

DOUGH FERMENTATION PROPERTIES AS A
FUNCTION OF PHYSICAL AND CHEMICAL
CHANGES

By

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TABLE OF CONTENTS

Chapter	Page
I. INTRODUCTION	1
Statement of problem.....	1
Purpose of study.....	2
Hypothesis.....	2
Assumption	3
II. REVIEW OF LITERATURE.....	4
Gluten protein	4
Effect of Mixing, Water, NaCl and Yeast.....	5
Effect of emulsifiers.....	6
Effect of Ascorbic Acid, Urea and DTT.....	8
Recording the fermentation properties.....	9
Cited references	13
III. EFFECT OF REDUCING SURFACE TENSION OF DOUGH ON FERMENTATION PROPERTIES OF DOUGH.....	19
Abstract.....	19
1. Introduction.....	20
2. Materials and methods	21
3. Statistical analysis.....	23
4. Results and Discussion	23
5. Conclusions.....	33

Chapter	Page
6. Cited references	34
IV. EFFECT OF OXIDIZING STATE ON FERMENTATION PROPERTIES OF DOUGH.	54
Abstract	54
1. Introduction.....	55
2. Materials and methods	56
3. Statistical Analysis.....	58
4. Results and Discussion	58
5. Conclusions.....	68
6. Cited references	69
V. EFFECT OF DISRUPTION OF HYDROGEN AND HYDROPHOBIC BONDS ON FERMENTATION PROPERTIES OF DOUGH.....	86
Abstract	86
1. Introduction.....	87
2. Materials and methods	88
3. Statistical Analysis.....	90
4. Results and Discussions.....	90
6. Conclusions.....	99
7. Cited references	100

Chapter	Page
VI. EFFECT OF DISRUPTION OF DISULFIDE BONDS ON FERMENTATION	
PROPERTIES OF DOUGH.....	115
Abstract.....	115
1. Introduction.....	116
2. Materials and methods	117
3. Statistical Analysis.....	119
4. Results and Discussion	119
6. Conclusions.....	128
7. Cited references	129
VII. CONCLUSIONS	145
VIII. FUTURE STUDIES.....	148
APPENDIX 1.....	149

LIST OF TABLES

Table	Page
CHAPTER III. EFFECT OF REDUCING SURFACE TENSION OF DOUGH ON FERMENTATION PROPERTIES OF DOUGH.	
1. Definitions of fermentation variables.....	37
2. Definitions of visco-elastic, mixing and baking terms.....	38
3. Proximate analysis of flours (means \pm SD, n=2) obtained from sites A and B.....	39
4. Fermentation properties in six commercial wheat flours treated with DATEM levels. Means (n=2) with same superscripts in a column are not significantly different ($P > 0.05$). The standard deviations of means are shown in parenthesis.....	40
5. Change of percent of fermentation properties of six commercial wheat flours treated with DATEM levels. Values with * are significantly different with control samples. Percentages are calculated from values in Table 4. % change = (Sample treated with additive - control sample)/control sample * 100.....	42
6. Explained variance (%) in PCA of fermentation variables with negative control in flours treated with DATEM.....	43
7. Explained variance (%) in PCA of fermentation variables with flour protein in flours treated with DATEM.....	43
8. Explained variance (%) in PCA of fermentation variables without negative control in flours treated with DATEM.....	44
9. Explained variance (%) in PCA of fermentation variables with flour protein and without negative control in flours treated with DATEM.....	44
10. Explained variance (%) in PCA of fermentation variables when compared with visco-elastic, mixing and baking variables in flours treated with DATEM. Definitions of fermentation, visco-elastic, mixing and baking variables explained in Table 2.....	45
11. Explained variance (%) in PCA of fermentation variables when compared with baking variables in flours treated with DATEM.....	46
CHAPTER IV. EFFECT OF OXIDIZING STATE ON FERMENTATION PROPERTIES OF DOUGH.	
1. Fermentation properties in six commercial wheat flours treated with ascorbic acid levels. Means (n=2) with same superscripts in a column are not significantly different ($P > 0.05$). The standard deviations of means are shown in parenthesis.....	71
2. Change (percent) of fermentation properties of six commercial wheat flours treated with ascorbic acid levels. Values with * are significantly different with control samples. Percentage calculated values are from Table 1 and % change = (Sample treated with additive - control sample)/control sample * 100.....	74
3. Explained variance (%) in PCA of fermentation variables with negative control in flours treated with ascorbic acid.....	75

4. Explained variance (%) in PCA of fermentation variables with flour protein in flours treated with ascorbic acid.....	75
5. Explained variance (%) in PCA of fermentation variables without negative control in flours treated with ascorbic acid	76
6. Explained variance (%) in PCA of fermentation variables with flour protein and without negative control in flours treated with ascorbic acid	76
7. Explained variance (%) in PCA of fermentation variables when compared with visco-elastic, mixing and baking variables in flours treated with ascorbic acid. Definitions of fermentation, visco-elastic, mixing and baking variables explained in Table 2.....	77
8. Explained variance (%) in PCA of fermentation variables when compared with baking variables in flours treated with ascorbic acid	78

CHAPTER V. EFFECT OF DISRUPTION OF HYDROGEN AND HYDROPHOBIC BONDS ON FERMENTATION PROPERTIES OF DOUGH.

1. Fermentation properties in six commercial wheat flours treated with urea levels. Means with same superscripts in a column are not significantly different (P > 0.05). The standard deviations of means are shown in parenthesis.....	101
2. Change (percent) of fermentation properties of six commercial wheat flours treated with urea levels. Values with * are significantly different with control samples. Percentage calculated values are from Table 1 and % change = (Sample treated with additive - control sample)/control sample * 100.....	103
3. Explained variance (%) in PCA of fermentation variables with negative control in flours treated with urea	104
4. Explained variance (%) in PCA of fermentation variables with flour protein in flours treated with urea.....	104
5. Explained variance (%) in PCA of fermentation variables without negative control in flours treated with urea	105
6. Explained variance (%) in PCA of fermentation variables with flour protein and without negative control in flours treated with urea	105
7. Visco-elastic, mixing and baking variables in flours treated with urea. Definitions of fermentation, visco-elastic, mixing and baking variables explained in Table 2	106
8. Explained variance (%) in PCA of fermentation variables when compared with baking variables in flours treated with urea.....	107

CHAPTER VI. EFFECT OF DISRUPTION OF DISULFIDE BONDS ON FERMENTATION PROPERTIES OF DOUGH.

1. Fermentation properties in six commercial wheat flours treated with DTT levels. Means with same superscripts in a column are not significantly different (P > 0.05). The standard deviations of means are shown in parenthesis.....	131
2. Change (percent) of fermentation properties of six commercial wheat flours treated with DTT levels. Values with * are significantly different with control samples.	

Percentage calculated values are from Table 1 and % change = (Sample treated with additive - control sample)/control sample * 100.....	133
3. Explained variance (%) in PCA of fermentation variables with negative control in flours treated with DTT.....	134
4. Explained variance (%) in PCA of fermentation variables with flour protein in flours treated with DTT	134
5. Explained variance (%) in PCA of fermentation variables without negative control in flours treated with DTT	135
6. Explained variance (%) in PCA of fermentation variables with flour protein and without negative control in flours treated with DTT.....	135
7. Visco-elastic, mixing and baking variables in flours treated with DTT. Definitions of fermentation, visco-elastic, mixing and baking variables explained in Table 2	136
8. Explained variance (%) in PCA of fermentation variables when compared with baking variables in flours treated with DTT	137

LIST OF FIGURES

Figure	Page
CHAPTER III. EFFECT OF REDUCING SURFACE TENSION OF DOUGH ON FERMENTATION PROPERTIES OF DOUGH.	
1. A graphical representation of gaseous curve of a) control sample from flour 3B and b) sample containing 0.6% DATEM (3B0.6). Blue tracings are the total volume and the red is the volume retained.....	47
2. A graphical representation of dough development of a) control sample from flour 3B and b) sample containing 0.6% DATEM (3B0.6).....	47
3. Loading plot of first two principal components based on fermentation properties with negative control of six commercial wheat flours, added with four levels of DATEM. Definitions of fermentation, visco-elastic, mixing and baking variables explained in Table 2 and 3. Flour protein content (%), 1A = 7.95, 2A = 11.19, 3A = 13.68, 1B = 10.4, 2B = 10.59 and 3B = 11.38, respectively.....	48
4. Loading plot of first two principal components based on fermentation properties with flour protein of six commercial wheat flours added with four levels of DATEM....	49
5. Loading plot of first two principal components based on fermentation properties without negative control of six commercial wheat flours added with four levels of DATEM.....	50
6. Loading plot of first two principal components based on fermentation properties with flour protein of six commercial wheat flours containing four levels of DATEM. Negative control samples were removed.....	51
7. Loading plot of first two principal components based on fermentation, baking, visco-elastic and dough properties of six commercial wheat flours added with four levels of DATEM.....	52

8. Loading plot of first two principal components based on fermentation and baking properties of six commercial wheat flours added with four levels of DATEM.....53

CHAPTER IV. EFFECT OF OXIDIZING STATE ON FERMENTATION PROPERTIES OF DOUGH.

1. A graphical representation of gaseous curve of a) control sample from flour 3B and b) sample containing 200 ppm ascorbic acid (3B200). Blue tracings are the total volume and the red is the volume retained79
2. A graphical representation of dough development of a) control sample from flour 3B and b) sample containing 200 ppm ascorbic acid (3B200).79
3. Loading plot of first two principal components based on fermentation properties with negative control of six commercial wheat flours, added with five levels of ascorbic acid. Definitions of fermentation, visco-elastic, mixing and baking variables explained in Table 2 and 3.80
4. Loading plot of first two principal components based on fermentation properties with flour protein of six commercial wheat flours added with five levels of ascorbic acid.....81
5. Loading plot of first two principal components based on fermentation properties without negative control of six commercial wheat flours added with five levels of ascorbic acid82
6. Loading plot of first two principal components based on fermentation properties with flour protein of six commercial wheat flours containing five levels of ascorbic acid. Negative control samples were removed.....83
7. Loading plot of first two principal components based on fermentation, baking, visco-elastic and dough properties of six commercial wheat flours added with five levels of ascorbic acid84
8. Loading plot of first two principal components based on fermentation and baking properties of six commercial wheat flours added with five levels of ascorbic acid85

CHAPTER V. EFFECT OF DISRUPTION OF HYDROGEN AND HYDROPHOBIC BONDS ON FERMENTATION PROPERTIES OF DOUGH.

1. A graphical representation of gaseous curve of a) control sample from flour 3B and b) sample containing 1M urea (3B1). Blue tracings are the total volume and the red is the volume retained.....108
2. A graphical representation of dough development of a) control sample from flour 3B and b) sample containing 1M urea (3B1).....108
3. Loading plot of first two principal components based on fermentation properties with negative control of six commercial wheat flours, added with four levels of urea. Definitions of fermentation, visco-elastic, mixing and baking variables explained in Table 2 and 3.109
4. Loading plot of first two principal components based on fermentation properties with flour protein of six commercial wheat flours added with four levels of urea110

5. Loading plot of first two principal components based on fermentation properties without negative control of six commercial wheat flours added with four levels of urea	111
6. Loading plot of first two principal components based on fermentation properties with flour protein of six commercial wheat flours containing four levels of urea. Negative control samples were removed.....	112
7. Loading plot of first two principal components based on fermentation, baking, visco-elastic and dough properties of six commercial wheat flours added with four levels of urea	113
8. Loading plot of first two principal components based on fermentation and baking properties of six commercial wheat flours added with four levels of urea	114

CHAPTER VI. EFFECT OF DISRUPTION OF DISULFIDE BONDS ON FERMENTATION PROPERTIES OF DOUGH.

1. A graphical representation of gaseous curve of a) control sample from flour 3B and b) sample containing 0.5 mM DTT (3B0.5). Blue tracings are the total volume and the red is the volume retained.....	138
2. A graphical representation of dough development of a) control sample from flour 3B and b) sample containing 0.5 mM DTT (3B0.5).	138
3. Loading plot of first two principal components based on fermentation properties with negative control of five commercial wheat flours, added with four levels of DTT. Definitions of fermentation, visco-elastic, mixing and baking variables explained in Table 2 and 3.	139
4. Loading plot of first two principal components based on fermentation properties with flour protein of five commercial wheat flours added with four levels of DTT.....	140
5. Loading plot of first two principal components based on fermentation properties without negative control of five commercial wheat flours added with four levels of DTT	141
6. Loading plot of first two principal components based on fermentation properties with flour protein of five commercial wheat flours containing four levels of DTT. Negative control samples were removed.....	142
7. Loading plot of first two principal components based on fermentation, baking, visco-elastic and dough properties of five commercial wheat flours added with four levels of DTT.....	143
8. Loading plot of first two principal components based on fermentation and baking properties of five commercial wheat flours added with four levels of DTT.....	144

CHAPTER 1
INTRODUCTION

Statement of Problem

Wheat and its products play a dominant role in the food consumption of humans. The type and quality of wheat to be used is decided based on the properties desired in the end food product. Wheat is generally preferred to other cereals because wheat flour forms cohesive dough which results in easy aeration during the pre-baking processing steps. Wheat contains two types of proteins, namely gluten and non-gluten proteins. Gluten strength determines the extent of aeration in dough. Gluten proteins are divided into monomeric and polymeric gluten proteins, which affect the viscosity and elastic properties of dough. Gluten proteins are primarily responsible for the rheological properties of the dough. They also affect the fermentation properties of the dough. Gas production, gas retention and dough development are the main aspects during fermentation. Fermentation time and yeast activity in turn affect the extent of aeration. However, detailed studies of the effect of additives and quality and quantity of protein on dough fermentation properties are limited. Emulsifiers and surfactants added to a dough system can affect the fermentation properties. The effect of these emulsifiers and surfactants in the fermentation properties of dough are poorly studied. Several instruments are available to measure these properties of dough during fermentation. These include the conventional Maturograph, Oven rise recorder, Gasograph and Rheofermentometer.

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Monomeric and polymeric proteins interactions include hydrophobic and hydrophilic non-covalent as well as covalent (disulfide) bonds. Surfactants, oxidizing and reducing agents modify surface tension and affect disulfide bonds. More studies are needed to understand the role of oxidizing agents like ascorbic acid on the baking and aerating properties of dough. Urea disrupts the hydrophilic and hydrophobic bonds and the interaction of polar amino acids with water affects the viscosity and elasticity of gluten. Urea can be used to elucidate the role of non-covalent bonds in gas retention properties. It is of interest to investigate the specific change in fermentation properties as a function of a decreasing number of disulphide bonds, the use of dithiothreitol can assist in the reduction of disulfide bonds in dough.

Purpose of the study

The objectives of the study are:

- 1) To study the effect of reducing surface tension [Chapter III (17)], the oxidized state [Chapter IV (51)], disruption of hydrogen and hydrophobic bonds [Chapter V (84)] and disruption of disulfide bonds [Chapter VI (113)] on fermentation properties of dough made from commercial hard red winter wheat flours with different protein content, and
- 2) To identify possible correlations between fermentation and visco-elastic, mixing and baking properties of dough.

Hypotheses

The following null hypothesis will be tested. There is no significant effect on fermentation properties between control flours and flours treated with DATEM to reduce the surface tension. There is no significant effect on fermentation properties between control flours and flours treated with ascorbic acid to oxidize the dough. There is no significant effect on fermentation properties between control flours and flours treated with urea and DTT to disrupt the hydrogen and

hydrophobic bonds and disulphide bonds. If the null hypotheses are rejected, the effects of the mentioned factors (surface tension and bonds) will be explained from possible structural changes that occurred in dough or differences in the nature of the proteins present in the gluten.

Assumptions

DATEM is an anionic oil-in-water emulsifier. DATEM is produced by the reaction of mono and diacetyl tartaric acid with monoglycerols or mixtures of mono and diacylglycerols derived from edible fats. The effect of DATEM varies on its components; when DATEM is added to dough, it will enhance the strength and elasticity of the dough and improve gas retention. We assume that DATEM interacts with proteins, especially glutenin to improve the gas retention of dough. Ascorbic acid in the form of dehydroascorbic acid (DHA) reacts with glutathione (GSH) converting it to its oxidized form (GSSG), increasing the dough strength. We assume that ascorbic acid will strength the dough will increase the retention capacity of the dough and loaf volume. Urea and DTT will disrupt the hydrogen, hydrophobic and disulphide bonds when they react with gluten and decrease dough strength. We also assume that urea and DTT will reduce the gas retention capacity of the dough and loaf volume.

CHAPTER II

REVIEW OF LITERATURE

Gluten protein

Protein quality and quantity in flour are of specific importance in the bread making performance (Goesaert and Brijis, 2005). Gluten proteins determine the rheological properties of the optimally mixed dough which contribute to the gas retention properties of the fermenting dough (Fig. 1) (Gan et al., 1995). These gas retention properties will affect the loaf volume which is one of the key factors evaluated in yeast-fermented products. Gluten consists of gliadins and glutennins. Gliadins are monomeric low molecular weight proteins linked by interchain disulfide bonds whereas glutenins are mixture of low and high molecular weight proteins. Two main factors that affect the protein quality are gluten protein quality and gliadin/glutenin protein ratio (Goesaert and Brijis, 2005). When flour is mixed with water those gluten proteins form a cohesive visco-elastic gluten protein network (Singh and MacRitchie, 2001). This gluten protein network undergoes changes and retains carbon dioxide produced during fermentation (Graveland et al., 1980; Veraverbeke et al., 1999).

