution of 5 ppm to 25 ppm (5, 10, 15, 20 and 25ppm) the kneaded dough was immersed in the distill water for one hour and then the dough was placed under the constant stream of water to isolate the gluten. The obtained gluten was freeze dried and was analyzed for various physicochemical properties. The result revealed the optimum dose to be 10 ppm on the basis of the improvement in the oil binding capacity, water holding capacity, sedimentation value, foaming volume and emulsifying capacity. The non-enzyme treated (control) and the optimized enzyme treated gluten were then incorporated in the pizza base dough of 147 and Lokwan whole wheat flour. For the conformation of the modification on morphological basis, FE-SEM images were taken of non-modified gluten and enzyme treated freeze dried gluten. Obtained dough and the pizza base dough were compared on the basis of Brabender's Farinograph and Extensograph. The result concluded improvement in the pizza base dough as well pizza base prepared from the optimized enzyme treated gluten as compared to control.

**Keywords:** Gluten, enzyme, farinograph, extensograph, physicochemical, visco-elastic.

---

### **I. Introduction**

The Durum Wheat is the preferred raw material for the production of pasta and bread worldwide and some specialty bread common in parts of Italy and Mediterranean region [1].

Gluten and soy protein are extensively being used as basic components for vegetarian food products especially in many Asian countries. Wheat gluten protein is an important raw material in the manufacture of foods for breakfast, infant, snack and pasta products. Wheat gluten is an economically important co-product of the wheat starch industry [2]. 'Vital Wheat Gluten' protein is now a significant ingredient in the food industry and important item of world trade [3, 4]. Its rheological properties are the basis of the functional uses of vital gluten [5]. Bakery industry has a wide application of vital wheat gluten. The prospects for utilization of wheat gluten are expanding due to the availability of gluten on the market at a relatively low cost. However, the expanding utilization of wheat gluten in food and nonfood industries [6, 7] has been limited by a general lack of some desirable functional properties, such as solubility, foaming and rheological properties. Efforts to improve the functional properties of wheat gluten are unfolding challenges.

Gluten is a protein macro-polymer in wheat flour which is a mixture of more than 100 heterogeneous poly-peptides, that are formed in hydrated flour during dough mixing and Gluten is composed of two main storage proteins, namely, Gliadins and glutenins. Gluten plays a major role in viscoelastic properties of bread-making which is highly correlated to the quality of end products. Glutenins and gliadins are the polymeric and monomeric protein components of gluten, respectively. Glutenins (with molecular mass of 69 to 88 kDa based on SDS-PAGE) [8] are responsible for elastic behavior, whereas gliadins (with molecular mass of 30 to 50 kDa) [9] are responsible for viscous flow properties of the foods.

For the generation of dough, the disulphide bonds play an important role and they are the most prominent linkages in biology of gluten network formation [10]. The use of alpha amylase (Diastase) led to improvement in loaf shape. The softener effect of the enzyme had justified and had proved to be useful for reducing amylopectin retrogradation and the firming rate of wheat bread crumb [11].

In present investigation the study was carried out with the aim to improve the functional and rheological properties of the gluten from Durum 206A Wheat variety by the diastase enzyme treatment.

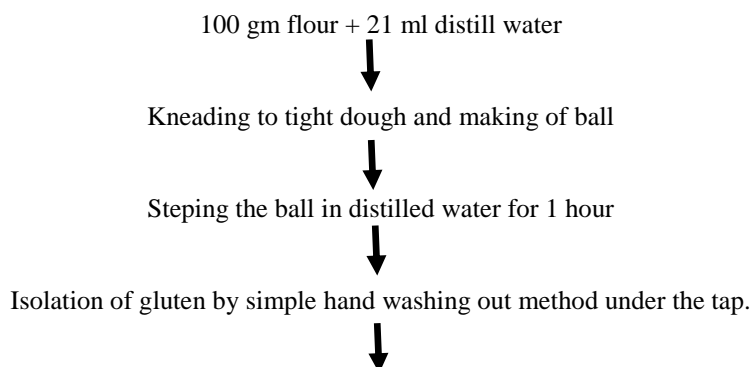
### **II. Materials and Methods**

The Durum 206A wheat was collected from the Agriculture Department, Government of Madhya Pradesh. The 147 wheat and Lokwan was purchased from the Local Super market. All the three wheat were

milled to flour at local milling center, by traditional milling method. All the chemicals used for analysis purpose were of analytical grade and of standard brand.

