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EFFECT OF ASSOCIATION OF DOUGH CHARACTERISTICS WITH STORAGE PROTEINS OF WHEAT AND GRAM FLOURS ON TEXTURE OF THE LEAVENED FLAT BREAD (NAAN)

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ABSTRACT

The flours of Pakistani wheat varieties/lines were evaluated for their dough characteristics (farinographic) and protein subunits (PAGE) associated with the texture of the end-product (naan). Based on these studies varieties LU-26 and LU-31 were selected for supplementation with 5, 10, 15, 20 and 25% gram (chickpea) flour to improve protein. The composite flours were further evaluated for protein subunits and associated with the texture of the composite flour naans. The storage protein subunits ω-gliadin, and Low Molecular Weight (LMW) albumins and globulins showed a positive correlation with the water absorption and softening of the dough. The texture of the product also showed a strong relationship with the glutenin, gliadin and albumin subunits of simple wheat flours. The addition of the gram flour into wheat flour caused proportionate increase in intensity of all protein subunits which exhibited a negative correlation with the texture of the composite flour naans for both varieties.

Key words: Flour, Chickpea, Farinograph, Proteins, Bread, Texture.

INTRODUCTION

Wheat flour when mixed with water is unique in viscoelastic dough forming properties due to the formation of gluten which is a storage protein consisting of monomeric gliadins and polymeric glutenins. Gliadins and glutenins are responsible for the viscoelastic properties of wheat flour dough, wherein gliadins contribute to the extensibility and glutenins to the elasticity and strain hardening behavior of gluten (Sissons, 2008). The protein quality and content influence the dough characteristics which could be associated with the storage proteins in order to determine flour bread quality. The rheological characteristics of flour vary between wheat varieties/cultivars. Various attempts have also been made to establish relationships between rheological properties of flour and high molecular weight (HMW) glutenin subunits (Sliwinsk *et al*., 2004).The variation in dough rheology and bread making performance between wheat cultivars is largely determined by differences in protein quantity and composition. Storage proteins have significant correlation with rheological characteristics of flour (Rehman, 1994; Pierre *et al*., 2007).

Wheat protein is deficient in some essential amino acids (lysine, methionine and threonine). Among legumes, gram (chickpea) contributes relatively higher quantity of essential amino acids (i.e. lysine, threonine and tryptophan) than wheat flour. Composite flour made up by mixing wheat and gram flour could make up amino acid deficiency thereby improving the quality of the leavened flat bread (naan) (Sabanis *et al*., 2006; Khatkar, 2006 and Iqbal *et al*., 2006). Earlier studies have emphasized the importance of the balance between aggregating chains of glutenin and monomeric gliadin polypeptides (Sliwinski *et al*., 2004). Therefore, quantification of types of proteins of composite flour and rheological properties was determined with reference to the end-use quality (texture of the leavened flat breads).

There are so many different aspects of food texture but external surface appearance (e.g. grainy, smooth or dry) in case of the naan is very important from acceptability point of view. In a pursuit to supplement wheat four with the gram flour to enhance nutritive value, the surface appearance of the naan gets grainy or dry due to difference in the particle size and moisture levels of the flours. Therefore, the main objective of the study was to first associate the farinographic characteristics of flours of wheat varieties/lines with their protein subunits for determination of effect on texture/appearance of the naans. Based on this information, ranking of the wheat varieties/lines was further aimed for supplementation with different levels of gram flour to improve protein and evaluate its effect on texture/appearance of the composite flour naans.

MATERIALS AND METHODS

1. Raw materials: Samples of seven wheat (*Triticum aestivum*) varieties/lines (LU-26, LU-31, Lines

4072, 4770, 4943, 5039 and 6500) were procured from the department of Plant Breeding and Genetics, University of Agriculture, Faisalabad, Pakistan. Varieties LU-26 and 31 were selected on the basis of their better dough behavior which is highly suitable for naan making The split gram (*Cicer arietinum*) was procured from local market.

2. Farinographic characteristics of flour: The straight grade flour samples of each wheat variety/line were run through Brabender Farinograph equipped with a bowl of 50 g capacity. The dough behaviour was determined according to the procedure described in AACC (1983).

3. Naan preparation and sensory evaluation: The traditional plain naan was prepared from the wheat flour (with and without gram flour). The simple and composite flour doughs containing instant yeast were kneaded manually. The dough balls (100 g each) were flatted with the hands to get naan dough disc (7.5″ dia) and baked in a *tandoor* (mud oven) by following the local method. Sensory evaluation for texture of the naans was performed on 10 hedonic scales (Land and Shephred, 1988) by an expert panel of judges.