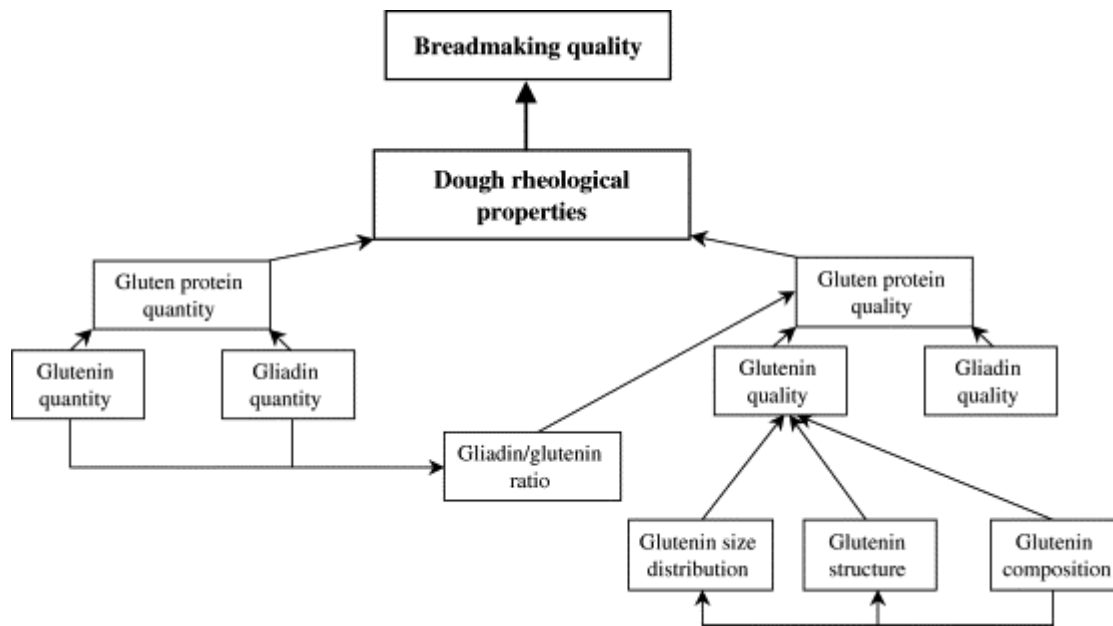


Figure 1. Factors governing bread making quality and wheat dough rheological properties (adapted from Veraverbeke and Delcour, 2002).

Effect of Mixing, Water, NaCl, and Yeast

The basic ingredients to form dough are wheat flour, water, NaCl and yeast. When these are mixed together a visco-elastic dough is formed. The function of mixing is to blend the ingredients into a homogeneous mass, to develop the dough into a three-dimensional visco-elastic structure with gas-retaining properties and to incorporate air which will form nuclei for gas bubbles that grow during dough fermentation (Bloksma et al., 1990; Collado and Leyn, 2000; Dobraszczyk and Morgenstern, 2003; Hosney and Rogers, 1990; Naeem et al., 2002).

The primary step in dough formation is addition of water which hydrates the proteins and forms cohesive and visco-elastic dough. Water acts as a plasticizer and solvent (Hosney et al., 1994). Rheological properties of dough are affected by the amount of water added to the flour (Ganet

al., 1994). Addition of water affects the consistency of dough and hydration time (Farahnaky and Hill, 2007).

Salt is added either as an aqueous solution or a dry powder (Kent and Evers, 1994). When salt is added to dough, it improves the flavor and also toughens the gluten and gives less sticky dough (Farahnaky and Hill, 2007). It has a strengthening effect on the gluten protein in the dough, Kojima et al., (1995) proved that when 1.5% salt was added to wheat dough the physical characteristics were affected. When salt is not added, dough mixes and rises faster and is more sticky. Larsson (2002) showed that doughs with NaCl had greater strength compared to doughs without NaCl. Salt increases the mixing tolerance but decreases the consistency of dough (Harinder and Bains, 1990). Salt increases the machinability of dough (Salovaara et al., 1982). Salt toughens the protein and increases mixing tolerance producing more stable and stiff dough (Galal et al., 1978; Shiu and Yeh, 2001).

When yeast is added to flour and mixing starts, yeast converts available sugars into CO₂ and ethanol under anaerobic conditions. The yeast produced CO₂ migrates into tiny cells formed during mixing by increasing internal gas pressure and subsequent expansion of dough (Hui and Corke, 2006).

Effect of Emulsifiers

Emulsifiers are fatty substances with hydrophilic and lipophilic properties. They will help to form an emulsion by reducing the surface tension of two immiscible phases (Dziezak et al., 1988; Flack et al., 1987; Krog et al., 1981). Classifications of emulsifiers are based on origin, solubility properties, presence of functional groups, hydrophilic/lipophilic balance (HLB) and potential for ionization (Artz et al., 1990). HLB index is defined as relative percentage of hydrophilic to lipophilic groups within the emulsifier molecule (Artz et al., 1990). Based on

ionization potential; surfactants are classified as either ionic and nonionic. Due to the presence of non-covalent bonds, nonionic substances do not dissociate in water (Stampfli and Nersten, 1995). Emulsifiers are divided into dough strengtheners and crumb softeners based on required properties in bread making. However some emulsifiers show both properties (Stampfli and Nersten, 1995). Diacetyl tartaric acid esters of monodiglycerides (DATEM), sodium stearyl-2-lactylate (SSL) and calcium stearyl-2-lactylate (CSL) are commonly used surfactants in bread making.

Actual mechanisms of these emulsifiers in dough strengthening are not fully understood. But theories have suggested that these dough strengtheners will form liquid films of lamellar structure in the interface between the gluten strands and the starch that improve the ability of gluten to form a film which retains the gas produced by the yeast (Krog et al., 1981; Stampfli et al., 1995). Emulsifiers increase the dough height by forming complexes with gluten proteins and protein-protein aggregates which increases the strength of gluten matrix (Gomez et al., 2004).

When diacetyl tartaric anhydride reacts with monoacyl glycerol with stearic acid as a main hydrophobic component, DATEM is formed. The carboxyl group of DATEM has an influence on the visco-elastic properties of dough and gluten (Koehler et al., 2001a). According to Koehler, DATEM at 0.1% w/w flour basis did not improve the loaf volume and above 0.5% w/w flour basis has no change in the visco-elasticity, dough properties and baking (Kohler and Grosch, 1999). Some researchers suggested that gluten, by slowing the diffusion of gas through dough phase contributes to gas retention. CO₂ which is produced by yeast fermentation diffuses to the gas cells and evaporates to generate excess pressure which leads to dough expansion (Gan et al., 1994).

Punching is done to remove large bubbles formed during mixing. Emulsifiers help in reducing the surface tension of bubbles which contributes to fine crumb structure (Campbell and Mougnet, 1999). DATEM from 0.4% to 0.7 % will break the bubbles formed during mixing by increasing the surface area for mass transfer which helps in dough expansion (Campbell et al., 2001).

Effect of Ascorbic Acid, Urea and DTT

Gluten plays a major role in bread making. The function of gluten depends on molecular weight of gluten, formation of covalent and non-covalent bonds between glutenin molecules and interactions between glutenin and other flour constituents (Goesacrt et al., 2005). Disulphide bonds hold the glutenin subunits together. Oxidizing and reducing agents will affect these glutenin subunits which lead to changes in rheological properties of dough (Fitchett and Frazier, 1986).

Dehydroascorbic acid oxidizes the sulphhydryl groups in gluten proteins. Ascorbic acid reacts with oxygen and forms deascorbic acid. L-ascorbic acid (L-AA) reacts with oxygen and forms L-dehydroascorbic acid (L-DHAA). L-DHAA acts as an oxidizing agent by promoting disulfide bonds which increase the loaf volume (Tsen et al., 1965). L-AA showed greater dough strengthening effects in low quality wheat flour than high quality (Aamodt et al., 2003). Ascorbic acid addition at 100 ppm showed a strong effect on dough rheology, mixing, and baking (Every et al., 2008).

During mixing, by addition of water hydrogen bonding increases the hydration of gluten. Hydrogen bonds break when gluten is deformed on extension. Formation of new hydrogen bonds occur when the stress is released (Belton et al., 1995). When urea (1 to 5 M) is added to gluten, it increases the elasticity by disruption of hydrogen bonding (Inda et al., 1999). DTT at 500 ppm decreases elasticity in gluten. But one study proved that when strong and weak gluten were treated with DTT at 500 ppm, elasticity decreased 60% in strong gluten and 42% in weak gluten (Khatkar et al., 2005).

Recording the fermentation properties

To observe and record the changes that occur during dough development, equipment which can continuously measure and record the changes is used. Maturograph, Oven rise recorder and Gasograph instruments have been used to measure the gas retention properties of dough but are not extensively used (Czuchajowska and Pomeranz, 1993).

The Rheofermentometer continuously records dough rise, gas formation and gas retention (Fig. 2).

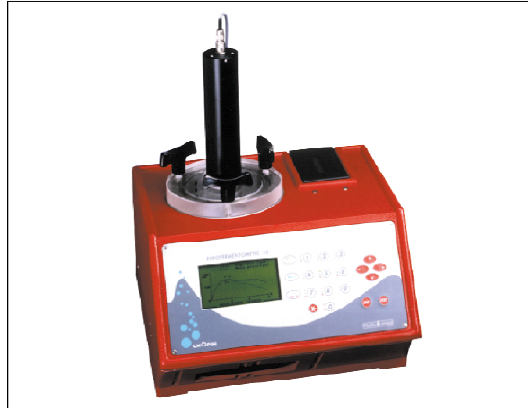
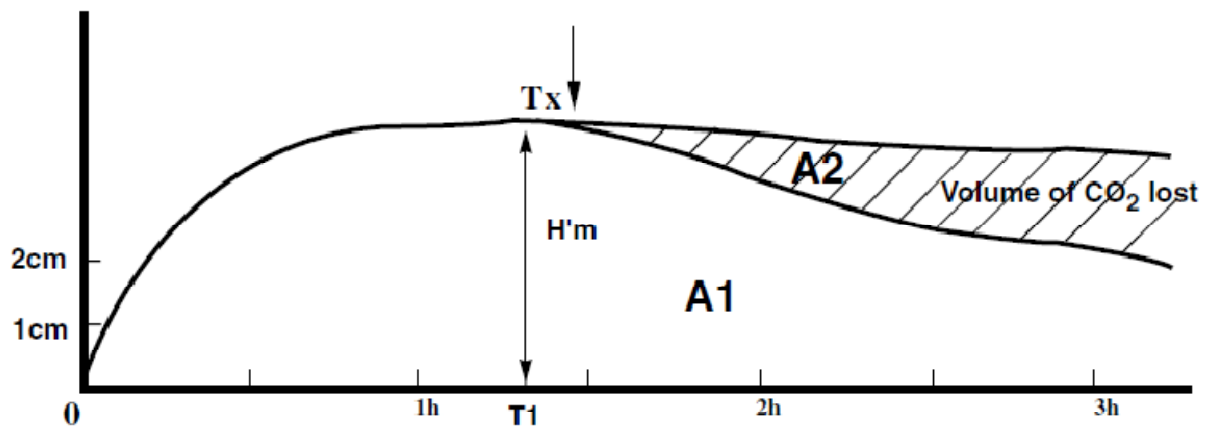


Figure 2. F3 Rheofermentometer (Source: Tripette & Renaud Chopin, 2004)

Rheofermentometer along with the alveograph as mixer have been used to test the quality of flour at relatively low and fixed water absorption (Czuchajowska and Pomeranz, 1993). The Rheofermentometer measures the fermentation properties of a dough sample when weight is placed on the sample and the development of dough is measured by a height sensor and gas development of the dough by a pressure sensor. The results are a gaseous release curve and dough development curve at the end of the test (Fig. 3) (Tripette & Renaud, 2004).

a)



b)

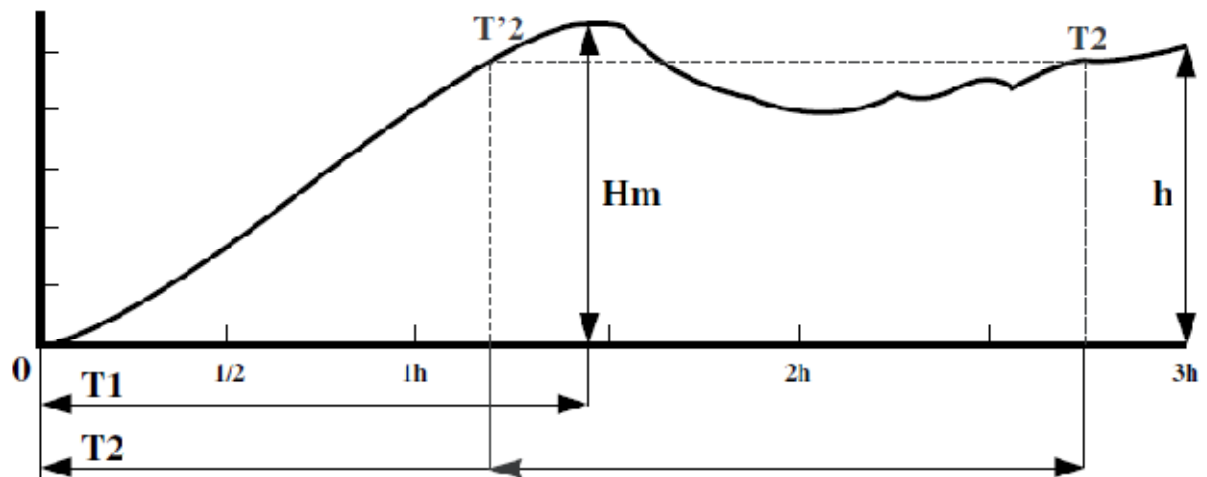


Figure 3. Gaseous release curve (a) and dough development curve (b) (Tripette & Renaud Chopin, 2004). Parameters obtained are explained in Table 1.

Table 1. Definitions of fermentation variables.

Abbreviations	Definitions	Units
Hm	Height of maximum dough development.	mm
h	Height of dough development at the end of the test.	mm
T1	Time of maximum rise.	h
(Hm-h)/Hm	Lowering of the development percentage after 4 h compared to time of maximum rise.	%
H'm	Maximum height of the gaseous curve.	mm
TV (A1 + A2)	Total volume of gaseous curve.	mL
VL (A2)	The carbon dioxide volume released by the dough during the fermentation test.	mL
VRt (A1)	Carbon dioxide retained in the dough at the end of the test.	mL
RC	Retention volume divided by the total gaseous release.	%
T'1	Time spent to reach maximum rise.	h

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CHAPTER III

EFFECT OF REDUCING SURFACE TENSION OF DOUGH ON FERMENTATION

PROPERTIES OF DOUGH

ABSTRACT

The objective of the study is to quantify the effect of reducing surface tension on fermentation properties of dough and to analyze possible correlation of fermentation and visco-elastic, mixing and baking properties of dough. Four levels of surface tension states were obtained by the addition of diacetyl tartaric acid ester of monoglyceride (DATEM) (0, 0.3%, 0.6% and 1.0% w/w, flour basis). Six commercial hard red winter wheat flours of different protein quantity and quality were used. Flours with no treatment were used as controls and flours with no yeast and no treatment were used as negative controls. Fermentation properties of dough were measured by Rheofermentometer F₃. Addition of DATEM increased the dough development and volume of gas retained. The levels of 0.3 and 0.6% DATEM increased the maximum height of dough development whereas 1% DATEM decreased it ($P < 0.05$). Fermentation variables explained more variance (69.2%) than the fermentation variables combined with visco-elastic, mixing and baking variables (47.9%). The ratio of dough heights $[(H_m - h)/H_m]$ is closely related to gluten elastic properties (Sep and RCY). Volume lost (VL) is closely related to gluten viscous (J-Jr, TCR) and negatively related to elastic properties. Maximum height of the dough (H_m) and dough height (h) are closely related to baking properties (LV and SV).

1. INTRODUCTION

It is well accepted that the genetic makeup and environment affect the end quality of wheat flour. The quality and quantity of the gluten present in the dough has direct influence on the fermentative and rheological properties of dough (Peterson et al., 1992; Baenziger et al., 1985; Bassett et al., 1989; Busch et al., 1969). Additives like emulsifiers and surfactants enhance the fermentative ability of dough. Surfactants are used to reduce the interfacial tension by enhancing stability and controlling destabilization of dough between oil and water interfaces. They interact with gluten proteins and enhance rheological characteristics at solid/liquid interface (Krog et al., 1991). Surfactants aid the incorporation and subdivision of air into the liquid phase which promotes foam formation and generally functions at the gas/liquid interface. Reducing surface tension favors foam formation. Stability of the foam is dependent on the stability of the film of water between air bubbles (Krog et al., 1990). One such emulsifier is DATEM. It is chemically an anionic oil-in-water emulsifier which helps to increase the volume of bread. DATEM can also enhance the gas-retention properties of the dough, thereby minimizing the chances of dough collapse (Zhang Xiujin et al., 2006). However, the influence of DATEM on dough properties varies with its chemical composition (Kohler et al., 2001a). For instance, DATEM with hydrophilic radicals increases the water retention capacity of dough and rheology of gluten (Kohler et al., 2001b). The gas retention capacity of dough is highly improved when DATEM interacts with gluten proteins and starch by forming inter-lamellar films in between starch and gluten (Zhang et al., 1993b; Stampfli et al., 1995). Several instruments are available to measure the rheological and gas retention properties of dough during fermentation. The Rheofermentometer (Chopin, Tripette and Renaud, France) is used in the study of the

behavior of flour during fermentation. The parameters measured are maximum dough height (H_m) in mm, maximum height of gaseous release (H'_m) in mm, CO₂ production in ml.