### 2.1 Preparation of Dough and Enzyme Treatment

The dough was prepared by method described by American Association of Cereal Chemists (AACC) and gluten was isolated by hand washing method as detailed in AACC (2000a) method No. 38-10 [12]. Following is the method for non-modified and freeze dried gluten powder.



Washing of the gluten with distill water and freeze dry at -110°C for 24 hours in freeze drier as per method suggested by N.Singh et. al., 2005 [13].

### 2.2 Isolation of Gluten and its Freeze Drying

Method for enzyme treatment and modification was followed and obtained from V. Kolpakova et. al., 2014 [14] with some minor modification by changing the enzyme and its concentration. 100 gm of the Durum Wheat flour was Kneaded by adding the 5 ppm concentrated Diastase enzyme solution of 28 ml, after kneading it was kept immersed in the water for 1 hour. After 1 hour, the gluten was isolated by the hand washing method. The similar process was repeated for the every concentration from 10 ppm to 25 ppm. On the completion of isolation of gluten from the treated dough which was modified by the alpha-amylase enzyme and gluten was freeze dried in (Lypolizer- SCANVAS- SANSKAM Technologies Pvt. Ltd.). The by-products such as bran; germ and starch mixture and pure starch were recovered.

### 2.3 Characterization of Isolated Modified Gluten and Non-Modified Gluten

The isolated gluten was characterized for various physical and chemical parameters using standard methods of analysis and instruments given in Table 2.1.

**Table 2.1:** Methods for the Physicochemical Analysis and Instruments

Name of Physicochemical Analysis	Method and Instrument
Bulk Density (gm/mL)	M. Olu et. al., 2012 [15], Measuring Cylinder
Moisture content (%)	S. Ranganna 1995 [16], Hot air oven (Hindustan Apparatus Manufacturing Company)
Ash content (%)	S. Ranganna 1995, Muffle Furnace (Tempo Instruments and Equipment Pvt. Ltd.)
Acid Insoluble Ash (%)	S. Ranganna 1995, Ash less filter paper
Sedimentation (mL)	AACC 56-61A, 2000b [17], Measuring Cylinder
Dispersibility (%)	
Solvent Retention Capacity (SRC) (%)	Anonymous, Kansas State University, 2008 [18], Centrifuge (REMI-R4C, Laboratory Centrifuge)
Water holding capacity (WHC) (%)	L.R. Beuchat 1977 [19], Centrifuge (REMI-R4C, Laboratory Centrifuge)
Oil binding capacities (OBCs) (%)	P. Chakraborty et. al., 1986 [20], Centrifuge (REMI-R4C, Laboratory Centrifuge)
Emulsifying capacity (EC) (%)	O. Zorba et. al., 1993 [21], magnetic Stirrer (REMI-1RL)
Foaming Volume (mL)	E. Makri et al. 2005 [22], Measuring Cylinder
Solubility (%)	M. M. Bradford, 1976 [23], UV-Double Beam Spectrophotometer (Systronics, UV-Double Beam Spectrophotometer 2205).

## III. Results and Discussions

The Table 3.1 is showing the physicochemical results for non-modified freeze dried gluten powder. The bulk density of the freeze dried gluten powder was 0.75. The results of the chemical analysis of the freeze dried gluten powder were all in limits which were prescribed by Food Safety Standards Regulations, 2011 [24].