4.Polyacrylamide gel electrophoresis (SDS-PAGE - discontinuoussystem): Protein composition i.e subunits were determined (Laemmli, 1970) using the SDS-PAGE system with a discontinuous Tris-glycine buffersystem using standard extraction procedure (Ng and Bushuk, 1987).

5. Statistical analysis: All the experiments were repeated three times and the data obtained was statistically analyzed using completely randomized design, multivariate factor and correlation analysis techniques (SAS; Minitab, 2008). Differences among the variables were analyzed using Duncan's Multiple Range and T-test applications.

RESULTS AND DISCUSSION

Farinographic properties of wheat flour: Physical dough characteristics like water absorption, arrival time, dough development, dough stability, dough resistance, tolerance index and softening of the dough were determined by farinographic study (Table 1). The differences in farinographic properties of flours depend on the wheat cultivars (Sliwinski *et al*., 2004). Farinographs are commonly used for determining the water absorption of flour, especially in industrial settings (Mondal and Datta, 2012). High water absorption of LU-31 (58%) as compared to other varieties/lines is an indication of better dough development for flat bread (naan) production.This result is in line with a similar study (Mohammed *et al*., 2012) where 58.8 ± 0.13% water absorption was reported. The major factors contributing to the farinograph absorption include protein content, starch, damaged starch, pentosans and gluten strength (Vizitiuand Danciu, 2011).

The arrival time varied slightly with the highest value 2.00 minutes for wheat variety LU-26 and Line 6500. This result is very close to some of the cultivars/lines as reported in Agronomy Progress Report (Mayo *et al*., 2009). Moreover, it showed that these samples (LU-26 and Line 6500) have the characteristics of durum hard wheat. On the other hand, dough development time varied significantly among all the wheat varieties/lines. It varied from 2.00 to 3.75 minutes for Line 4070 to variety LU-31 respectively. This result is in range of other researchers finding who reported 2.1 ± 0.23 (Mohammed *et al*., 2012), 1.53 ± 0.21 minutes (Paraskevopoulou *et al*., 2010) and 1.8 minutes (Voicu *et al*., 2012). Dough development showed a positive correlation with water absorption which might be due to the starch swelling leading to the changes in rheological properties especially in case of LU-31.

As far as resistance of the dough is concerned, longer the time, stronger will be the flour. The wheat variety LU-26 reflected very strong gluten content showing highest time (19.75 minutes) for resistance of the dough. This indicates that as the dough is rest, a cohesive gluten network was developed through inter- and intra-molecular bonding, which render the dough stronger and less stretchable (Sim*et al*., 2009). Dough stability also varied (1.50 to 17.25 minutes) among all the wheat varieties/lines. In the present study, wheat lines showed dough stabilities in range with other researchers findings who reported 1.40 ± 0.40 (Paraskevopoulou *et al*., 2010) and 6.1 ± 0.43 minutes (Mohammed *et al*., 2012).

The values of the softening of the dough also differed for all the wheat varieties/lines. In general, the flours which have low softening value are stronger and the ones having higher softening values are weaker. In the present study, wheat variety LU-26 was considered stronger as it possessed low softening value (40 BU). Another study (Mohammed *et al*., 2012) has reported degree of softening as 44± 3.12 BU, which is very close to the results of the present study. Tolerance index is the difference in Brabender units (BU) from the top of the curve measured 5 minutes after the peak is reached. In general, flours which have good tolerance to mixing have low tolerance and the higher the tolerance index value, the weaker the flour. The results of the present study are similar to the results of researchers (Ciacco and D'Appolonia, 1982) who recorded 30 BU for sound wheat flour which is similar to the result of the wheat Line-4072. The overall rheological characteristics of wheat flour ranked wheat variety LU-26 at the top followed by LU-31 having the strongest flour characteristics for optimum dough development, suitable for leavened flat bread (naan).

Texture of the simple wheat naans: Texture being the main attribute of sensory characteristics for the leavened flat bread (naan) was evaluated in order to select the top ranked varieties/lines for gram flour supplementation. Mean scores (Table 2) for texture of the naans prepared from simple flours of different wheat varieties/lines varied significantly where variety LU-26 got the maximum score (8.20) followed by LU-31 (8.00).