The objectives of this study were:

- 1) To study the effect of reducing surface tension of the dough using the surfactant DATEM on the fermentation properties of dough.
- 2) To analyze possible correlation of fermentation and visco-elastic, baking and mixing properties of dough.

2. Materials and Methods

a. Materials and Labeling

Six commercial hard red wheat flours were obtained from two different milling supplies A and B. They differ in protein content. DATEM (Caravan Ingredients, Lenexa, KS) was added to the flours at 0, 0.3, 0.6 and 1.0% w/w flour basis. Instant active dry yeast was from Lesaffre Yeast Corporation (Milwaukee, WI) and sodium chloride from Fisher Scientific (Fair Lawn, NJ). Flours with no DATEM were used as control (0) and flours with no DATEM and no yeast were used as negative control (N). Thus site A flours were labeled as 1A0 (positive control), 1AN (negative control), 1A0.3, 1A0.6, 1A1; 2A0, 2AN, 2A0.3, 2A0.6, 2A1; 3A0, 3AN, 3A0.3, 3A0.6 and 3A1. The 0, 0.3, 0.6 and 1 represent the percentage of DATEM added to the flour. Similarly site B flours were labeled as 1B0, 1BN, 1B0.3, 1B0.6, 1B1; 2B0, 2BN, 2B0.3, 2B0.6, 2B1; 3B0, 3BN, B0.3, 3B0.6 and 3B1.

b. Methods

Dough Preparation

Dough was prepared as described in the Chopin protocol using Chopin AlveoConsistograph. The ingredients consisted of 250 g of flour, 3 g of dry yeast and 5g of sodium chloride. DATEM was added to the flours at 0.3, 0.6 and 1.0 % w/w flour basis. The quantity of deionized water added depended on the moisture content of the flour and it was given by the reference table published by the International Association for Cereal Science and Technology (ICC) as described in the Chopin Protocol. The sodium chloride was dissolved in water prior to the addition to dough. Instant dry yeast and DATEM were blended with 250 g of flour in the kneader bowl. Salt water was progressively added to the flour at the beginning of the first minute of the mixing period. After one minute, the mixing was stopped to remove the flour sticking to the walls and ensure a homogeneous hydration. The mixing process was continued for 6 minutes. A sample size of 315 g of dough was used for each treatment.

Fermentation Test

Rheofermentometer was used to study the fermentation properties of dough. The dough (315 g) obtained from AlveoConsistograph was placed in the bottom of the aluminum basket and packed it down with hands. The height of the dough in the basket must be leveled out just below the lowest holes. The piston with a 2000 g weight was placed on top of the dough and temperature should stabilize to 28.5° C. The basket placed in the F3 Rheofermentometer bowl. Displacement sensor was placed and the whole system was tightly closed and the test was run for a total of 4 h. This time represents 1 h longer than the Chopin Protocol as it was determined experimentally with the samples and treatments in this study.

The F3 Rheofermentometer analyzes the development of a dough sample placed in the bowl. The piston placed on the dough rises. The piston is directly linked to a displacement sensor which will calculate the dough rising. Rheofermentometer is also linked to a pressure sensor through a pneumatic circuit that measures the pressure increase in the fermenting dough. The three curves are dough development, speed of CO₂ release and quantity produced and volume of CO₂ retained in dough. Fermentation variables are defined in Table 1 and visco-elastic, mixing and baking terms are defined in Table 2.

3. STATISTICAL ANALYSIS

A factorial design within a randomized block design was implemented. Five levels of DATEM and 3 levels of flour protein were compared in a 5 X 3 factorial. The significant differences in means were compared using Analysis of Variance (ANOVA) with Tukey's comparisons ($\alpha=0.05$) using SAS (Version 9.1 SAS Institute Inc., Cary, NC). Principal Component Analysis (PCA) is a mathematical algorithm that reduces the dimensionality of the data (Ringer, 2008). PCA is performed using Canoco for windows 4.5 (Biometris, Plant Research International, Wageningen, the Netherlands).

4. Results and Discussion

Protein, moisture and ash content of the flour samples are reported in Table 3. Typical dough fermentation property curves and the parameters obtained are illustrated in Figures 1 and 2 showing results for sample 3B control and 3B containing 0.6% DATEM. The volume of CO₂ lost (VL) is decreased when the surface tension of the sample is reduced (Fig. 1). Volume of retention of gas was improved for sample with reduced surface tension when compared with control sample. From the dough development curves we can observe that the height of dough is

improved when surface tension of sample is reduced (Fig. 2). A summary of the definition of fermentation properties of all samples is found in Table 1.

Maximum height of the dough (Hm)

Hm is the maximum height of dough development. As expected, the control sample without yeast shows no development (Table 4). The effect of reducing surface tension on Hm appears to be flour specific and could be attributed to differences in quality. Observations that significantly increased Hm ($P < 0.05$) were 1A flours with 0.3 and 0.6% levels and high protein B flours (at all levels) compared to the controls. Observations that significantly decrease Hm ($P < 0.05$) were 3A (high protein) flour with all treatments and 2A1 compared to the control. The effect of reducing surface tension on Hm does not appear to cause a general trend, which means it can detect quality differences in the flours. This means that there appears to be an optimum stability of the different phases of the dough (example gas-liquid, gas-solid) and this can be achieved with or without the addition of DATEM, depending on the quality of the wheat. Passing that optimum stability of such phases, the effect will be deleterious for the fermentation properties. So, it is possible that flour 3A does not need improvement of the stability of the different phases in the dough, thus, the addition of DATEM is not beneficial. The control sample has good fermentation properties without reducing the surface tension of its phases. Overall highlights are: highest value of Hm was shown by 3B1 (47.9 mm) and lowest by 1A1 (22.10 mm) (Table 4). The change of the fermentation properties (%) is reported in Table 5. High percentage (59%) increment in maximum height was observed in the lowest protein sample with 0.3% DATEM (1A0.3). A 28.1% decrease in maximum height was observed in the sample 3A (13.7% protein) with 1% DATEM (3A1) (Table 5). The overall effect of decrease in surface

tension on Hm was to decrease except for 3B with all the DATEM levels and 1A and 1B with 0.3 and 0.6 levels which show significant increase.

Height of the dough development (h)

The height of the dough development (mm) at the end of the test was denoted by h. As expected, negative controls showed no development. Overall, the effect of reducing surface tension on height of the dough at the end of the test is the same as the observed on maximum height of the dough. Lowest value of height was observed in 1B0 (19.85 mm) and highest value was observed in 3B1 (46.8 mm) (Table 4). For A flours, 0.3% and 0.6% of DATEM level increased the height of the dough when compared to control sample whereas 1% of DATEM decreased the height (Table 4). For B flours, h increased by increasing the % level of DATEM (Table 4). The highest increase (58%) of h was observed in 1A0.3 and highest (27.6%) decrease in 3A1 (Table 5).

Lowering of development percentage [(Hm-h)/Hm]

$(Hm-h)/Hm$ is the ratio of dough height at the end of the fermentation test in percentage. A large percent means the dough has maintained its height during fermentation. Effect of reducing surface tension on $(Hm-h)/Hm$ appear to be flour specific. The single observation that significantly increased $(Hm-h)/Hm$ was made for 1A flour (8% protein) with 0.3% level. Observations that significantly decreased $(Hm-h)/Hm$ were B flours (10.4% protein) at all DATEM levels. Overall highlights of trends are: high value was observed in 1B0 (39.1%) and lowest value in 3B0 (0) (Table 4). Highest percentage increase was observed in 2B0.6 (400%) and highest percentage decrease in 2A0.3 (59.1%) (Table 5). This also has the same effect as Hm and h as it shows increase with most of the 1A flours and no change in 3B flours.

Maximum height of the gaseous curve (H'm)

H'm is the maximum height of the gaseous release curve. Effect of decrease in surface tension on H'm also appears to be flour specific. H'm significantly increases in 1A with 0.3 and 1% DATEM and 3B flours with all levels of DATEM. While H'm significantly decreases in 3A with 0.3 and 1% DATEM and in 1B flour with all DATEM levels (Table 4 and 5). Overall highlights are: high value of H'm was shown by 3B0.3 (69.75%) and lowest value of the sample (with yeast) was shown by 1A0 (47.5%) (Table 4). Highest percentage (28.7%) increment (desirable) in maximum height was observed in 3B0.3 and 18.1% decreased by 1B0.6 (Table 5). H'm is a critical parameter in fermentation and is related to Hm and h. The overall trend of decreasing the surface tension of dough on H'm is the same as on Hm and h.

Total Volume (TV)

TV is the total volume under the gaseous curve. Observations that significantly increased total volume are 1A with 0.3 and 1% and 3B flour with 0.3 and 0.6% of DATEM. Observations that significantly decreased TV are 2A and 2B with 1% and 3A with 0.3 and 0.6% of DATEM (Table 4 and 5). Overall highlights of trends are: high value of total volume was observed in 3B0.3 (1914 mL) and lowest in 1B0 (1412 mL) (Table 4). An increase in total volume (22.4%) was observed in 1A0.3 and 14.3% of total volume decreased in 3A1 (Table 5). We observe an increase of 1A and 1B flours (low protein) and 3B flours with most of the DATEM levels.

Volume lost (VL)

VL is the carbon dioxide volume released by the dough during the fermentation test. Overall trend of VL is to decrease when the surface tension is reduced. All observations (all levels) were significantly decreased volume lost when compared to controls (Table 4 and 5). This means reducing surface tension positively effects the fermentation properties by decreasing the volume lost. Overall highlights are: high value was observed in 2B0 (567 mL) and lowest in 1A1 (24 mL) (Table 4). Highest percentage (16.4%) of volume lost was observed in 3B0.3 and lowest percentage (7) in 2B1 (Table 5).

Volume retained (VRt)

VRt is the carbon dioxide remaining in the dough at the end of the test. The overall trend of volume retention was to increase as the surface tension was reduced. All observations (all levels) were significantly increased when compared to controls except 3A with 1%. Overall highlights of trends are: highest value was observed in 3B0.6 (1875 mL) and lowest in 1B0 (135 mL) (Table 4). Highest percentage (52%) of volume retained was observed in 1A0.3 and lowest percentage (7.3%) in 3A1 (Table 5). Volume retained shows a maximum volume and then decreases and depends on DATEM levels. Flours 1A and 3B showed highest % of volume retained (Table 5).

Retention Coefficient (RC)

Retention coefficient (RC) is the retention volume divided by the total gaseous release. Overall trend of RC is to increase by reducing the surface tension. All observations (all levels) were significantly increased when compared to controls. This means that reducing surface tension positively affects the fermentation properties by increasing the retention coefficient. Overall highlights of trends are: highest value was observed in 1A1 (98.6%) and lowest value in

2B0 (70.5%) (Table 4). Highest percentage (38.4%) of retention coefficient was observed in 2B1 and lowest percentage (21.5%) in 1B0.6 (Table 5). The effect of reducing the surface tension on retention coefficient is an overall reduction. Some flours show high percentage of retention coefficient increase (2B) and the others low (1B).

Time of maximum rise (T1)

T1 is the time taken by the dough to reach maximum height during dough development. Observations that are significantly decreased T1 ($P < 0.05$) were all high protein B flours with all levels, 1A with 0.3 and 1%, 2A with 0.6 and 1% and 2B with 0.6% of DATEM. Time decreases when the surface tension was reduced by adding DATEM to the flours (Table 4). Overall highlights of trends are: high value was observed in 1A1 (3.9 h) and lowest in 1B0 (1.5 h) (Table 4). Highest percentage (20.3%) of time taken to reach maximum height of dough development was observed in 1B0.6 and time decreased 50% in 2A1 (Table 5). The effect of a reduced surface tension on T1 varied. Half of the samples showed a decrease which means they took less time to reach maximum height of dough development. The other half of the samples showed an increase in T1 taking more time to reach maximum height of the dough.

Time of maximum rise (T'1)

T'1 is the time spent to reach maximum rise during gaseous release. The overall trend of T'1 is to increase. Observations that significantly increased T'1 were 2B and 3B flours with all levels and 2A with 0.3% of DATEM. Overall highlights of trends are: highest value was observed in 3B1 (4 h) and lowest in 2B0 (1.38 h) (Table 4). Highest increase (188%) of time taken to reach maximum height of gaseous curve was observed in 2B1 and the only decrease of 9.5% in 1A0.6 (Table 5). Overall, the effect of reducing surface tension increased the time to reach maximum height of gaseous release.

Campbell (2001) proved that 0.4 to 0.7% DATEM increases the gas retention properties. Koehler and Grosch (1999) said that concentrations above 0.5% w/w flour basis produce no significant change in dough properties. Treating the flours with DATEM showed increment in dough development and in percentage of gas retained (Tables 4 and 5). DATEM of levels 0.3% and 0.6% showed larger increment when compared to flour treated with 1% of DATEM.

PCA results

Principal component analyses were performed on the data sets obtained from fermentation parameters.

Fermentation variables Vs fermentation variables with flour protein

PCA were performed on the data sets, to assess the relationship of flour protein and fermentation properties (Fig. 3 and 4). Figure 3 represents the fermentation properties alone for all the samples. Principal component axis 1 (PC1) explained 58.9% variance and principal component axis 2 (PC2) explained 18.8% variance. Total explained variance is 77.7% (Table 6). Among fermentation properties, the highest contribution of variance (96.8%) was volume of retention (VRt) in PC1 whereas in PC2 the highest contribution of variance (80.9%) was volume lost (VL) (Table 6). Figure 4 displays the relation of fermentation properties plus flour protein. Principal component axis 1 (PC1) explained 53.5% variance and principal component axis 2 (PC2) explained 17.5% variance. Total explained variance is 71% (Table 7). Among fermentation properties with flour protein, the highest contribution of variance (97.4%) was maximum height of gaseous release (H'm) on PC1 whereas on PC2 highest contribution of variance (72.7%) was volume lost (VL) (Table 7). Only 0.12% of explained variance was contributed by flour protein on PC1 and 7.32% on PC2 (Table 6). This suggests that the variation

of protein is weakly related to the volume lost and its contribution to the variance is very small when compared with samples with changes in their surface tension. In both graphs (Fig. 3 and 4), most of the fermentation variables are on PC1. Flours treated with DATEM are very close to PC1 when compared with control and negative samples. All control samples are closely related among themselves and to volume lost. They are well separated from the flours with surface tension changes. Negative controls are also closely related among themselves and well separated from the samples with changes in surface tension. So negative controls are removed from the data sets and PCA was compared. It also suggests that the samples with reduced surface properties are closely related to the maximum height and the volume of the gas retained by the dough during fermentation in the first component. The samples are negatively related to differences in their gas volume lost by their dough's in the second component. This means that all the samples with reduced surface tension lost less gas compared to the control samples.

Fermentation variables without negative control Vs fermentation variables with flour protein and without negative control

PCA were performed to assess the relationship of fermentation variables and protein without the negative controls (Fig. 5 and 6). From the fermentation properties on Figure 5, principal component axis 1 (PC1) explained 42.6% variance and principal component axis 2 (PC2) explained 26.6% variance (Fig. 5). Total explained variance is 69.2% (Table 8). Among fermentation properties, the highest contribution of variance (75.6%) was volume of retention (VRt) on PC1 whereas on PC2 the highest contribution of variance (88%) was volume lost (VL) (Table 8). In comparison, when flour protein was included (Fig. 6), principal component axis 1(PC1) explained 38.8% variance and principal component axis 2 (PC2) explained 24.6% variance. Total explained variance is 63.4% (Table 9). Among fermentation properties with flour

protein, the highest contribution of variance (75.8%) was volume of retention (VRt) on PC1 whereas on PC2 the highest contribution of variance (82.6%) was volume lost (VL) (Table 8). Only 0.04% of explained variance was contributed by flour protein on PC1 and 8.07% on PC2 (Table 9). As the total explained variance of fermentation variables (69.2%) is 5.8 units of percentage higher than fermentation variables with flour protein (63.5%), we can say that compared to changes in surface tension in this set, flour protein appears to have a small effect and is marginally correlated to other fermentation variables. The fact that there are two distinct groups suggests that the decrease of surface tension separates the differences in dough fermentation properties. One group is closely associated to PC1, highly influenced by the gas retained and the maximum dough height. The second group is less associated with the same two properties mentioned. They are negatively associated with volume lost but strongly and positively associated with the retention coefficient. In both the graphs, most of the A flours are associated to lowering development percentage ($[Hm-h]/Hm$) and B flours are associated to time to reach maximum height of gaseous release ($T'1$) and Volume of retention (VRt) (Fig. 5 and 6). Control samples are positively associated with volume lost and negatively associated with retention coefficient. They are separated from the flours with lower surface tension (treated with DATEM). Flours which are treated with DATEM are closely related to PC1 and PC2 (Fig. 5 and 6).