**Table 3.1:** Results of the Physicochemical Analysis of Non-Modified Freeze Dried Gluten of Durum Wheat

Parameter	Result
Moisture (%)	7.332 ± 0.21
Ash (%)	0.66 ± 0.06
Acid Insoluble Ash (%)	0.089 ± 0.005
Bulk Density (gm/mL)	0.75 ± 0.02
Dispersibility (%)	70 ± 0.01

Sedimentation Value (mL)	32 ± 0.06
Water Absorption Capacity (gm/gm)	1.86 ± 0.05
Oil Holding Capacity (gm/gm)	1.00 ± 0.04
Emulsification Capacity (%)	11.225 ± 0.36
Foaming Volume (mL)	13.20 ± 0.05
Water Solubility Index	3.46 ± 0.05
Solvent Retention Capacity	
Deionized Water (%)	235.48 ± 0.03
Sucrose 50 %	421.80 ± 0.04
Lactic Acid 5 %	527.08 ± 0.05
Sodium Carbonate 5 %	384.38 ± 0.03

All values are means of three determinations ± standard deviation (SD)

The ash content, moisture content and acid insoluble ash content of the freeze dried gluten powder were in the prescribed limit Food Safety Standards Regulations, 2011, ISO 1666 [25] and ISO 3593 [26]. The dispersibility was 70 % and the sedimentation value was 32 mL. The water absorption capacity was 1.86 %. The oil holding capacity was of 1 %. The oil holding capacity and water holding capacity of the gluten powder was same as prescribed by J.S. Charalle et. al., 2013 [27]. It showed good emulsification capacity of 11.25 %. The foaming volume was of 13.20 mL. The gluten powder showed 421.8073 % of Sucrose retention and 384.380 % of sodium carbonates retention. The lactic acid retention was 527.08 %, which showed the gluten was of good quality.

Results of the physicochemical analysis of diastase modified gluten are shown in Table 3.2. As per the result obtained from the chemical and physical analysis of the enzyme modified freeze dried gluten powder. There was no significant change in the moisture, ash, acid insoluble ash, bulk density. It remained as before the same and identical to the values before the modification and after the enzyme modification.

**Table 3.2:** Results of the Physicochemical Analysis of Diastase Modified Freeze Dried Gluten Powder

Parameter	G <sub>1</sub>	G <sub>2</sub>	G <sub>3</sub>	G <sub>4</sub>	G <sub>5</sub>
Moisture (%)	7.32 ± 0.07	7.33 ± 0.09	7.32 ± 0.07	7.33 ± 0.08	7.32 ± 0.08
Ash (%)	0.66 ± 0.01	0.65 ± 0.02	0.66 ± 0.05	0.67 ± 0.08	0.66 ± 0.01
Acid Insoluble Ash (%)	0.083 ± 0.03	0.081 ± 0.03	0.089 ± 0.02	0.083 ± 0.07	0.089 ± 0.02
Bulk Density (gm/mL)	0.72 ± 0.01	0.72 ± 0.01	0.73 ± 0.01	0.73 ± 0.05	0.74 ± 0.05
Dispersibility (%)	70 ± 0.21	70.2 ± 0.18	70.6 ± 0.14	71.4 ± 0.16	71.6 ± 0.12
Sedimentation Value (mL)	30 ± 0.15	32.8 ± 0.14	29.4 ± 0.11	28.6 ± 0.13	28.2 ± 0.13
Water Absorption Capacity (gm/gm)	2.25 ± 0.11	2.35 ± 0.07	2.26 ± 0.11	2.28 ± 0.11	2.24 ± 0.11
Oil Holding Capacity (gm/gm)	1.36 ± 0.11	1.56 ± 0.07	1.24 ± 0.11	1.03 ± 0.16	1.01 ± 0.11
Emulsification Capacity (%)	10.67 ± 0.11	10.75 ± 0.13	10.56 ± 0.15	10.45 ± 0.16	10.21 ± 0.13
Foaming Volume (mL)	13.2 ± 0.09	15.8 ± 0.08	14.2 ± 0.09	12.7 ± 0.11	12.2 ± 0.05
Water Solubility Index	3.48 ± 0.08	3.78 ± 0.04	3.27 ± 0.05	3.21 ± 0.08	3.11 ± 0.11
Solvent Retention Capacity					
Deionized water	237.5 ± 0.06	238.6 ± 0.03	239.5 ± 0.06	234.8 ± 0.04	229.3 ± 0.04
Sucrose 50 %	401.2 ± 0.04	409.8 ± 0.05	390 ± 0.07	386 ± 0.05	378.5 ± 0.06
Lactic Acid 5 %	527 ± 0.04	534.5 ± 0.03	490 ± 0.08	485 ± 0.07	462 ± 0.05
Sodium Carbonate 5 %	385 ± 0.05	380 ± 0.02	378 ± 0.03	375 ± 0.03	372 ± 0.05