Protein Acrylamide Gel Electrophoreses (PAGE) of simple and composite flours: SDS-PAGE of simple flours of various wheat varieties/lines and gram (chickpea) flour was done in order to study the resultant peptide bands. Typical scans (not shown) of the electrophoretic bands showed variation in the band intensity calculated on area percent basis (Table 3). Then, based on the physicochemical characteristics of the simple flours of various wheat varieties/lines and subsequent evaluation of the texture of the naans made from their flours, the top ranked varieties LU-26 and LU-31 were selected for supplementation with 5, 10, 15, 20 and 25% gram (chickpea) flour. The composite flours of both the varieties (LU-26 and 31) were also subjected to SDS-PAGE, typical scans (not shown) of which showed significant variation in band intensities corresponding to the increased proportions of gram supplementations as calculated on area percent basis (Tables 4 and 5 respectively). The addition of gram flour increased the protein content of the composite flours. This increase in the protein content was depicted by the increased intensity of the electrophoretic bands. A proportionate increase in the bands intensity took place with an increase in the additional levels of gram flour ranging from 5 - 25%.

Molecular weight distribution of proteins of wheat varieties/lines with and without different levels of gram flour supplementation was determined with the help of standard molecular markers on SDS-PAGE gels. The storage proteins of flours were quantified through gel-electrophoresis and scanning densitometry. Differences in the intensity and distance traveled by each band among the wheat flour and composite flour samples reflected differences in protein content. The patterns were divided into five groups of bands, A1 to A5 according to their molecular weights in the descending order. Relative molecular ranges were assigned corresponding to each area on the basis of previous studies where A1 to A3 areas mainly composed of glutenin protein subunits, gliadin protein subunits also constitute somewhat less than half of the area A3 and ω-gliadin are slightly present in area A2. The high molecular weight (HMW) glutenin subunits are clearly differentiated into group A1 $\geq 70 \times 10^3$ (70 KD); A2 group is mainly ω-gliadin, 70 – 60 KD; A3 mainly low-molecular-weight (LMW) glutenin subunits from 60–40 KD. Certain higher molecular weight gliadins have mobilities in the A3 range. Group A4 corresponds mainly to α- and β-gliadins but certain LMW appear in A4 region. Area A5 is referred to globulins and it has polypeptide molecular weight range <28K (Urminská and Bašista, 2005; van Eckert *et al*., 2010 and MacRitchie *et al*., 1991).

Wheat variety LU-26 had highest ratio of glutenins (0.39) and Line 4072 lowest (0.26) among all wheat varieties/lines (Table 3). The results of the simple wheat flour protein fractions are close to the findings of Singh *et al*. (2011) where the analysis of polymeric proteins showed glutenins, gliadins and glutenin to gliadin ratios between 28.14 and 40.44%, 45.33 and 55.83% and 0.50 and 0.89, respectively. While, in case of the composite flours, the findings of the present study support an increase in the proportion of gliadin with increase in protein content as the gliadins are the dominant fractions in the wheat endosperm. The bands are further strengthened by an increase in the percent of gram flour to wheat flour. Area A5 corresponds to LMW albumins and globulins, ≤ 28 KD. Different protein entities in groups A3, A4 and A5 overlap somewhat. For protein molecular weights near 66 KD and 45 KD there was apparent aggregation of 2-3 bands resulting in an unexpectedly high value. Quantifications performed are percentages of total area, and also related to the amount of dye bound by each polypeptide. Generally, the proportions of proteins increased with increase in protein content.

Association among protein bands, farinographic properties and texture of the leavened flat breads (naans) from simple flours of various wheat varieties/lines: Application of multivariate factor analysis provided information about interrelationships among the studied parameters. A two dimensional loading plot was generated to elucidate relationships among these variables. The resultant plot is presented in Fig. 1. According to this plot, variables situated together far from the origin are positively correlated. They are considered as negatively correlated if present on opposite sides of the origin. It is evident from the plot that not all of the bands were positively correlated with each parameter. There existed a balance between the tolerance index and texture of the leavened flat breads. Water absorption and softening of the dough were also inversely related. The bands in the areas A1, A3 and A4 showed a significant relationship with texture of the breads. Water absorption and softening of the dough also correlated significantly with the bands A2 and A5. Furthermore, dough development, arrival time, resistance to dough, dough stability and texture of the breads had an inverse relationship with the water absorption and softening of the dough and a negative correlation with the polypeptides A2 and A5. The subunits of proteins in the area A5 reflected a negative correlation with the texture of the breads.