Fermentation properties Vs Fermentation properties with visco-elastic, mixing, baking properties

The relationship of fermentation variables with visco-elastic, mixing and baking properties was investigated (Fig. 7). From Figure 6, principal component axis 1 (PC1) explained 38.8% variance and principal component axis 2 (PC2) explained 24.6% variance. Total

explained variance is 63.4% (Table 9). From Figure 7, principal component axis 1(PC1) explained 27.5% variance and principal component axis 2 (PC2) explained 20.4% variance. Total explained variance is 47.9% (Table 10). Among all properties, the highest contribution of variance (73.5%) was specific volume of baked loaves (SV) on PC1 whereas on PC2 the highest contribution of variance (84.6%) was delta compliance (J-J_r) (Table 10). Flour protein explained 60.6% of variance on PC1 and 4.8% on PC2 (Table 10). As the total explained variance of fermentation variables (69.2%, Fig. 5) is more than fermentation variables with visco-elastic, mixing and baking variables (47.9%, Fig. 7), we can say that fermentation properties were able to discriminate better the effect of reducing the surface tension and contributed to explained higher variance. In Figure 7, low protein A flours are separated from other flours. All control samples are closely related and positively correlated with delta compliance (J-J_r) and volume lost (VL). They are negatively correlated to lowering development percentage ([Hm-h]/Hm) retention coefficient (RC). Flours with reduced surface tension are closely related to loaf height and specific volume. From Table 10, the highest contribution of explained variance was observed by baking properties. All baking properties show greatest contribution of explained variance on PC1. So a PCA analysis is performed on flour protein, fermentation properties and baking properties.

Relationship of flour protein, Fermentation and baking properties

PCA were performed on the data sets of flour protein, fermentation variables and baking properties (Fig. 8). PCA analyses of fermentation properties with flour protein were already performed (Fig. 6 and Table 9). From Figure 6, principal component axis 1 (PC1) explained 38.8% variance and principal component axis 2 (PC2) explained 24.6% variance. Total explained variance is 63.4% (Table 9). From Figure 8, principal component axis 1 (PC1)

explained 35.3% variance and principal component axis 2 (PC2) explained 21.6% variance. Total explained variance is 56.9% (Table 11). The highest contribution of explained variance (72.3%) was height of baked loaves (LH) on PC1 whereas on PC2 the highest contribution of variance (59.8%) was volume of retention (VRt) (Table 11). Flour protein explained 23.3% variance on PC1 and 29.3% on PC2 (Table 11). As the total explained variance of fermentation variables (63.4%) is more than fermentation variables with baking variables (56.9%), we can say fermentation properties explain more variance than baking properties. Ratio of dough heights $[(H_m-h)/H_m]$ is closely related to elastic properties (Sep and RCY). Volume lost (VL) is closely related to viscous properties (J-Jr, TCR) and negatively related to elastic properties. Maximum height of the dough (H_m) and dough height (h) are closely related to baking properties (LV and SV). All control samples were negatively associated with gas retained (VRt) and positively related with loaf height (LH) and specific volume (SV). Samples 1A1, 1A0.6 and 1B1 are negatively associated with loaf height and specific volume. This suggests as the reduction of surface tension increased on these samples it negatively affected their baking performance. The height of dough at the end of the fermentation test is the most closely related to loaf height and specific volume. A third fermentation parameter worth mentioning is the total volume of gas produced which is associated with loaf volume and specific volume.

5. Conclusions

Null Hypothesis is rejected as there is significant effect on fermentation properties between control flours and flours treated by reducing surface tension (addition of DATEM). By reducing the surface tension of the dough, the height of the dough is significantly improved in 1A and 3B flours and the retention volume of the gas is increased (11.3-52.1%). Volume of gas lost is reduced (7.3-16.4%) and retention coefficient is increased (21-38%).

Fermentation variables explained more variance (69.2%) than the fermentation variables combined with visco-elastic, mixing and baking variables (47.9%). The ratio of dough heights [(Hm-h)/Hm] is closely related to gluten elastic properties (Sep and RCY). Volume lost (VL) is closely related to gluten viscous (J-Jr, TCR) and negatively related to elastic properties. Maximum height of the dough (Hm) and dough height (h) are closely related to baking properties (LV and SV).

6. Cited references

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Table 1. Definitions of fermentation variables.

Abbreviations	Definitions	Units
Hm	Height of maximum dough development.	mm
h	Height of dough development at the end of the test.	mm
T1	Time of maximum rise.	h
(Hm-h)/Hm	Lowering of the development percentage after 4 h compared to time of maximum rise.	%
H'm	Maximum height of the gaseous curve.	mm
TV	Total volume of gaseous curve.	mL
VL	The carbon dioxide volume released by the dough during the fermentation test.	mL
VRt	Carbon dioxide retained in the dough at the end of the test.	mL
RC		%
T'1	Retention volume divided by the total gaseous release.	h
	Time spent to reach maximum rise.	

Table 2. Definitions of visco-elastic, mixing and baking terms (adapted from Ambardekar, 2009)

Abbreviations	Definitions	Units
Visco-elastic		
J-J_r	Delta compliance defined as the difference in compliance of creep and recovery at 100 s. An increase in delta compliance suggests that the viscous component is higher than elastic component by either an increase in viscosity or decrease in elasticity of the gluten structure at 100 s.	Pa ⁻¹
SeP	Separation time is time at which the creep and recovery split and no longer stay superimposed. An increase in separation time suggests that the elastic component is higher than viscous component by either an increase in elasticity or decrease in viscosity of the gluten structure.	S
RCY	Percent recoverability is the elastic ability of gluten to recover to its original state after the stress is removed.	%
TCC	Rate at which the deformation of gluten reaches its equilibrium. Higher the value of TCC slower the rate of deformation of gluten.	S
TCR	Rate at which the elastic recovery of gluten reaches its equilibrium. Higher the value if TCR, slower the rate of recovery of gluten.	S
Mixing		
WA	Ability of flour to absorb water in order to form a convened dough consistency at 500 FU.	%
DT	Time required for the flour to develop into dough of convened consistency during mixing.	Min
ST	Time for which the developed dough remains stable during mixing.	Min
BT	Time at which the dough starts breaking down after mixing.	Min
Baking		
LV	Volumes of baked loaf measured at 10 min.	cm ³
LH	Heights of baked loaves.	Mm
PH	Heights of loaves after proofing.	Mm
OSP	Increase in height of loaves in the oven during baking.	Mm
SV	Specific volume of baked loaves.	cm ³ /g
FP	Flour protein.	%

Table 3. Proximate analysis of flours (means \pm SD, n=2) obtained from sites A and B (adapted from Ambardekar, 2009).

Flours	Protein (%)	Moisture (%)	Ash (%)
1A	7.95 \pm 0.05	11.69 \pm 0.02	0.29 \pm 0.01
2A	11.19 \pm 0.07	10.51 \pm 0.03	0.38 \pm 0.01
3A	13.68 \pm 0.02	10.14 \pm 0.02	0.41 \pm 0.00
1B	10.40 \pm 0.10	12.54 \pm 0.02	0.47 \pm 0.00
2B	10.59 \pm 0.07	12.57 \pm 0.00	0.48 \pm 0.01
3B	11.38 \pm 0.01	12.98 \pm 0.04	0.58 \pm 0.01

Table 4. Fermentation properties in six commercial wheat flours treated with DATEM levels. Means (n=2) with same superscripts in a column are not significantly different (P > 0.05). The standard deviations of means are shown in parenthesis.

TRT	Fermentation Properties									
	Hm (mm)	h (mm)	(Hm-h) /Hm (%)	H'm (mm)	TV (mL)	VL (mL)	VRt (mL)	RC (%)	T1 (h)	T'1 (h)
1A0	25.0 ^{lmn} (1.3)	24.55 ^{lmn} (1.3)	1.80 ^{fgh} (0.3)	47.50 ^k (2.5)	1507.5 ^{hijk} (76.5)	323 ^{de} (37)	1184.5 ^{mn} (39.5)	78.65 ^{cdef} (1.3)	3.93 ^a (0.02)	3.17 ^{abc} (0.15)
1AN	0 ⁿ (0)	0 ^o (0)	0 ^h (0)	5.0 ^l (0.1)	12.5 ^l (0.5)	2 ^g (0)	11 ^o (0)	83.25 ^{bcd} (2.05)	4.00 ^a (0)	0.12 ^l (0)
1A0.3	39.75 ^{cd} (0.05)	38.80 ^{bc} (0.8)	2.40 ^{bc} (1.9)	60.5 ^{cd} (0.6)	1845 ^{abc} (116)	44 ^{fg} (5)	1801 ^{abc} (121)	97.6 ^a (0.4)	2.16 ^{defg} (0.01)	3.81 ^{ab} (0.19)
1A0.6	29.45 ^{ijkl} (7.1)	27.95 ^{ijkl} (6.0)	4.45 ^{efgh} (2.6)	50.9 ^{ghijk} (1.7)	1544.5 ^{fghijk} (98.5)	35.5 ^{fg} (3.5)	1509.5 ^{fghi} (94.5)	97.7 ^a (0.1)	2.93 ^{abcdef} (1.03)	2.87 ^{bcde} (1.05)
1A1	22.10 ^m (1.0)	21.65 ^{mn} (0.6)	2.0 ^{fgh} (1.5)	55.3 ^{efg} (2.1)	1709 ^{cde} (35)	24 ^{fg} (3)	1685 ^{cde} (38)	98.6 ^a (0.2)	3.93 ^a (0.02)	3.76 ^{ab} (0.01)
2A0	32.15 ^{ghijk} (0.5)	31.45 ^{ghij} (1.1)	2.20 ^{fgh} (1.9)	53.10 ^{ghi} (1.0)	1695.5 ^{de} (27.5)	389.5 ^c (11.5)	1306 ^{klm} (16)	77.05 ^{ef} (0.35)	3.68 ^a (0.28)	1.51 ^h (0.16)
2AN	0 ⁿ (0)	0 ^o (0)	0 ^h (0)	4.90 ^l (1.3)	12 ^l (3)	3.5 ^g (0.5)	9 ^o (3)	70.2 ^g (6)	4.00 ^a (0)	0.10 ^l (0)
2A0.3	31.90 ^{hijk} (1.80)	31.60 ^{fghij} (1.5)	0.90 ^{gh} (0.9)	55.40 ^{efg} (1.2)	1647.5 ^{defgh} (28.5)	48.5 ^{fg} (16.5)	1599 ^{ef} (45)	97.05 ^a (1.05)	3.98 ^a (0.02)	3.83 ^{ab} (0)
2A0.6	31.85 ^{hijk} (0.8)	31.25 ^{ghijk} (0.6)	1.85 ^{fgh} (0.5)	52.20 ^{ghij} (1.4)	1628 ^{efgh} (54)	33 ^{fg} (0)	1595.5 ^{ef} (54.5)	98 ^a (0.1)	2.13 ^{defg} (0.07)	1.84 ^{efgh} (0.08)
2A1	27.25 ^{klm} (1.2)	26.35 ^{klm} (0.6)	3.25 ^{fgh} (2.0)	49.80 ^{hijk} (1.2)	1520 ^{ghijk} (38)	28.5 ^{fg} (2.5)	1491.5 ^{fghi} (40.5)	98.1 ^a (0.2)	1.84 ^{efg} (0.13)	1.98 ^{defgh} (0.07)
3A0	39.40 ^{cde} (2.2)	39.15 ^{bc} (2.4)	0.65 ^h (0.6)	54.3 ^{fgh} (0.1)	1669 ^{def} (13)	362.5 ^{cd} (13.5)	1307 ^{klm} (0)	78.3 ^{def} (0.6)	3.98 ^a (0.02)	1.66 ^{gh} (0.04)
3AN	0 ⁿ (0)	0 ^o (0)	0 ^h (0)	4.35 ^l (0.7)	17.5 ^l (3.5)	2.5 ^g (0.5)	15.5 ^o (4.5)	85.5 ^b (6.5)	4.00 ^a (0)	0.11 ^l (0.01)
3A0.3	34.10 ^{fghi} (1.1)	33.70 ^{defg} (1.1)	1.15 ^{fgh} (0.05)	48.55 ^{ijk} (1.9)	1483 ^{ijk} (58)	28.5 ^{fg} (3.5)	1455 ^{ghij} (61)	98.1 ^a (0.3)	3.85 ^a (0.10)	2.01 ^{defgh} (0.06)
3A0.6	32.65 ^{ghij} (0.3)	32.50 ^{efghi} (0.4)	0.45 ^h (0.1)	52.55 ^{ghij} (1.4)	1606.5 ^{efghi} (45.5)	35.5 ^{fg} (2.5)	1571 ^{efg} (43)	97.8 ^a (0.1)	3.96 ^a (0.01)	1.83 ^{efgh} (0.02)
3A1	28.50 ^{ijkl} (0.5)	28.35 ^{hijkl} (0.3)	0.50 ^h (0.5)	48.40 ^{jk} (0.7)	1430.5 ^k (17.5)	28 ^{fg} (2)	1402.5 ^{ijkl} (19.5)	98.05 ^a (0.15)	3.98 ^a (0.02)	2.23 ^{cdefgh} (0.03)

Table 4. Continued

TRT	Fermentation Properties									
	Hm	H	(Hm-h)/Hm	H'm	TV	VL	VRt	RC	T1	T'1
	(mm)	(mm)	(%)	(mm)	(mL)	(mL)	(mL)	(%)	(h)	(h)
1B0	33.2 ^{ghij} (2.3)	19.85 ⁿ (3.75)	39.1 ^a (15.5)	59.55 ^{de} (1.75)	1412 ^k (32)	277 ^e (15)	1135 ⁿ (17)	80.4b ^{cde} (0.6)	1.58 ^g (0.17)	1.41 ^h (0.01)
1BN	0 ⁿ (0)	0 ^o (0)	0 ^h (0)	5.5 ^l (1.8)	18.5 ^l (6.5)	3 ^g (2)	15.5 ^o (4.5)	83.35 ^{bcd} (4.65)	4.00 ^a (0.00)	0.12 ⁱ (0.00)
1B0.3	34.15 ^{fghi} (0.45)	24.85 ^{lmn} (1.75)	27.15 ^b (6.05)	49.5 ^{ijk} (1.6)	1479.5 ^{ijk} (22.5)	32 ^{fg} (4)	1447.5 ^{ghij} (18.5)	97.85 ^a (0.25)	1.76 ^{fg} (0.14)	1.70 ^{fgh} (0.05)
1B0.6	34.45 ^{efghi} (0.15)	27.3 ^{ijkl} (0.7)	20.75 ^b (2.35)	48.75 ^{ijk} (1.15)	1474 ^{ijk} (27)	34 ^{fg} (1)	1439.5 ^{ghij} (28.5)	97.7 ^a (0.1)	1.90 ^{efg} (0.00)	1.82 ^{efgh} (0.05)
1B1	32.6 ^{ghij} (1.9)	24.75 ^{lmn} (0.75)	23.95 ^b (2.15)	49.8 ^{hijk} (1.7)	1458.5 ^{jk} (34.5)	31.5 ^{fg} (2.5)	1428 ^{hijk} (32)	97.9 ^a (0.1)	1.74 ^{fg} (0.03)	1.70 ^{fgh} (0.10)
2B0	43.3 ^{abc} (0.5)	42.75 ^{ab} (0.05)	1.25 ^{fgh} (1.05)	61.15 ^{cd} (4.15)	1911.5 ^{ab} (113.5)	567 ^a (102)	1344.5 ^{jkl} (11.5)	70.55 ^g (3.55)	3.41 ^{abc} (4.00)	1.38 ^h (0.13)
2BN	0 ⁿ (0)	0 ^o (0)	0 ^h (0)	5.2 ^l (0)	23 ^l (0)	1 ^g (0)	21 ^o (0)	94.6 ^a (0)	0.51 ^a (0)	0.12 ⁱ (0)
2B0.3	41.5 ^{bcd} (1)	39 ^{bc} (2.8)	6.1 ^{defgh} (4.5)	61.85 ^{cd} (2.55)	1780.5 ^{bcd} (85.5)	44 ^{fg} (1)	1736.5 ^{bcd} (84.5)	97.5 ^a (0.1)	2.33 ^{cdefg} (0.33)	3.93 ^{ab} (0.02)
2B0.6	45 ^{ab} (2)	42.2 ^{ab} (2)	6.25 ^{defgh} (0.25)	64.85 ^{bc} (1.05)	1887.5 ^{ab} (19.5)	48.5 ^{fg} (2.5)	1839 ^{ab} (17)	97.45 ^a (0.15)	2.14 ^{d^{efg}} (0.03)	3.95 ^a (0.00)
2B1	43.1 ^{abc} (2.7)	42.65 ^{ab} (2.25)	1 ^{gh} (1)	59 ^{de} (3.3)	1701.5 ^{de} (105.5)	40 ^{fg} (8)	1661.5 ^{de} (97.5)	97.65 ^a (0.35)	3.96 ^a (0.04)	3.98 ^a (0.02)
3B0	36.85 ^{defgh} (0.75)	36.85 ^{cde} (0.75)	0 ^h (0.4)	54.2 ^{fgh} (1.1)	1737 ^{cde} (46)	462.5 ^b (20.5)	1275 ^{lm} (25)	73.4 ^{fg} (0.5)	4.00 ^a (0.00)	2.09 ^{defgh} (1.03)
3BN	0 ⁿ (0)	0 ^o (0)	0 ^h (0)	4.9 ^l (0.6)	14 ^l (1)	2.5 ^g (0.5)	12 ^o (1)	84 ^{bc} (1.2)	2.11 ^{defg} (1.89)	0.12 ⁱ (0.00)
3B0.3	44.4 ^{abc} (0)	40.45 ^{bc} (0.05)	8.9 ^{cdefgh} (0.1)	69.75 ^a (2.95)	1914 ^{ab} (136)	76 ^f (26)	1838 ^{ab} (162)	95.95 ^a (1.65)	2.12 ^{defg} (0.10)	3.96 ^a (0.04)
3B0.6	46.8 ^a (3.9)	43.85 ^{ab} (4.25)	6.4 ^{defgh} (1.3)	69.15 ^{ab} (0.55)	1924 ^a (10)	49 ^{fg} (2)	1875 ^a (8)	97.45 ^a (0.05)	2.41 ^{bcdefg} (0.06)	3.98 ^a (0.03)
3B1	47.9 ^a (1.3)	46.8 ^a (0.2)	2.25 ^{fgh} (2.25)	61.5 ^{cd} (1.2)	1717.5 ^{cde} (35.5)	46 ^{fg} (16)	1670.5 ^{cde} (51.5)	97.3 ^a (1)	3.45 ^{abc} (0.55)	4.00 ^a (0.00)

Table 5. Change (percent) of fermentation properties of six commercial wheat flours treated with DATEM levels. Values with * are significantly different (P<0.05) from control samples. Percentages are calculated from values in Table 4. % change = (Sample treated with additive - control sample)/control sample * 100.