All values are means of three determinations ± standard deviation (SD)

The sample G<sub>a2</sub> had shown the high Solvent retention capacity for the lactic acid retention of 504.5 % showing the enhancement in the gluten property. But as the concentration of the enzyme was increased, there was a negative deviation in all, the functional properties. The foam volume was of 15.8 ml per gram of the modified freeze dried gluten powder. It was highest among all the samples which were treated with the enzyme.

SRC for varies gluten samples which were treated with Diastase enzyme. The best result is shown by the sample G<sub>a2</sub>, which was treated by 10 ppm alpha amylase for the time period of 1 hour. The lactic acid retention of the sample G<sub>a2</sub> is 534.5 %, which was highest in all the samples. The sodium carbonate retention was lowest in the said sample. As the concentration of the enzyme was increased up to 10 ppm, it gave the positive result, after the 15 ppm onwards it showed the negative deviation in the Solvent retention capacities. The quality of the gluten protein was eroded due to the concentration of ranging from 15 ppm to 25 ppm. But the enzyme gives positive effect on the gluten when it is used in the lower concentration. G<sub>a2</sub> sample was accepted for the further incorporating in the wheat flours of both the Indian local varieties of wheat flour. The values for the foaming volume, water holding capacity and the oil holding capacities are comparable to the findings of the O. David, 2007 [28].

The dispersibility and the lactic acid sedimentation value for each of the gluten samples which were modified by using the Diastase Modified Gluten. As the concentration of Diastase enzyme was increasing there was increase in the Sedimentation value up to a concentration of 10 ppm but as concentration was above 15 ppm

there was decrease in the Sedimentation value. This showed that Diastase had affected the quality of the gluten and improved it. As the modification was done for the lower concentration it gave good result but as concentration was increased the quality of gluten decreased. The samples which was modified by using the 10 ppm of enzyme showed the highest sedimentation value; sample Ga<sub>2</sub> was accepted for the further incorporating in the wheat flours of both the Indian local varieties of wheat flour.

The sample G<sub>02</sub> was selected to add in the whole wheat flour of 147 and lokwan at 5 %, and then the rheological results were obtained. The farinograph and extensograph analysis were done by the Stern Ingredients Lab Pvt. Ltd. Andheri west, Mumbai.

The Table 3.3 is showing the Farinograph results of mixing characteristics of dough. The highest arrival time of 7.67 minutes was recorded in the flour of Lokwan with enzyme treated gluten followed by 4.33 minutes in 147 whole wheat flour containing enzyme treated gluten powder. The highest departure time was observed in the flour of Lokwan with enzyme treated gluten and the lowest was obtained from the flour of 147 with non-modified gluten. The departure time ranged from 15.10 to 21.33 min among different wheat varieties.