From the above results, it is exhibited that out of the five band areas only two band areas (A2 and A5) showed a positive correlation with the two parameters of farinographic characteristics (water absorption and softening of the dough). All other parameters of farinograph showed a negative correlation. Water addition required for optimum dough development of wheat cultivars correlates positively with gluten protein content indicating that all glutens require similar amounts of water for proper hydration. There exists strong dependence of rheological properties on molecular weight and molecular weight distribution for polymers (Sliwinski *et al*., 2004). The water addition correlates positively with protein content ($r = 0.87$) and with gluten protein content ($r = 0.97$). Dough development correlates negatively ($r = -0.80$, $P<0.01$) with group A2+A4+A5 (gliadins, albumins and globulins, respectively) (Rehman, 1994). This indicated that these protein fractions had adverse effects on the strength of flour. Peak development time decreased as the proportion of glutenin decreased. Dough stability showed significant negative correlation ($r=-0.66$, $P<0.05$) with gliadins (A2+A4), reflecting weakening of flours with an increase in the gliadins content. The area A2 is related to glutenin and gliadin subunits with polypeptide molecular weight from 60-70K. The main factor determining the rheological behavior of hydrated gluten is the glutenin to gliadin ratio. These protein fractions (glutenin and gliadin) must be present for optimal gluten network development in a specific ratio (Mohammed *et al*., 2012).

Dough resistance showed negative correlation with the protein subunits. This result is close to the finding (Rehman, 1994) who found that resistance to extension showed weak negative correlations with gliadin contents (A2+A4) and α+β gliadins content (A4), which was correlated significantly ($r = 0.75$, $P<0.05$) with dough stability (DS). Similarly, in another study no significant correlation was found between total protein content and bread making characteristics during quality classification of 13 Iranian wheat cultivars (Peighambardoust *et al*., 2011).

The polypeptide bands in the areas A1, A3 and A4 affected the texture of the naans positively. These bands are concerned to the glutenin, gliadin and albumin subunits. The polypeptides A1 have a molecular weight of >70KD. The protein subunits in the areas A3 and A4 have a range of 28 to 60KD molecular weight. As, these polypeptides are related to the strength of the dough, the texture of the bread is also improved due to the dough strength.

Association between protein bands and texture of the leavened flat breads obtained from composite flours of the wheat varieties LU-26 and LU-31: The increased levels of gram flour supplementation caused proportionate hardness of the naan texture for both varieties which resulted in lower score but still acceptable. Most people who have tried bread from chickpea-wheat flour mixes have found the texture to be appealing (Mohammed *et al*., 2012). In overall rating, variety LU-26 got higher score (5.72) than LU-31 (5.54) (Table 6).This result is referenced to a study where hardness in the texture could be attributed primarily to the gluten fraction, which decreases upon being diluted by the added gram (chickpea) protein (Sabanis *et al*., 2006). Flour ccharacteristics contributing to dough rheology also vary from variety to variety and sometimes among cultivars (Farooq and Boye, 2011).

The protein bands (A1-A5) of the composite flours of wheat varieties LU-26 and LU-31were correlated with the texture of the naans. Application of multivariate factor analysis made it convenient to establish a correlation between the protein subunits and texture of the naans. A two dimensional loading plot was generated to elucidate relationships between these variables. It is evident from the Fig. 2 that all the polypeptide bands showed a negative correlation with the texture of the naans for both the wheat varieties. The reason for this negative correlation might be the harder naan texture due to low moisture content of gram flour. This finding is supported by another study (Paraskevopoulou *et al*., 2010) where incorporation of lupin protein isolate into wheat flour significantly impaired the texture of the baked breads.

Another reason for this inverse relationship might be that with the added levels of gram flour into wheat flour, the dough rheology changed which is the direct measurement of the texture of the product. The presence of chickpea flour in wheat flour dough affects bread quality in terms of texture (Mohammed *et al*., 2012).The result of present study is related to a previous study in which exposure of both gliadins and glutenins to reducing agents altered the mixing behavior of these fractions (Skerritt*et al*., 1996). The variation in the particle size of the gram flour and wheat flour could be another potential reason for non-correlation of the texture of the naans with the polypeptide units of the proteins of the enriched flours. Likewise, in another study, dough rheology, firmness, cohesiveness, rollability and some physical properties of tortillas were negatively affected with the increase (15, 25 and 35%) in small red, black, pinto or navy bean flour regardless of the bean cultivar (Anton *et al*., 2008). Correlations between the quantity of certain polypeptides components and texture of the breads would require further study before relationships can be established.