TRT	Fermentation properties									
	Hm (%)	h (%)	(Hm-h) /Hm (%)	H'm (%)	TV (%)	VL (%)	VRt (%)	RC (%)	T1 (%)	T'1 (%)
1A0.3	59.0*	58.0*	33.3*	27.4*	22.4*	-13.6*	52.1*	24.1*	-45.0*	20.2
1A0.6	17.8*	13.9	147.2	7.2	2.5	-11*	27.4*	24.2*	-25.5	-9.5
1A1	-11.6	-11.8	11.1	16.4*	13.4*	-7.4*	42.3*	25.4*	0.0*	18.6
2A0.3	-0.8	0.5	-59.1	4.3	-2.8	-12.4*	22.4*	26.0*	8.2	153.6*
2A0.6	-0.9	-0.6	-15.9	-1.7	-4.0	-8.5*	22.1*	27.2*	-42.1*	21.9
2A1	-15.2*	-16.2*	47.7	-6.2	-10.4*	-7.3*	14.2*	27.3*	-50.0*	31.1
3A0.3	-13.5*	-13.9*	76.9	-10.6*	-11.1*	-7.9*	11.3*	25.3*	-3.3	21.1
3A0.6	-17.1*	-17.0*	-30.8	-3.2	-3.7	-9.8*	20.2*	24.9*	-0.5	10.2
3A1	-28.1*	-27.6*	-23.1	-10.9*	-14.3*	-7.7*	7.3	25.2*	0.0	34.3
1B0.3	2.9	25.2	-30.7*	-16.9*	4.8	-11.5*	27.5*	21.7*	11.4	20.6
1B0.6	3.8	37.5*	-46.9*	-18.1*	4.4	-12.3*	26.8*	21.5*	20.3	29.1
1B1	-1.8	24.7	-38.8*	-16.48	3.3	-11.4*	25.8*	21.8*	10.1	20.6
2B0.3	-4.2	-8.8	388.0	1.1	-6.9	-7.8*	29.2*	38.2*	-31.7	184.8*
2B0.6	3.9	-1.3	400.0	6.1	-1.3	-8.5*	36.8*	38.1*	-37.2*	186.2*
2B1	-0.5	-0.2	-20.0	-3.5	-11.0*	-7.0*	23.68*	38.4*	16.1	188.4*
3B0.3	20.5*	9.8	0.0	28.7*	10.2*	-16.4*	44.2*	30.7*	-47.0*	89.5*
3B0.6	27.0*	19.0*	0.0	27.6*	10.8*	-10.6*	47.1*	32.8*	-39.8*	90.4*
3B1	30.0*	27.0*	0.0	13.5*	-1.1	-9.9*	31.0*	32.6*	-13.8*	91.4*

Table 6. Explained variance (%) in PCA of fermentation variables with negative control in flours treated with DATEM.

DATEM	Axes	PC1	PC2	1+2
	PC (%)	58.9	18.9	77.8
Fermentation	Hm	94	2	96
	H	90	4	94
	(Hm-h)/Hm	6	11	17
	H'm	97	1	98
	TV	95	2	98
	VL	5	80	85
	VRT	97	0	97
	RC	20	62	82
	T1	13	25	38
	T'1	72	1	73

Table 7. Explained variance (%) in PCA of fermentation variables with flour protein in flours treated with DATEM.

DATEM	Axes	PC1	PC2	1+2
	PC (%)	53.5	17.5	71
Fermentation	Hm	94	2	96
	H	89	5	95
	(Hm-h)/Hm	6	16	22
	H'm	97	0	98
	TV	95	2	98
	VL	5	73	77
	VRT	97	0	97
	RC	20	55	75
	T1	13	30	43
	T'1	72	1	73
Flour Protein	FP	0	7	7

Table 8. Explained variance (%) in PCA of fermentation variables without negative control in flours treated with DATEM.

DATEM	Axes	PC1	PC2	1+2
	PC (%)	42.6	26.6	69.2
Fermentation	Hm	62	7	69
	h	68	16	84
	(Hm-h)/Hm	9	11	20
	H'm	73	3	76
	TV	71	14	85
	VL	6	88	94
	VRT	76	18	93
	RC	10	83	93
	T1	1	21	22
	T'1	50	6	56

Table 9. Explained variance (%) in PCA of fermentation variables with flour protein and without negative control in flours treated with DATEM.

DATEM	Axes	PC1	PC2	1+2
	PC (%)	38.8	24.6	63.4
Fermentation	Hm	61	8	69
	h	68	18	86
	(Hm-h)/Hm	9	13	23
	H'm	73	3	76
	TV	71	12	83
	VL	6	83	89
	VRT	76	17	93
	RC	10	78	87
	T1	1	24	26
	T'1	50	7	58
Flour Protein	FP	0	8	8

Table 10. Explained variance (%) in PCA of fermentation variables when compared with visco-elastic, mixing and baking variables in flours treated with DATEM. Definitions of fermentation, visco-elastic, mixing and baking variables explained in Table 2.

DATEM	Axes	PC1	PC2	1+2
	PC (%)	27.5	20.4	47.9
Fermentation	Hm	41	1	42
	h	55	3	58
	(Hm-h)/Hm	15	36	50
	H'm	20	0	20
	TV	22	8	30
	VL	01	24	25
	VRT	10	2	12
	RC	0	24	24
	T1	7	31	38
	T'1	1	2	3
Visco-elastic	SeP	5.	68	73
	J-J_r	0	85	85
	RCY	0	50	50
	TCR	0	23	23
	TCC	0	52	53
Mixing	WA	53	27	80
	DT	31	0	32
	ST	59	0	60
	BT	38	1	40
Baking	PH	26	39	65
	LH	73	0	73
	SV	74	0	74
	OSP	24	27	51
	LV	72	1	73
Flour Protein	FP	61	5	65

Table 11. Explained variance (%) in PCA of fermentation variables when compared with baking variables in flours treated with DATEM.

DATEM	Axes	PC1	PC2	1+2
	PC (%)	35.3	21.6	56.9
Fermentation	Hm	56	6	62
	h	70	3	72
	(Hm-h)/Hm	15	1	17
	H'm	49	19	68
	TV	53	10	63
	VL	0	46	46
	VRT	31	60	90
	RC	0	49	49
	T1	0	17	18
	T'1	11	50	61
Baking	PH	11	0	12
	LH	72	13	86
	SV	69	14	83
	OSP	38	11	50
	LV	64	16	80
Flour Protein	FP	23	29	53

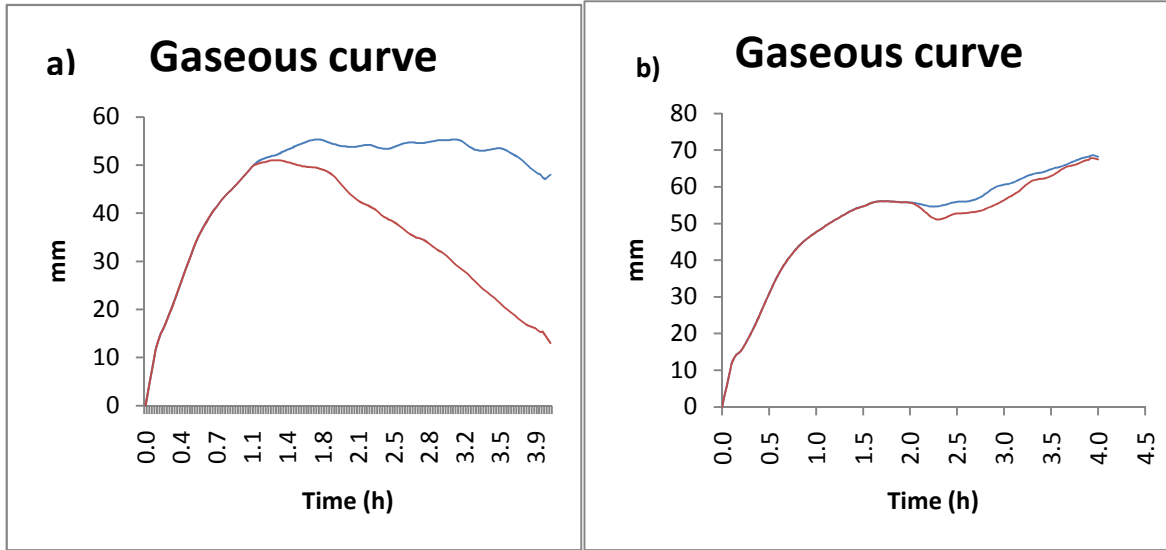


Figure 1. A graphical representation of gaseous curve of a) control sample from flour 3B and b) sample containing 0.6% DATEM (3B0.6). Blue tracings are the total volume and the red is the volume retained.

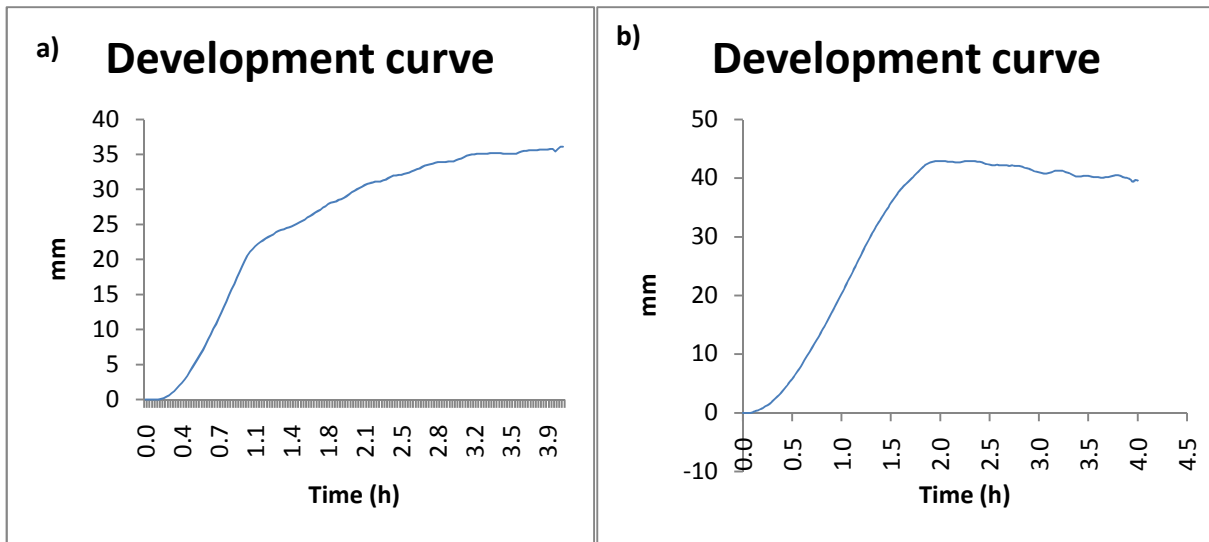


Figure 2. A graphical representation of dough development of a) control sample from flour 3B and b) sample containing 0.6% DATEM (3B0.6).

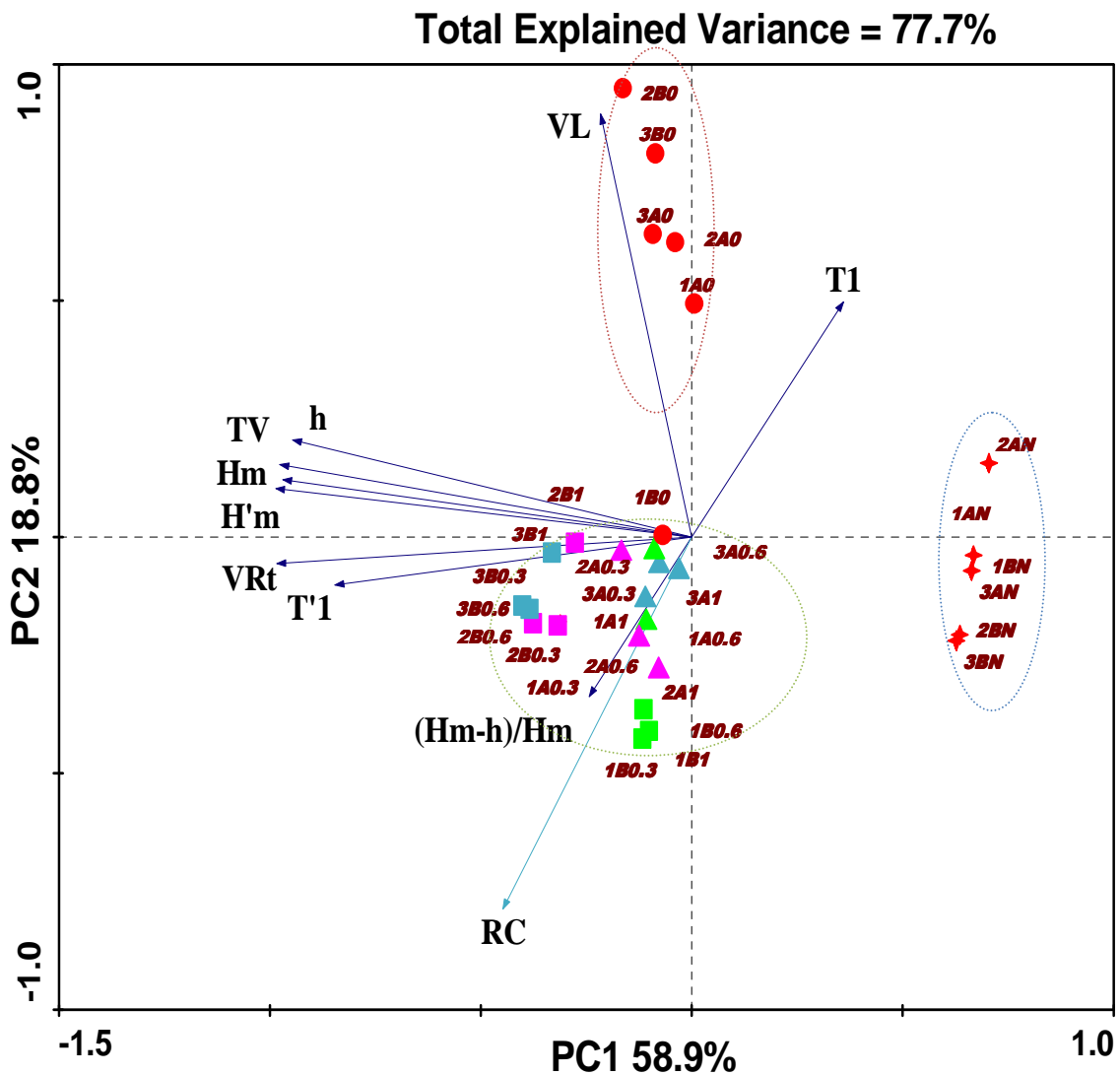


Figure 3. Loading plot of first two principal components based on fermentation properties with negative control of six commercial wheat flours, added with four levels of DATEM. Definitions of fermentation, visco-elastic, mixing and baking variables explained in Table 2 and 3. Flour protein content (%), 1A = 7.95, 2A = 11.19, 3A = 13.68, 1B = 10.4, 2B = 10.59 and 3B = 11.38, respectively. Symbols and definitions: ● - Control samples, ★ - Negative controls, ■ - Low protein B flours, ■ - Medium protein B flours, ■ - High protein B flours. ▲ - Low protein A flours, ▲ - Medium protein A flours, ▲ - High protein A flours.