**Table 3.3:** Farinograph Result - Mixing Characteristics of Dough

Name of Dough	AT (min)	PT (min)	DT (min)	MTI (B.U.)
147 with non-modified gluten	3.25 ± 0.20	11.00 ± 0.96	15.10 ± 1.22	440
147 with enzyme treated gluten	4.33 ± 0.29	13.83 ± 1.10	16.17 ± 1.41	490
Lokwan with non-modified gluten	4.30 ± 0.19	8.40 ± 0.85	15.20 ± 1.09	470
Lokwan with enzyme treated gluten	7.67 ± 0.23	13.50 ± 1.10	21.33 ± 1.41	505

(AT- Arrival Time, PT- Peak Time, DT- Departure Time, MTI- Mixing Tolerance Index)

The mixing tolerance index ranged from 440 to 505 brabender units (B.U.). The highest mixing tolerance index of 505 was found in the Lokwan whole wheat flour with enzyme treated gluten. The lowest mixing tolerance index was observed in 147 with non-modified gluten. The whole wheat flour of Lokwan with enzyme treated gluten had shown highest peak time of 13.50 which concludes that the gluten which was modified has been successfully done. While the whole wheat flour of 147 with non-modified gluten and whole wheat flour of Lokwan with non-modified gluten had the lower peak time. Higher peak time indicates good quality protein while lower peak time shows lower quality protein. The results from farinographs of wheat flours are comparable with the early findings of I. Ahmad 1993 [29], Q. Islam *et. al.*, 1998 [30], F.M. Anjum and C.E. Walker, 2000 [31] and M.S. Butt *et. al.*, 2001[32].

The Table 3.4 is showing the Extensograph results. found to be 26.25 and the highest resistance was recorded for lokwan whole wheat flour added with enzyme modified gluten powder. There was substantial increment in the resistance of the dough which were added with the modified gluten as compared to the dough's which were added with a non modified gluten powder.

**Table 3.4:** Results of Extensograph for Fortified 147 Whole Wheat Flour

Parameter	147 Non -Modified Gluten	147 Enzyme Modified Gluten	Lokwan Non - Modified Gluten	Lokwan Enzyme Modified Gluten
R	13	26.25	16	31
E	20	18	13	20
R/E	0.65	1.45	1.20	1.55
Area under curve (cm <sup>2</sup> )	90	160	110	190

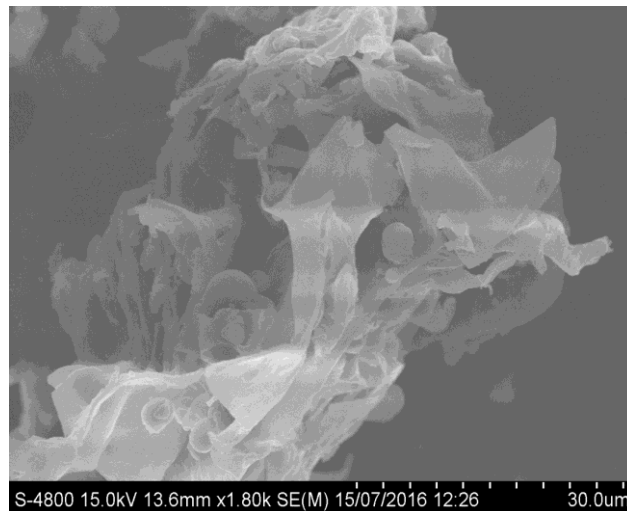
(R- Resistance, E- Extensibility, R/E- Resistance to Extensibility Ratio)

The resistance of the 147 whole wheat flour which was fortified with enzyme modified gluten powder was The extensibility was recorded highest for the Lokwan whole wheat flour with enzyme modified gluten. The least extensibility was found for the Lokwan with non modified gluten powder. The R/E ranged between 0.65 to 1.55. The R/E ration gives us information regarding resistance to extensibility ratio of the dough, which was highest 1.55 for Lokwan whole wheat flour added with enzyme modified gluten powder. According to the suggested data by B.S. Khatkar [33] , the area under the curve gives us information regarding strength of the flour. The area more than 200cm<sup>2</sup> suggests strongest flour strength. Area between more than 120 to 200 suggest strong flour. While area less than 80 & more than 120 is the medium strong. Area less than 80 is the weak strength flour. 147 & Lokwan whole wheat flour added with enzyme modified gluten powder fall in the category of strong flour, while 147 & Lokwan whole wheat flour added with non modified gluten powder are in the medium strong flour category. The results pertaining to the physical dough properties obtained from extensograph are comparable with Cereal Varieties, 2003 [34]; S. Kalnina *et. al.*, 2015 [35].