Table 1.Farinographic characteristics of various wheat varieties/lines.

Characteristics	Variety LU-26	Variety LU-31	Line 4072	Line 4070	Line 4943	Line 5039	Line 6500
Water absorption (%)	55.30 e	58.00 a	57.30 b	56.20d	54.80f	56.60c	54.40g
Arrival time (min.)	2.00	1.75	1.50	1.50	1.50	1.75	2.00
Dough development (min.)	2.50 b	3.75 a	2.25 b	2.00 b	2.25 b	2.50 b	2.50b
Resistance to dough (min.)	19.75 a	11.00 c	10.45c	15.00b	4.00 d	5.50 d	4.00 d
Dough stability (min.)	17.25 a	7.25 c	8.20 c	13.00b	1.75de	3.00 d	1.50 e
Softening of dough (BU)	40.00 b	50.00 b	80.00 a	40.00b	50.00b	50.00b	40.00b
Tolerance index (BU)	40.00 bc	40.00bc	30.00 c	20.00c	60.0ab	40.0bc	70.00a

Means sharing similar letter(s) are statistically non-significant at 5% probability.

Table 2. Texture scores for the sensory evaluation of simple flour naans made from various wheat varieties/lines.

Characteristic	Variety LU-26	Variety LU-31	Line 4072	Line 4070	Line 4943	Line 5039	Line 6500
Texture	8.20 a	8.00 a	6.73 b	6.53 b	6.93 b	6.57 b	6.53 b

Means sharing similar letter(s) are statistically non-significant at 5% probability.

Table 3. Electrophoretic band areas of various wheat varieties/lines and gram flour

	Area of bands (%)					
Variety/line	**A1**	**A2**	**A3**	**A4**	**A5**	**RT**
LU-26	10.10	15.50	18.40	32.30	23.70	0.39
LU-31	8.30	18.20	15.90	30.40	27.20	0.32
Line 4072	6.10	15.70	14.80	29.90	33.50	0.26
Line 4770	6.95	16.70	15.30	30.30	30.75	0.28
Line 4943	6.87	16.00	15.90	29.90	31.33	0.29
Line 5039	7.55	17.20	16.40	28.90	29.95	0.31
Line 6500	7.65	17.30	16.50	29.50	29.05	0.31
Gram flour	13.40	16.30	19.20	33.10	18.00	0.48

RT = Ratio of glutenins (A1+A3) to gliadins, albumins and globulins (A2+A4+A5).

Table 4. Electrophoretic band areas of composite flour of wheat variety LU-26

Treatments	Area of bands (%)					
	A1	**A2**	**A3**	**A4**	**A5**	**RT**
LU-26	10.10	15.50	18.40	32.30	23.70	0.39
5% gram flour	10.60	16.30	19.30	33.90	19.80	0.43
10% gram flour	11.10	17.00	20.20	35.50	16.00	0.46
15% gram flour	11.60	17.80	21.10	37.10	12.30	0.49
20% gram flour	12.10	18.60	22.00	38.70	8.40	0.52
25% gram flour	12.60	19.40	23.00	40.40	4.60	0.55
Gram flour	13.40	16.30	19.20	33.10	18.00	0.48

RT = Ratio of glutenins (A1+A3) to gliadins, albumins and globulins (A2+A4+A5).

Table 5. Electrophoretic band areas of composite flour of wheat variety LU-31

	Area of bands (%)					
Treatments	**A1**	**A2**	**A3**	**A4**	**A5**	**RT**
LU-31	8.30	18.20	15.90	30.40	27.20	0.32
5% gram flour	8.70	19.10	16.90	31.90	23.50	0.34
10% gram flour	9.10	20.00	17.40	33.40	19.90	0.36
15% gram flour	9.50	20.90	18.20	34.90	16.30	0.38
20% gram flour	9.90	21.80	19.00	36.50	12.60	0.41
25% gram flour	10.40	22.70	19.80	38.00	9.00	0.43
Gram flour	13.40	16.30	19.20	33.10	18.00	0.48

RT = Ratio of glutenins (A1+A3) to gliadins, albumins and globulins (A2+A4+A5).

Table 6. Score of the texture of the composite flour naans of varieties LU-26 and 31

Characteristic	LU-26	LU-31
Texture	5.72 a	5.54 b

Means sharing similar letter(s) are statistically non-significant at 5% probability.