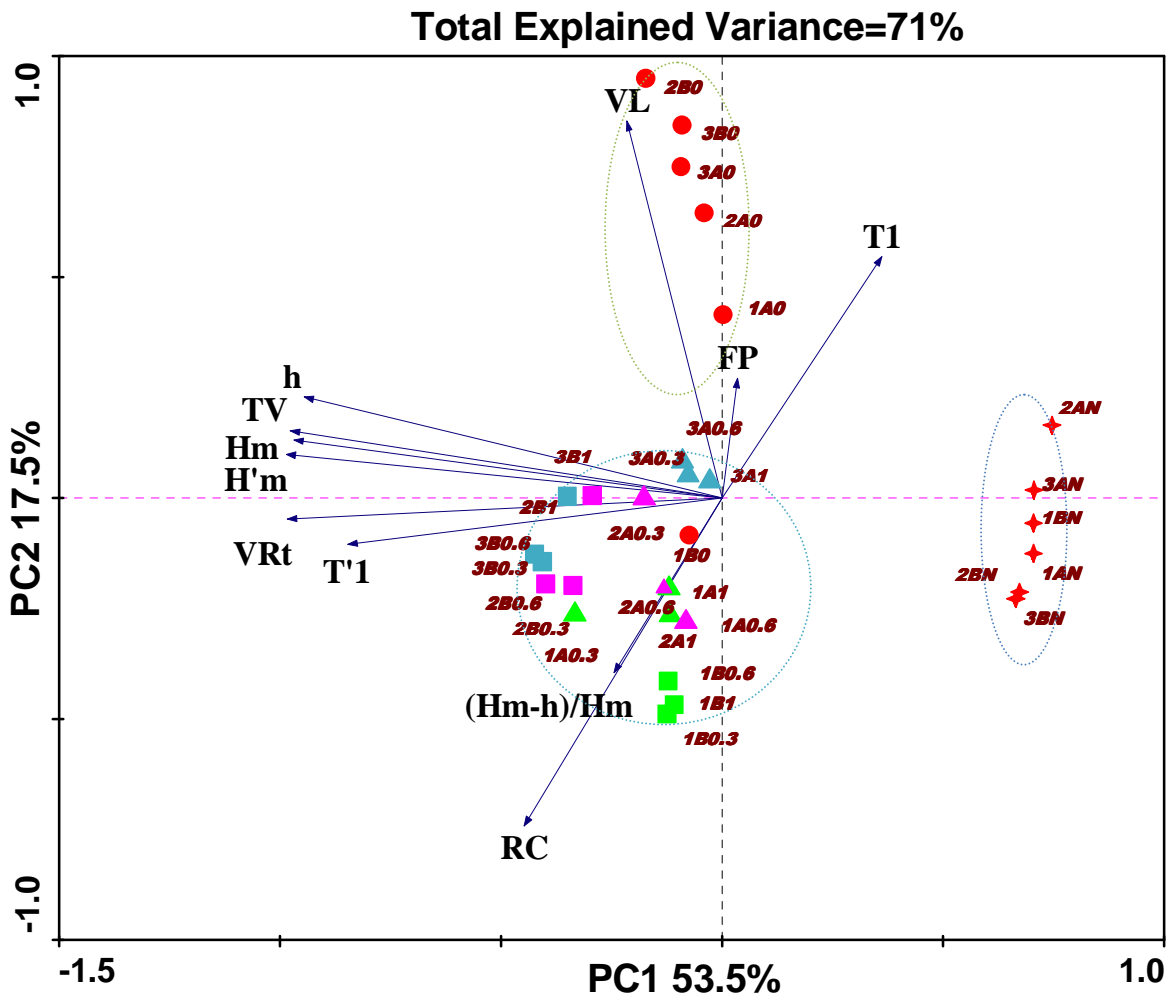


Figure 4. Loading plot of first two principal components based on fermentation properties with flour protein of six commercial wheat flours added with four levels of DATEM. Symbols and definitions: ● -Control samples, ★ - Negative controls, ▲ - Low protein A flours, ▲ - Medium protein A flours, ▲ - High protein A flours, ■ - Low protein B flours, ■ - Medium protein B flours, ■ - High protein B flours.

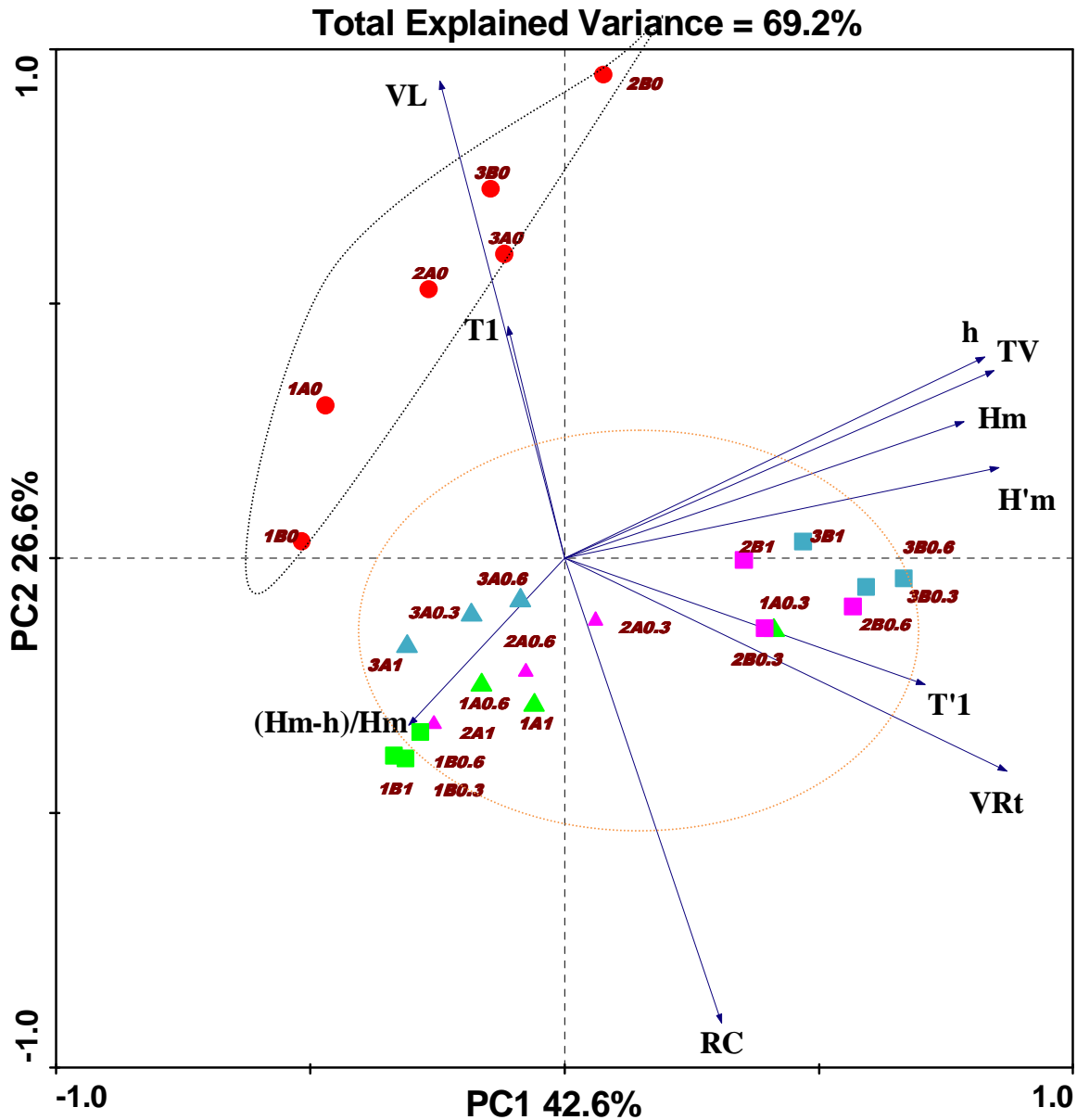


Figure 5. Loading plot of first two principal components based on fermentation properties without negative control of six commercial wheat flours added with four levels of DATEM. Symbols and definitions: ● -Control samples, ▲ - Low protein A flours, ▲ -Medium protein A flours, ▲ - High protein A flours. ■ - Low protein B flours, ■ - Medium protein B flours, ■ - High protein B flours.

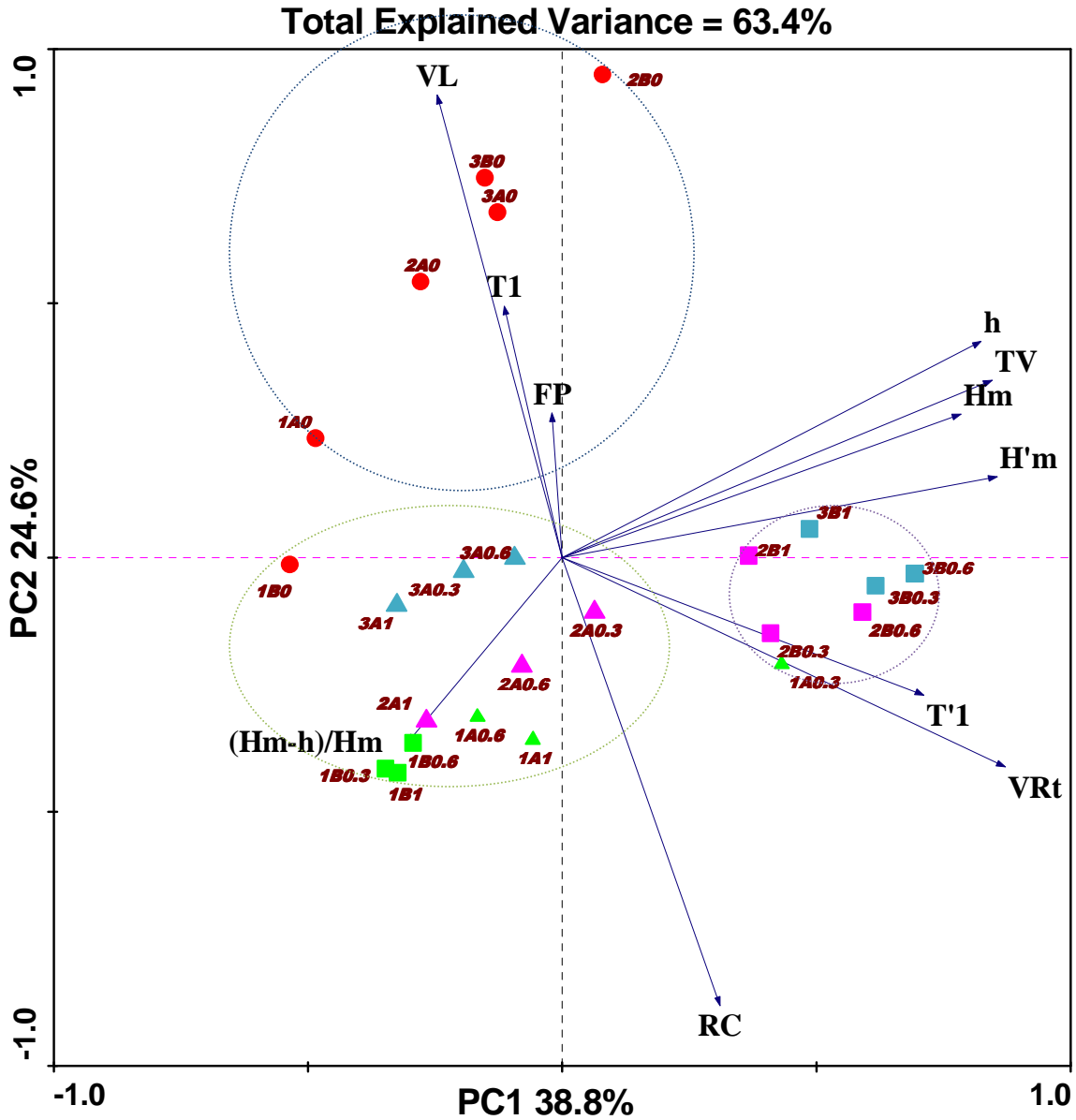


Figure 6. Loading plot of first two principal components based on fermentation properties with flour protein of six commercial wheat flours containing four levels of DATEM. Negative control samples were removed. Symbols and definitions: ● -Control samples, ▲ - Low protein A flours, ▲ -Medium protein A flours, ▲ - High protein A flours. ■ - Low protein B flours, ■ - Medium protein B flours, ■ - High protein B flours.

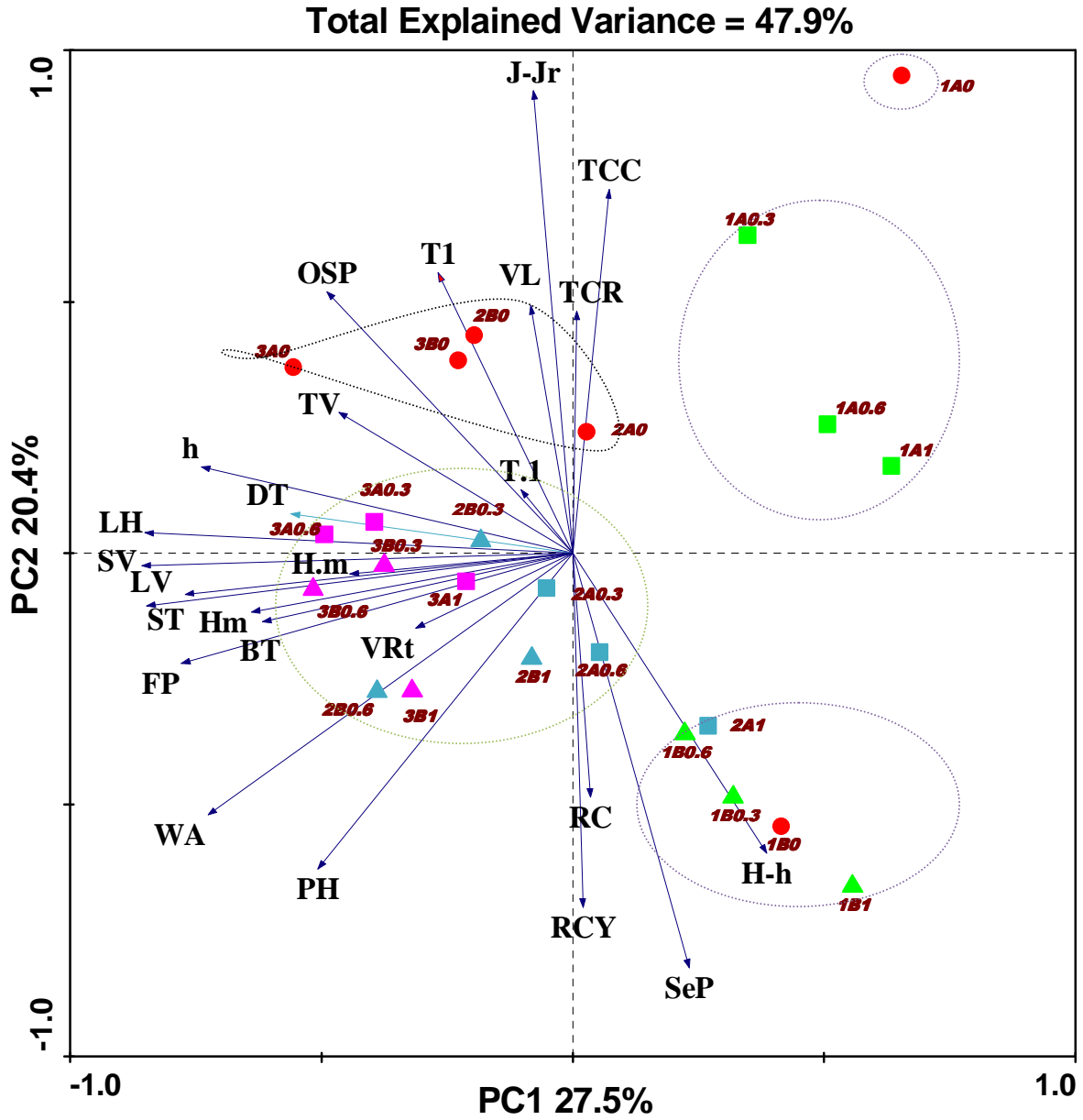


Figure 7. Loading plot of first two principal components based on fermentation, baking, visco-elastic and dough properties of six commercial wheat flours added with four levels of DATEM. Symbols and definitions: ● -Control samples, ▲ - Low protein A flours, ▲ -Medium protein A flours, ▲ - High protein A flours, ■ - Low protein B flours, ■ - Medium protein B flours, ■ - High protein B flours.