Fig.. 1 shows the FE-SEM image of non modified & freeze dried gluten protein in the stretched formed. The Fig. 2 shows FE-SEM image of enzyme modified & freeze dried gluten protein matrix along with the center located starch granule.

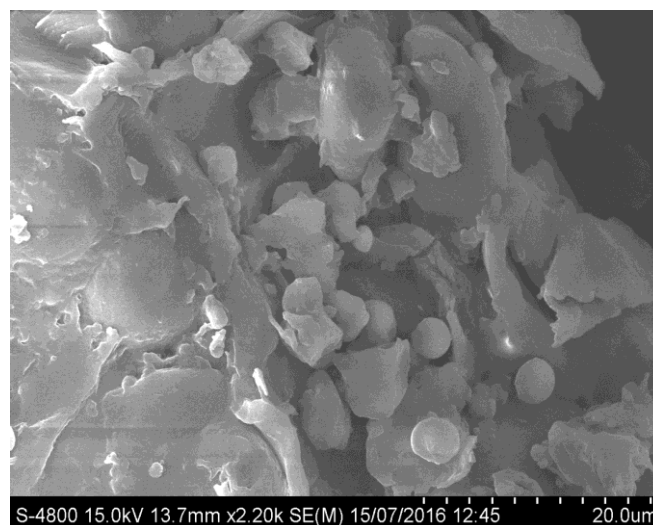


**Figure 1:** Non-modified freeze dried gluten powder



**Figure 2:** Modified freeze dried gluten powder

The Fig 3 is showing FE-SEM image of modified freeze dried gluten powder incorporated in the 147 whole wheat flour. The base of the image is showing the protein sheet, while the round granules are the starch granules. The Fig. 4 is showing the FE-SEM image of the enzymatically modified protein incorporated in the lokwan whole wheat flour.



**Figure 3:** Modified freeze dried gluten powder in 147 whole wheat flour

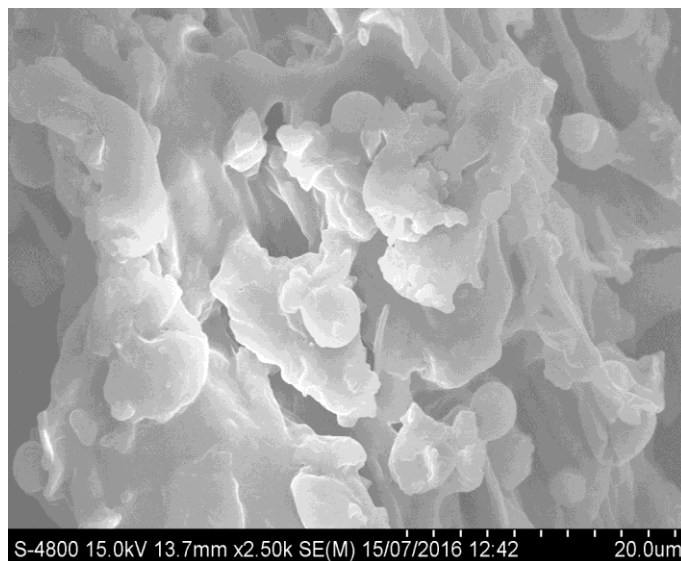


Figure 4: Modified freeze dried gluten powder in lokwan whole wheat flour

#### IV. Conclusion

The study concludes that the treatment to wheat flour gluten by diastase enzyme helps to transform the functional and rheological properties. The condition optimized for enzyme treatment was found to be 10 ppm dose. Enzyme treatment had improved the Water Holding Capacity, Oil Holding Capacity and Foaming Volume of gluten. The addition of the modified gluten to the whole wheat flour (147 and lokwan) brought improvement in the dough extensibility, elasticity, Mixing Tolerance Index, dough gluten quality and quantity. It is possible to produce pasta (spaghetti) and pizza base from Indian whole wheat flour variety by incorporating the developed modified gluten in flour.