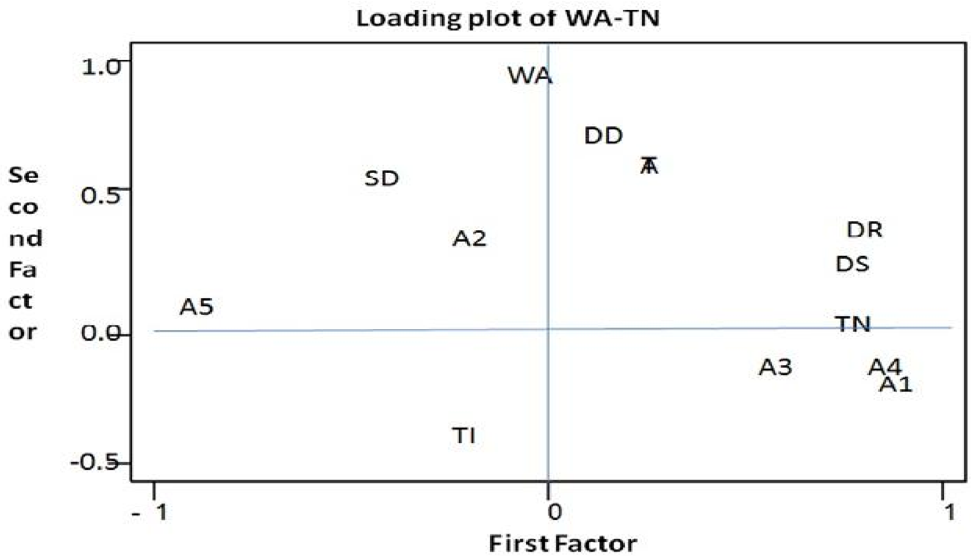

Fig. 1 Loading plot for association among protein bands, rheological properties & texture of the simple flour leavened flat breads (naans)

WA: Water absorption; AT: Arrival time; DD: Dough development time; DR: Resistance to dough; DS: Dough stability; SD: Softening of the dough; TI: Tolerance index; TN: Texture of the naan; A1-A5: Electrophoretic band areas occupied by protein subunit

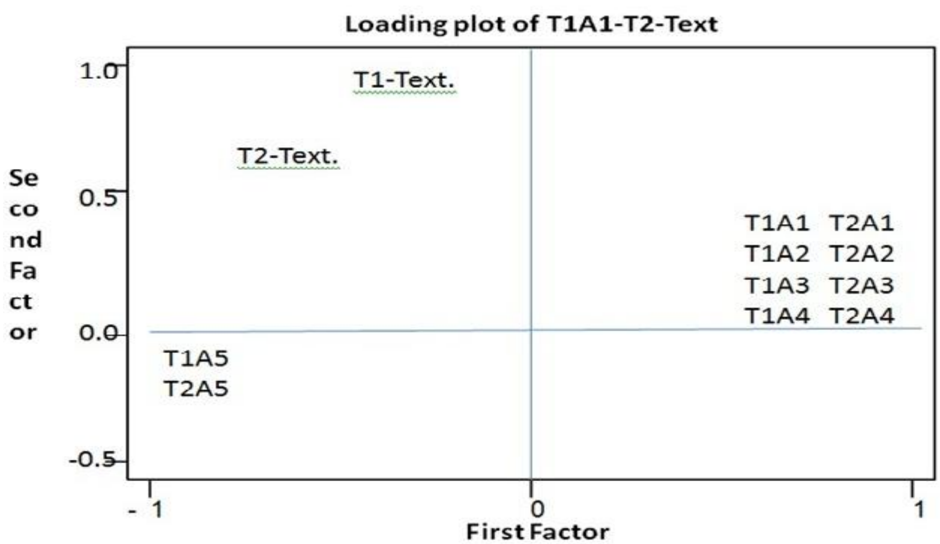

Fig. 2. Loading plot for association between the protein bands of composite flour and texture of the composite flour leavened flat breads (naans)

T1: Wheat variety LU-26; T2: Wheat variety LU-31; Text.: Texture of the naan; A1-A5: Electrophoretic band areas occupied by protein subunits

Conclusion: A definite association was noticed among farinographic properties, storage protein subunits and texture of the leavened flat breads (naans) obtained from simple flours. However, texture of the composite flour naans deteriorated with increased concentration of gram flour as the concentration of storage protein subunits increased. In future, it should be possible to use proteomics to predict quality and classify grain and flour samples by cultivar type or class. Moreover, mass spectrometry may also be applied to obtain a quantitative analysis of the distribution of protein types in a flour sample.

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