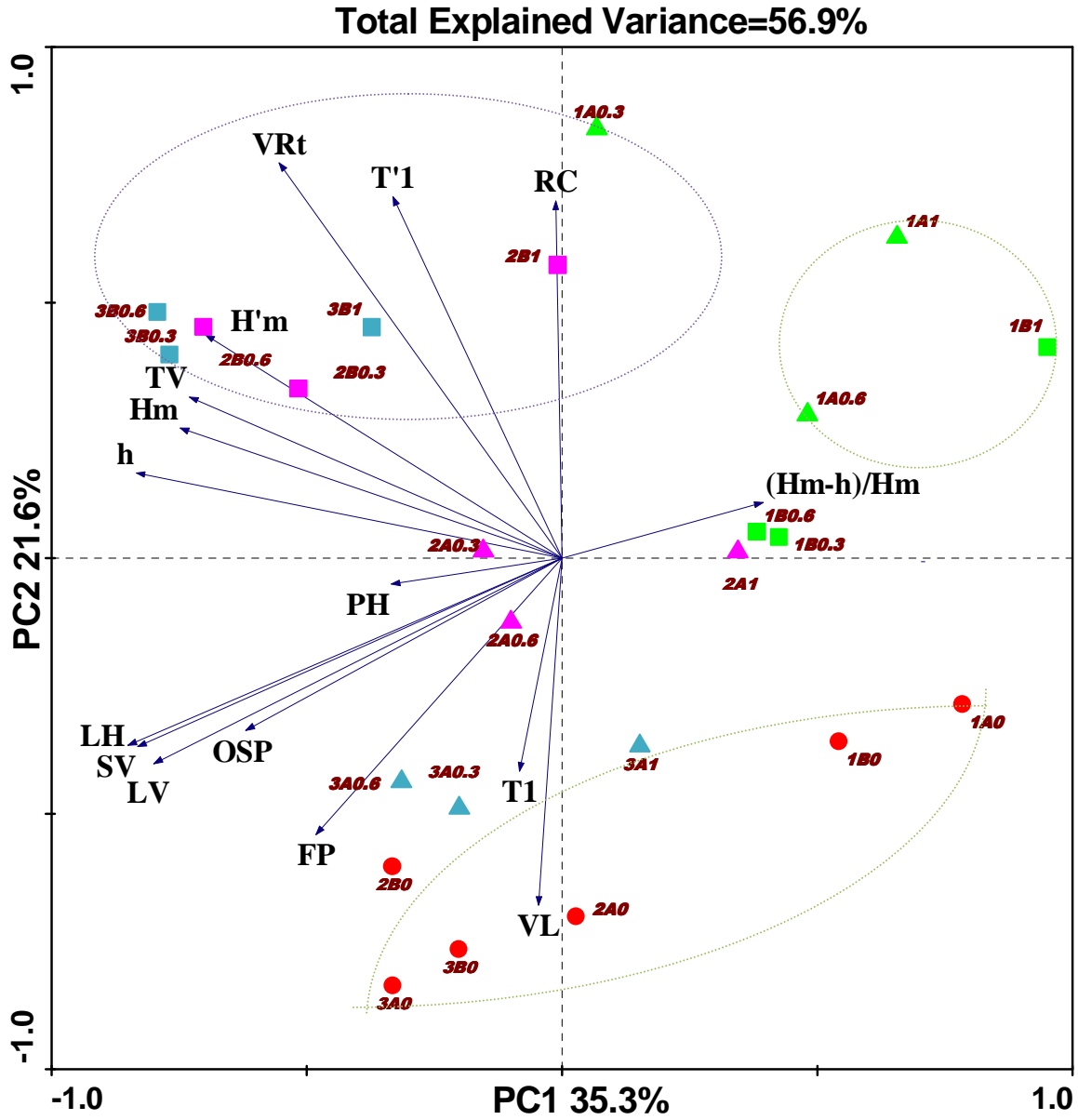


Figure 8. Loading plot of first two principal components based on fermentation and baking properties of six commercial wheat flours added with four levels of DATEM. Symbols and definitions: ● -Control samples ▲ – Low protein A flours, ▲ -Medium protein A flours, ▲ - High protein A flours. ■ – Low protein B flours, ■ - Medium protein B flours, ■ - High protein B flours.

CHAPTER IV

EFFECT OF OXIDIZED STATE ON FERMENTATION PROPERTIES OF DOUGH

ABSTRACT

The objective of the study is to investigate the effect of oxidized state on fermentation properties of dough and to analyze possible correlations of fermentation and visco-elastic, baking and mixing properties of dough. Five levels of oxidized states were obtained by the addition of ascorbic acid (0, 50, 100, 150 and 250 ppm). Six commercial hard red winter wheat flours with different protein content were used. Flours with no treatment were used as controls and flours with no yeast and no treatment were used as negative controls. Fermentation properties of dough were measured with a Rheofermentometer F₃. Oxidized dough showed increased dough development and volume of gas retained. Oxidizing levels increased the retention coefficient of gluten from most flours ($P < 0.05$). When fermentation properties are compared with visco-elastic, mixing and baking properties of dough, biplot of principal component analysis explained 51.5% of total variance. First principal component axis explained 27.2% variance and second component axis explained 24.3% variance. Fermentation properties alone without flour protein explained 61.7% of total variance. Fermentation variables explained more variance (61.7%) than the fermentation variables combined with visco-elastic, mixing and baking variables (51.5%). The ratio of dough heights $[(H_m - h)/H_m]$ and volume lost (VL) are closely related to gluten elastic properties (Sep and RCY). The time taken to reach maximum height of the dough (T1) is closely related to gluten viscous (TCC) and baking properties (OSP).

Maximum height of the dough (Hm) and dough height (h) are closely related to flour protein (FP) and baking properties (LH and LV).

1. INTRODUCTION

During mixing the gluten in the dough is stretched and pulled apart so that it can provide the needed strength and structure during proofing and baking. Oxidizing agents enhance gluten reformation and so are used to adjust dough strength, elasticity and tolerance. Oxidative dough improvers convert sulfhydryl groups of gluten proteins to disulfide linkages (Sullivan et al., 1940; Tsen and Bushuk 1963). Ascorbic acid is an oxidizing agent used in baking to improve dough elasticity, gas retention and water absorption. During mixing, ascorbic acid (L-AA) reacts with oxygen and forms dehydroascorbic acid (L-DHA) which oxidizes the sulphhydryl groups in gluten protein. This specie in turn reacts with thiols to form disulfide and to regenerate ascorbic acid (Stauffer et al., 1990). Oxidation of glutathione (GSH) to oxidized disulfide derivative (GSSG) improves the effect of L-AA in dough properties. They are added to dough to improve the strength of the gluten structure to allow it to hold more CO₂ produced during fermentation. Ascorbic acid was effective in improving loaf volume in bread. Flour with less protein requires more ascorbic acid than a high protein flour to reach its optimum potential (Collins et al., 1966). Adding 50 ppm of ascorbic acid gives a tighter strength and dough tightness increases during fermentation (Hoseney et al., 1980). Addition of 150 ppm ascorbic acid to yeasted dough increased the effectiveness of incorporating oxygen from mixing atmosphere (Chamberlain and Collins, 1979). Several instruments are available to measure the rheological and gas retention properties of dough during fermentation. The Rheofermentometer (Chopin, Tripette and Renaud, France) is used in the study of the behavior of flour during

fermentation. The parameters measured are maximum dough height (H_m) in mm, maximum height of gaseous release (H' m) in mm, CO₂ production in ml.

The objectives of the study were:

- 1) To study the effect of an oxidizing agent on the fermentation properties of dough using ascorbic acid.
- 2) To analyze possible correlations of fermentation and visco-elastic, baking and mixing properties of dough.

2. Materials and Methods

a. Materials and Labeling

The procurement of wheat flour samples were explained in the Materials and Methods section of Chapter III. Five levels (0, 50, 100, 150 and 250 ppm) of ascorbic acid (Mallinckrodt Baker Inc., Phillipsburg, NJ) were used. Flours with no ascorbic acid were used as control (0) and flours with no ascorbic acid and no yeast were used as negative control (N). Thus site A flours were labeled as 1A0 (positive control), 1AN (negative control), 1A50, 1A100, 1A150, 1A200; 2A0, 2AN, 2A50, 2A100, 2A150, 2A200; 3A0, 3AN, 3A50, 3A100, 3A150, and 3A200. The 0, 50, 100, 150 and 200 represent the parts per million of ascorbic acid added to the flours. Similarly site B flours were labeled as 1B0, 1BN, 1B50, 1B100, 1B150, 1B200; 2B0, 2BN, 2B50, 2B0100, 2B150, 2B200; 3B0, 3BN, B50, 3B100, 3B150, and 3B200.

b. Methods

Dough Preparation

Dough was prepared as described in the Chopin protocol using Chopin AlveoConsistograph. The ingredients consisted of 250 g of flour, 3 g of dry yeast and 5g of

sodium chloride. Ascorbic acid was added to the flours at 0, 50, 100, 150 and 200 ppm. For 50 ppm ascorbic acid, a stock solution of 100 ml was prepared containing 0.05 g of ascorbic acid. Then 25 ml of stock solution was added to 250 g of flour. In the same way, stock solutions were prepared for 100 ppm containing 0.1 g of ascorbic acid, 0.15 g of ascorbic acid for 150 ppm and 0.2 g for 200 ppm. From the described stock solutions, 25 ml was mixed with the water added to the flour to obtain each ascorbic acid addition. The quantity of deionized water added depended on the moisture content of the flour and it was given by the reference table published by the International Association for Cereal Science and Technology (ICC) as described in the Chopin Protocol. The sodium chloride is dissolved in water prior to the addition to dough. Instant dry yeast and ascorbic acid were blended with 250 g of flour in the kneader bowl. Salt water was progressively added to the flour at the beginning of the first minute of the mixing period. After one minute, the mixing was stopped to remove the flour sticking to the walls and ensure a homogeneous hydration. The mixing process was continued for 6 minutes. A sample size of 315 g of dough was used for each treatment.

Fermentation Test

Rheofermentometer was used to study the fermentation properties of dough. The dough (315 g) obtained from AlveoConsistograph was placed in the bottom of the aluminum basket and packed it down with hands. The height of the dough in the basket must be leveled out just below the lowest holes. The piston with a 2000 g weight was placed on top of the dough. The basket was placed in the F3 Rheofermentometer bowl. Displacement sensor was placed and the whole system was tightly closed and the test was run for a total of 4 h. This time represents 1 h longer than the Chopin Protocol as it was determined experimentally with the samples and treatments in this study.

The F3 Rheofermentometer analyzes the development of a dough sample placed in the bowl. The piston placed on the dough rises. The piston is directly linked to a displacement sensor which will calculate the dough rising. Rheofermentometer is also linked to a pressure sensor through a pneumatic circuit that measures the pressure increase in the fermenting dough. The three curves are dough development, speed of CO₂ release and quantity produced and volume of CO₂ retained in dough. Fermentation variables are defined in Table 1 and visco-elastic, mixing and baking terms are defined in Table 2 (Chapter III).

3. STATISTICAL ANALYSIS

A factorial design within a randomized block design was implemented. Six levels of ascorbic acid and 3 levels of flour protein were compared in a 6 X 3 factorial. The significant differences in means were compared using Analysis of Variance (ANOVA) with Tukey's comparisons ($\alpha=0.05$) using SAS (Version 9.1 SAS Institute Inc., Cary, NC). Principal Component Analysis (PCA) is a mathematical algorithm that reduces the dimensionality of the data (Ringer, 2008). PCA is performed using Canoco for windows 4.5 (Biometris, Plant Research International, Wageningen, the Netherlands).

4. Results and Discussion

Protein, moisture and ash content of the flour samples are reported in Table 3 (Chapter III). Typical dough fermentation property curves obtained are illustrated in Figure 1 and 2 showing results for sample 3B control and 3B containing 200 ppm ascorbic acid. The volume of CO₂ lost (VL) is decreased when the sample is oxidized with 200 ppm ascorbic acid (Fig. 1). The volume of retention of gas also improved in the oxidized sample when compared with control sample. From the dough development curves we can observe that the height of dough is

improved when it is oxidized with 200 ppm ascorbic acid (Fig. 2). A summary of definition of the fermentation properties of all samples is found in Table 1 (Chapter III).

Maximum height of the dough (Hm)

Hm is the maximum height of the dough development. As expected, the control sample without yeast shows no development (Table 1). The effect of oxidation on Hm does not appear to cause a general trend, which means it can detect quality differences in the flours. Observations that significantly increased Hm were 3B flours (high protein) with 50, 100 and 200 ppm of ascorbic acid. Observations that significantly decreased Hm were 3A flours (high protein) with 100 and 200 ppm of ascorbic acid (Table 1 and 2). Highest value of Hm was shown by 3B200 (50.8 mm) and lowest by 1A100 (21.7 mm) (Table 1). The change of the fermentation properties (%) is calculated in comparison to the control sample (with yeast) and reported in Table 2. Overall highlights of trends are: high percentage (37.9%) increment in maximum height was observed in sample 3B (11.4% protein) with 200 ppm ascorbic acid (3B200). A 19.2% decrease in maximum height was observed in the sample 3A100 which is the highest protein sample (13.7%) (Table 2). This suggests that the effect of oxidation in maximum height of dough during fermentation is sample specific.

Height of the dough development (h)

The height of the dough development (mm) at the end of the test was denoted by h. As expected, negative controls showed no development. The effect of oxidation on h is the same as the observed on maximum height of development except 1B flours with all levels. 1B flours with all oxidized levels significantly increased height of the dough development (Table 1 and 2). Overall highlights of trends are: lowest value of height was observed in 1B0 (19.9 mm) and highest value was observed in 3B200 (50.6 mm) (Table 1). The highest increase (55.7%) of h

was observed in 1B150 and decrease (18.8%) in 3A100 (Table 2). This observation also suggests that the samples differed in the oxidation of the disulfide bonds achieved with the same levels of ascorbic acid and thus have different protein quality.

Lowering of development percentage [(Hm-h)/Hm]

(Hm-h)/Hm is the ratio of dough height at the end of the fermentation test in percentage. A large percent means the dough has maintained its height during fermentation. The effect of oxidation on (Hm-h)/Hm appear to be flour specific. Observations of significant decrease of (Hm-h)/Hm were low protein B flours with all levels of oxidation (Table 1 and 2). Overall highlights of trends are: high value of (Hm-h)/Hm was observed in 1B0 (39.1%) and lowest value in 2A150 (0.15%) (Table 1). Highest percentage decrease was observed in 2A200, 3A50, 3A150 and 2B150 (100%) (Table 2). High protein B flours show no change.

Maximum height of the gaseous curve (H'm)

H'm is the maximum height of the gaseous release curve. The overall effect of oxidation was a decrease of H'm in most of the samples, except in four samples, 1A with 100 and 150 ppm ascorbic acid and 3B with 50 and 100 ppm ascorbic acid which showed a significant increase of H'm (28.1 and 15.8%, and 8.9 and 2.6% increase, respectively). H'm significantly decreases in 2A with 50 and 100 ppm, 3A with 50, 100 and 200 ppm of ascorbic acid and in 1B and 2B flours with all levels of ascorbic acid (Table 1 and 2). Overall highlights are: high value of H'm was shown by 1B0 (61.2 mm) and lowest value of the sample with yeast was shown by 1B150 (42.8 mm) (Table 1). Highest percentage (28.1%) increment (desirable) in maximum height was observed in 1A100 and 28.2% decreased by 1B150 (Table 2). The effect of oxidation on maximum height of the gaseous release did not give a linear response and varied according to the

sample (sample specific) and to the level of oxidation (level of ascorbic acid). But an overall trend of oxidization of dough was to decrease maximum height with few exceptions.

Total Volume (TV)

TV is the total volume under the gaseous curve. The effect of oxidation on total volume significantly increases in 1A with 100 ppm of ascorbic acid. TV significantly decreases in 2A and 3A with 50, 100 and 200 ppm, 2B flours with all levels and 3B with 150 and 200 ppm of ascorbic acid (Table 1 and 2). Overall highlights of trends are: high value of total volume was observed in 2B0 (1911.3 mL) and lowest in 1B150 (1347 mL) (Table 2). An increase in total volume (22.6%) was observed in 1A100 and 21.5% of total volume decreased in 2B150 (Table 2). The overall effect of oxidation on total volume was a decrease except for sample 1A at all levels of oxidation. Sample 1A had the lowest protein (8%), thus when the protein content is low the total volume is expected to be increased with oxidation. This observation agrees with Koehler (2003a) who reported different levels of ascorbic acid improve low protein flours better than high protein flours.

Volume lost (VL)

VL is the carbon dioxide volume released by the dough during the fermentation test. Overall trend of VL is to decrease by oxidation. All observations (all samples and levels) were significantly decreased volume lost when compared to controls (Table 1 and 2). Overall highlights are: high value was observed in 2B0 (567 mL) and lowest in 1A100 (16.5 mL) (Table 1). Highest percentage 1B100 (11.6%) of volume lost was observed in 1A100 and lowest percentage (5.1%) in 1A100 (Table 2). This observation also agrees with Koehler's (2003a) statement that ascorbic acid improves low protein flours better than high protein flours.

Volume retained (VRt)

VRt is the carbon dioxide retained in the dough at the end of the test. The overall trend of oxidation is to increase volume retention. Observations that significantly increased volume retention when compared to controls were 1A flours with all levels, 2A and 3A with 150 ppm, 1B with 50, 100 and 200 ppm and in 3B flours with 50, 100 and 150 ppm of ascorbic acid (Table 1 and 2). Overall highlights of trends are: high value of volume retention was observed in 1A100 (1831.5 mL) and lowest in 1B0 (1135 mL) (Table 1). Highest percentage (54.6%) of volume retained was observed in 1A100 and lowest percentage (2.2%) in 2A100 (Table 2).

Retention Coefficient (RC)

Retention coefficient (RC) is the retention volume divided by the total gaseous release. The overall trend of RC is to increase with oxidation. All observations (all levels) of RC were significantly increased when compared to controls (Table 1 and 2). This means that oxidation positively affects the fermentation properties by increasing the retention coefficient. Overall highlights of trends are: high value of RC was observed in 1A100 (99.1%) and lowest value in 2B0 (70.55%) (Table 1). Highest percentage (38.6%) of retention coefficient was observed in 2B200 and lowest percentage (21.5%) in 1B100 (Table 2). The effect of oxidation on retention coefficient is to increase. Retention coefficient and volume retained are the only two parameters that show a definitive increase with oxidation.

Time of maximum rise (T1)

T1 is the time taken by the dough to reach maximum height during dough development. The overall trend of time of maximum rise of dough development is to increase with oxidation. Observation of significant increase in T1 is 1B with 150 ppm of ascorbic acid (61.4% increase) when compared to the control (Table 1 and 2). Overall highlights of trends are: high value of T1

was observed in 2A200, 3AN, 3A50 and 3A150 (3.9 h). Lowest value of T1 was observed in 1B0 (1.5 h) (Table 1). Highest percentage (61.4%) of time taken to reach maximum height of dough development was observed in 1B150 and time decreased 1.5% in 1A150 (Table 2). The overall effect of oxidation on T1 is to cause an increase.