#### References

- [1]. M. Sissons, Role of durum wheat composition on the quality of pasta and bread. *Food-Global Science Books*, 2(2), 2008, 75-90.
- [2]. I. Toufeili, B. Ismail, S. Shadarevian, R. Baalbaki, B.S. Khatkar, A.E. Bell and J.D. Schofield, The role of gluten proteins in the baking of arabic bread. *Journal of Cereal Sciences*, 30(3), 1999, 255-265.
- [3]. V. Krishnakumar and I. Gordon, The world wheat gluten market. *International Food Ingredients*, (4), 1995, 41–45.
- [4]. M. Boland, G.W. Brester and M.R. Taylor, *Global and US wheat gluten industries: Structure, competition, and trade. Agricultural Marketing Policy Center briefing no. 76*. Bozeman: Montana State University, 2005.
- [5]. L. Day, M.A. Augustin, I.L., Batey and C.W. Wrigley, Wheat-gluten uses and industry needs, *Trends in Food Science & Technology*, 17(2), 2006, 82–90.
- [6]. B.S. Kayserilioglu, U. Bakir, L. Yilmaz and N. Akkas, Drying temperature and relative humidity effects on wheat gluten film properties. *Journal of Agricultural Food Chemistry*, 51(4), 2003, 964-968.
- [7]. C. Larre, C. Desserme, J. Barbot and J. Gueguen, Properties of deamidated gluten films enzymatically cross-linked. *Journal of Agricultural Food Chemistry*. 48(11), 2000, 5444-5449.
- [8]. O.D. Anderson, N.G. Halford, J. Forde, R. Yip, P.R. Shewry and F.C. Greene, Structure and analysis of the high-molecular-weight glutenin genes from *Triticum aestivum* cv Cheyenne, In: Miller TE, Koebner RMD (eds) *Proceedings 7<sup>th</sup> International Wheat Genet Symp*. Bath Press, 1988, 699–704.
- [9]. A.S. Tatham, A.F. Drake and P.R. Shewry, Conformational studies of synthetic peptides corresponding to the repetitive region of the high molecular weight (HMW) glutenin subunits of wheat, *Journal of Cereal Science*. 11, 1990, 189-200.
- [10]. J.A. Gerrard, Protein-protein crosslinking in food: methods, consequences, applications, *Trends in Food Science and Technology*, 13, 2002, 389-397.
- [11]. Y. Champenois, G. Valle, G. della, V. Planchot, A. Buleon, and P. Colonna, Influence of alpha-amylases on bread staling and on retrogradation of wheat starch models, *Sciences des Aliments*, 19(3-4), 1999, 471-486.
- [12]. AACC (American Association of Cereal Chemists), 10<sup>th</sup> Edition. Adapted from Method 38-10, 2000a.
- [13]. N. Singh, M. Kaur, and K. Sandhu, Physicochemical and functional properties of freeze-dried and oven dried corn gluten meals, *International Food Research Journal*, 21(3), 2005, 1219-1224.
- [14]. V. Kolpakova, L. Chumikina, A. Vasil'ev, L. Arabova, and A. Topunov, Wheat gluten proteolysis by enzyme preparations of directional action, *International Journal of Agronomy and Agricultural Research*, 5(2), 2014, 72-86.
- [15]. M. Olu, O.A.B. Ogunmoyela, O. Adekoyeni, O. Jimoh, S.O. Oluwajoba and M.O. Sobanwa, Rheological and Functional Properties of Soy-Poundo Yam Flour, *International Journal of Food Science and Nutrition Engineering*, 2(6), 2012, 101-107 DOI: 10.5923/j.food.20120206.01
- [16]. S. Ranganna, *Handbook of Analysis and Quality Control for Fruits and Vegetable Product*, Second Edition, Tata McGraw Hill Publication. Co. L.td. New Delhi. 1995.
- [17]. AACC (American Association of Cereal Chemists), 10<sup>th</sup> Edition Adapted from Method 56-61A, 2000b.
- [18]. Anonymous, "A Guide to Understanding Wheat and Flour Quality", Version 2, Kansas State University, 2008.
- [19]. L.R. Beuchat, 1977, Functional and electrophoretic characteristic of succinylated peanut flour protein. *Journal of Agriculture and Food Chemistry*, 25, 1977, 258-261.

- [20]. P. Chakraborty, M. Lemeguer and P. Jelen, Coconut proteins isolate by ultra filtration, *Food Engineering and Process Application*, 2, 1986, 308–315.
- [21]. O. Zorba, H.Y. Gokalp, H. Yetim and H.W. Ockerman, Salt, phosphate and oil temperature effects on emulsion capacity of fresh or frozen meat and sheep tail fat, *Journal of Food Science*, 58, 1993, 492–496.
- [22]. E. Makri, E. Papalamprou and G. Doxastakis, Study of functional properties of seed storage proteins from indigenous European legume crops (lupin, pea & broad bean) in admixture with polysaccharides, *Food Hydrocolloids*, 19, 2005, 583–594.
- [23]. M.M. Bradford, Method for quantitation of microgram quantities of protein utilizing the principle of protein-dye-binding. *Biochemistry*, 72, 1976, 248–254.
- [24]. Food Safety Standards Regulations, 2011.
- [25]. ISO (International Organization for Standardisation) 1666: 1996. Starch — Determination of moisture content — Oven-drying method. International Organization for Standardisation, 1666.
- [26]. ISO (International Organization for Standardisation) 3593: 1981. Starch -- Determination of ash. International Organization for Standardisation, 3593.
- [27]. J.S. Charelle, B. Dominique, S. Sedano and M. Noel, Functional Properties of Flours Prepared From Glucosinolate – Rich Vegetables: Alugbati (*Basella rubra*), *The Research Congress*, De La Salle University Manila, 2013.
- [28]. O. David, E. Arthur, S. Kwadwo, E. Badu and P. Sakyi, Proximate Composition and Some Functional Properties of Soft Wheat Flour, *International Journal of Innovative Research in Science, Engineering and Technology*, 4(2), 2007, 753-758.
- [29]. I. Ahmad, *Physico-Chemical and Functional Properties of Some New Pakistani Wheat Varieties*, M.Sc. Thesis, Department of Food Technology, University of Agriculture, Faisalabad–Pakistan, 1993.
- [30]. Q. Islam, F.M. Anjum, M.S. Butt and M. Hinnai, Suitability of Local Wheat Varieties for the Production of Pizza. *Pakistan Journal of Food Sciences*, 8, 1998, 8–11.
- [31]. F.M. Anjum and C.E. Walker, Grain, flour and bread making properties of eight Pakistani hard white spring wheat cultivars grown at three different locations for two years. *International journal of Food Science and Technology*, 35, 2000, 407–16.
- [32]. M.S. Butt, F.M. Anjum, D.J. Van-Zuilichem and M. Shaheen, Development of predictive models for end use quality through canonical analysis. *International Journal of Food Science and Technology*, 36, 2001, 433–40.
- [33]. B.S. Khatkar, *Quality Testing of Wheat Flour and Bakery Ingredients*, Diploma Thesis, Directorate of Distance Education Guru Jambheshwar University of Science and Technology Hisar – 125 001, India.
- [34]. Cereal Varieties, Agricultural Research Institute of the Hungarian Academy of Sciences, Martonvásár2003, 1-20.
- [35]. S. Kalnina, T. Rakejeva, D. Kunkulberga and R. Galoburda, Rheological properties of whole wheat and whole triticale flour blends for pasta production, *Agronomy Research*, 13(4), 2015, 948–955.