Time of maximum rise (T'1)

T'1 is the time spent to reach maximum rise during gaseous release. The overall trend of time of maximum rise of gaseous release is to increase with oxidation. Observations with significant increase of T'1 were 1A with 50, 150 and 200 ppm, 2A, 2B and 3B flours with all levels and 3A flour with 150 ppm of ascorbic acid (Table 1 and 2). Overall highlights are: high value of T'1 was observed in 2A150, 2A200 and 3B200 (4). Lowest value (1.4) of T'1 was observed in 2B0 (Table 1). Highest increase (185.5%) of time taken to reach maximum height of gaseous curve was observed in all medium protein B flours and lowest (15%) increase was observed in 1B200 (Table 2). Time to reach maximum height of gaseous release increases with oxidation.

In summary, from Table 4 and 5 we can say that maximum height of the dough development decreases for A flours and increases for B flours. Maximum height of gaseous release shows an increase with low protein A flours. Highest percentage of retention volume of gas was observed in low protein A flour (1A100). Flours treated with 100 ppm ascorbic acid improved gas retention properties better than other concentrations. Highest percentage of retention coefficient of gas was observed in medium protein flours. Koehler (2003a) reported that different levels of ascorbic acid improve low protein flours rather than high protein flours. But in our study we observed different levels of ascorbic acid improved medium and high protein flours as well. Chamberlain and Collins (1979) proved that yeasted dough ascorbic acid

at 150 ppm increases the effectiveness of dough. Our study showed that fermentation properties of yeasted dough from commercial hard red winter wheat flours with ascorbic acid at 100 ppm were more desirable than those obtained with 150 ppm.

PCA results

Principal component analyses were performed on the data sets obtained from fermentation parameters.

Fermentation variables Vs fermentation variables with flour protein

PCA were performed on the data sets, to assess the relationship of flour protein and fermentation properties (Fig. 3 and 4). Figure 3 represents the fermentation properties alone and all the samples. Principal component axis 1 (PC1) explained 54.7% variance and principal component axis 2 (PC2) explained 20.8% variance. Total explained variance is 75.5% (Table 3). Among fermentation properties, the highest contribution of variance (94.7%) was volume of retention (VRt) in PC1 whereas in PC2 the highest contribution of variance (70.8%) was volume lost (VL) (Table 3). Figure 4 displays the fermentation properties plus flour protein. Principal component axis 1 (PC1) explained 49.7% variance and principal component axis 2 (PC2) explained 18.9% variance. Total explained variance is 68.6% (Table 4). Among fermentation properties with flour protein, the highest contribution of variance (94.7%) was volume of retention (VRt) on PC1 whereas on PC2 highest contribution of variance (70.8%) was volume lost (VL) (Table 4). Only 0.07% of explained variance was contributed by flour protein on PC1 and 0% on PC2 (Table 4). This suggests that the variation of protein is weakly related to the volume lost and its contribution to the variance is very small when compared with samples with changes in their oxidizing state. In both graphs (Fig. 3 and 4), most of the fermentation variables are on PC1. Flours treated with ascorbic acid are very close to PC1 when compared with control

and negative samples. All control samples are closely related among themselves and to volume lost. They are well separated from the oxidized flours. Negative controls are also closely related among themselves and well separated from the samples with changes due to oxidation. So negative controls are removed from the data sets and PCA was compared. It also suggests that the oxidized samples are closely related to volume of the gas retained by the dough during fermentation in the first component. These samples are negatively related to volume lost and differences in their gas volume lost by their dough's in the second component. This means that all the oxidized samples lost less gas compared to the control samples.

Fermentation variables without negative control Vs fermentation variables with flour protein and without negative control

PCA were performed to assess the relationship of fermentation variables and protein without the negative controls (Fig. 5 and 6). From the fermentation properties on Figure 5, principal component axis 1 (PC1) explained 32.7% variance and principal component axis 2 (PC2) explained 29.0% variance (Fig. 5). Total explained variance is 61.7% (Table 5). Among fermentation properties, the highest contribution of variance (65.3%) was time taken to reach maximum height of gaseous curve (T'1) on PC1 whereas on PC2 the highest contribution of variance (75.8%) was total volume (TV) (Table 5). In comparison, when flour protein was included (Fig. 6), principal component axis 1 (PC1) explained 29.8% variance and principal component axis 2 (PC2) explained 26.5% variance. Total explained variance is 56.3% (Table 6). Among fermentation properties with flour protein, the highest contribution of variance (64.3%) was time taken to reach maximum height of gaseous curve (T'1) on PC1 whereas on PC2 the highest contribution of variance (62.8%) was maximum height of gaseous curve (Table 6). Only 0.16% of explained variance was contributed by flour protein on PC1 and 3.07% on PC2 (Table

6). As the total explained variance of fermentation variables (61.7%) is 5.4 units of percentage higher than fermentation variables with flour protein (56.3%), we can say that compared to changes in oxidation state in this set, flour protein appears to have a small effect and is marginally correlated to other fermentation variables. Controls are closely related to volume lost (VL) and are separated from the oxidized flours. Most of the A flours are closely related to time to reach maximum height of gaseous curve (T'1) and retention coefficient (RC). They are negatively correlated to total volume (TV) and maximum height of dough development (Hm). Most of the B flours are closely related to volume of gas retained (VRt) and negatively correlated to volume lost (VL) and lowering development percentage ($[Hm-h]/Hm$). By oxidizing the dough, we are bringing samples close to the axis thus by increasing the fermentation properties.

Fermentation properties Vs Fermentation properties with visco-elastic, mixing, baking properties

The relationship of fermentation variables with visco-elastic, mixing and baking properties was investigated (Fig. 7). From Figure 6, principal component axis 1 (PC1) explained 29.8% variance and principal component axis 2 (PC2) explained 26.5% variance. Total explained variance is 56.3% (Table 6). From Figure 7, principal component axis 1 (PC1) explained 27.2% variance and principal component axis 2 (PC2) explained 24.3% variance. Total explained variance is 51.5% (Table 7). Among all properties, the highest contribution of variance (84.7%) was delta compliance ($J-J_r$) and second major component that contributes high variance (83.0%) is flour protein (FP) on PC1 whereas on PC2 the highest contribution of variance (60.7%) was loaf volume (LV) (Table 7). As the total explained variance of fermentation variables (56.3%) is higher than fermentation variables with visco-elastic, mixing and baking variables (51.5%), we can say including all the variables from visco-elastic, mixing and baking do not improve the

explained variance. This means that all these analyses are not increasing the ability to separate the samples. Fermentation properties would give as much information as all the tests combined. All variables are closely associated. Low protein A flours and low protein B flours are separated from other flours. Ratio of dough heights $[(H_m-h)/H_m]$ and volume lost (VL) are closely related to elastic properties (Sep and RCY). Time taken to reach maximum height of the dough (T1) is closely related to gluten time constant of creep (TCC, viscous property) and oven spring (OSP, baking property). Maximum height of the dough (H_m) and dough height (h) are closely related to flour protein (FP) and baking properties (LH and LV). All control samples are closely related and positively correlated with volume lost (VL) and are negatively correlated to delta compliance ($J-J_r$) (viscous component) and retention coefficient (RC). Medium and high protein oxidized flours are closely related to loaf height and loaf volume. All baking properties show greatest contribution of explained variance on PC2. It was of interest to explore other possible correlations revealed by PCA analysis on flour protein, fermentation properties and baking properties.

Relationship of flour protein, Fermentation and baking properties

PCA were performed on the data sets of flour protein, fermentation variables and baking properties (Fig. 8). PCA analyses of fermentation properties with flour protein were already performed (Fig. 6 and Table 6). From Figure 6, principal component axis 1 (PC1) explained 29.8% variance and principal component axis 2 (PC2) explained 26.5% variance. Total explained variance is 56.3% (Table 6). From Figure 8, principal component axis 1 (PC1) explained 27.1% variance and principal component axis 2 (PC2) explained 24.9% variance. Total explained variance is 52% (Table 8). Highest contribution of explained variance (72.1%) was loaf volume (LV) and second highest variance (71.2%) was explained by height of loaf

volume (LH) on PC1 whereas on PC2 highest contribution of variance (59.8%) was volume of gas retained (VRt) (Table 8). Flour protein explained 45.6% variance on PC1 and 10.9% on PC2 (Table 8). As the total explained variance of fermentation variables (56.3%) is more than fermentation variables with baking variables (52%), we can say fermentation properties explain more variance than baking properties. Control samples were negatively associated with gas retained (VRt) and positively related with volume lost (VL). Weak flours are separated from the rest of the flour samples. The height of dough at the end of the fermentation test is the variable most closely related to loaf height and loaf volume.

5. Conclusions

Null Hypothesis is rejected as there is significant effect of the oxidized state of the dough (by adding ascorbic acid) on fermentation properties when compared to the control samples. By oxidizing the dough, the maximum height of gaseous release decreased in all flours except in low protein A flours. The highest percentage of retention volume of gas was observed in low protein A flour while the highest percentage of retention coefficient of gas was observed in medium protein B flour.

Fermentation variables explained more variance (61.7%) than the fermentation variables combined with visco-elastic, mixing and baking variables (51.5%). The ratio of dough heights $[(H_m-h)/H_m]$ and volume lost (VL) are closely related to gluten elastic properties (Sep and RCY). The time taken to reach maximum height of the dough (T1) is closely related to gluten viscous (TCC) and baking properties (OSP). Maximum height of the dough (Hm) and dough height (h) are closely related to flour protein (FP) and baking properties (LH and LV).

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Table 1. Fermentation properties in six commercial wheat flours treated with ascorbic acid levels. Means (n=2) with same superscripts in a column are not significantly different (P > 0.05). The standard deviations of means are shown in parenthesis.

TRT	Fermentation Properties									
	Hm (mm)	h (mm)	(Hm-h)/Hm (%)	H'm (mm)	TV (mL)	VL (mL)	VRT (mL)	RC (%)	T1 (hr)	T'1 (hr)
1A0	25 ^{mn} (1.3)	24.55 ^{kl} (1.35)	1.8 ^b (0.3)	47.5 ^{fghijklm} (2.5)	1507.5 ^{efghijk} (76.5)	323 ^{de} (37)	1184.5 ^{lm} (39.5)	78.65 ^{cd} (1.35)	3.93 ^a (0.02)	3.16 ^{bc} (0.15)
1AN	0 ^o (0)	0 ⁿ (0)	0 ^b (0)	5 ⁿ (0.1)	12.5 ^l (0.5)	2 ^f (0)	11 ⁿ (0)	83.25 ^{bc} (2.05)	4 ^a (0)	0.11 ^h (0)
1A50	23.6 ⁿ (0.9)	23.55 ^{lm} (0.85)	0.2 ^b (0.2)	51.8 ^{defghi} (0.8)	1555 ^{cdefghij} (35)	19.5 ^f (0.5)	1535.5 ^{bcdefg} (34.5)	98.75 ^a (0.05)	3.97 ^a (0.03)	3.97 ^a (0.02)
1A100	21.7 ⁿ (3.3)	21.45 ^{lm} (3.15)	1.05 ^b (0.55)	60.85 ^{ab} (7.45)	1848.5 ^{ab} (214.5)	16.5 ^f (1.5)	1831.5 ^a (216.5)	99.1 ^a (0.2)	3.95 ^a (0)	3.68 ^{ab} (0.31)
1A150	24.6 ^{mn} (3)	24.45 ^{klm} (2.85)	0.55 ^b (0.55)	55 ^{abcde} (2.1)	1634 ^{cdefg} (64)	27 ^f (3)	1607 ^{bcd} (61)	98.35 ^a (0.15)	3.87 ^a (0.13)	3.9 ^a (0.09)
1A200	23.35 ⁿ (2.85)	23.1 ^{lm} (2.6)	0.95 ^b (0.95)	50.45 ^{defghijkl} (1.95)	1565.5 ^{cdefghi} (91.5)	24.5 ^f (4.5)	1540.5 ^{bcdef} (87.5)	98.4 ^a (0.2)	3.97 ^a (0.03)	3.9 ^a (0.1)
2A0	32.15 ^{kl} (0.55)	31.45 ^{ij} (1.15)	2.2 ^b (1.9)	53.1 ^{cdefg} (1)	1695.5 ^{bcdef} (27.5)	389.5 ^c (11.5)	1306 ^{ijklm} (16)	77.05 ^{de} (0.35)	3.67 ^a (0.28)	1.50 ^{fg} (0.16)
2AN	0 ^o (0)	0 ⁿ (0)	0 ^b (0)	4.9 ⁿ (1.3)	12 ^l (3)	3.5 ^f (0.5)	9 ⁿ (3)	70.2 ^f (6)	4 ^a (0)	0.1 ^h (0)
2A50	29 ^{lm} (0.5)	28.45 ^{jk} (1.05)	1.95 ^b (1.95)	43.9 ^{lm} (2.1)	1369.5 ^{ijk} (46.5)	31 ^f (3)	1338.5 ^{hijkl} (49.5)	97.7 ^a (0.3)	3.97 ^a (0.03)	3.95 ^a (0.04)
2A100	28.95 ^{lm} (1.25)	28.65 ^{jk} (1.05)	1.05 ^b (0.65)	43.9 ^{lm} (0.4)	1357.5 ^{ijk} (25.5)	22 ^f (3)	1335 ^{hijkl} (23)	98.4 ^a (0.2)	3.71 ^a (0.10)	3.95 ^a (0.04)
2A150	31.15 ^{kl} (2.25)	31.1 ^{ij} (2.2)	0.15 ^b (0.15)	48.5 ^{efghijklm} (0.6)	1534.5 ^{defghijk} (40.5)	27.5 ^f (0.5)	1507.5 ^{cdefghi} (41.5)	98.2 ^a (0.1)	3.98 ^a (0.02)	4 ^a (0)
2A200	31.5 ^{kl} (1)	31.5 ^{ij} (1)	0 ^b (0)	46.35 ^{ghijklm} (3.45)	1429.5 ^{ghijk} (76.5)	23.5 ^f (5.5)	1406 ^{efghijk} (82)	98.3 ^a (0.5)	4 ^a (0)	4 ^a (0)

Table 1. continued

TRT	Fermentation Properties									
	Hm (mm)	h (mm)	(Hm-h)/Hm (%)	H'm (mm)	TV (mL)	VL (mL)	VRT (mL)	RC (%)	T1 (hr)	T'1 (hr)
3A0	39.4 ^{efgh} (2.2)	39.15 ^{efg} (2.45)	0.65 ^b (0.65)	54.3 ^{abcdef} (0.1)	1669 ^{bcdef} (13)	362.5 ^{cd} (13.5)	1307 ^{jklm} (0)	78.3 ^{cde} (0.6)	3.98 ^a (0.02)	1.65 ^{efg} (0.04)
3AN	0 ^o (0)	0 ⁿ (0)	0 ^b (0)	4.35 ⁿ (0.75)	17.5 ^l (3.5)	2.5 ^f (0.5)	15.5 ⁿ (4.5)	85.5 ^b (6.5)	4 ^a (0)	0.10 ^h (0.01)
3A50	34.8 ^{hijk} (0.8)	34.8 ^{ghi} (0.8)	0 ^b (0)	46.35 ^{ghijklm} (1.15)	1437.5 ^{ghijk} (44.5)	24 ^f (3)	1413.5 ^{efghijk} (47.5)	98.35 ^a (0.25)	4 ^a (0)	2.17 ^{de} (0.03)
3A100	31.85 ^{kl} (1.35)	31.8 ^{ij} (1.4)	0.15 ^b (0.15)	44.4 ^{klm} (1)	1381 ^{hijk} (35)	32 ^f (6)	1349.5 ^{efghijkl} (41.5)	97.7 ^a (0.5)	3.98 ^a (0.02)	2.05 ^{def} (0.04)
3A150	38.35 ^{fghi} (0.25)	38.35 ^{efg} (0.25)	0 ^b (0)	55.95 ^{abcd} (8.15)	1748 ^{abc} (277)	27 ^f (6)	1721 ^{ab} (271)	98.5 ^a (0.1)	4 ^a (0)	2.63 ^{cd} (0.67)
3A200	33.25 ^{jkl} (0.05)	33.1 ^{hij} (0.1)	0.45 ^b (0.45)	45.45 ^{hijklm} (0.35)	1415 ^{hijk} (14)	26.5 ^f (0.5)	1388.5 ^{efghijk} (13.5)	98.1 ^a (0)	3.97 ^a (0.03)	2.18 ^{de} (0.18)
1B0	33.2 ^{jkl} (2.3)	19.85 ^m (3.75)	39.1 ^a (15.5)	59.55 ^{abc} (1.75)	1412 ^{hijk} (32)	277 ^e (15)	1135 ^m (17)	80.4 ^{bcd} (0.6)	1.58 ^d (0.17)	1.40 ^g (0.01)
1BN	0 ^o (0)	0 ⁿ (0)	0 ^b (0)	5.5 ⁿ (1.8)	18.5 ^l (6.5)	3 ^f (2)	15.5 ⁿ (4.5)	83.35 ^{bc} (4.65)	4 ^a (0)	0.11 ^h (0)
1B50	32.15 ^{kl} (1.65)	30.2 ^{ij} (0.7)	5.95 ^b (2.65)	45.75 ^{hijklm} (0.25)	1429.5 ^{ghijk} (9.5)	31.5 ^f (2.5)	1398 ^{efghijk} (7)	97.85 ^a (0.15)	1.91 ^{cd} (0)	1.71 ^{efg} (0.05)
1B100	31.4 ^{kl} (0.3)	29.3 ^j (0)	6.7 ^b (0.9)	45 ^{ijklm} (1.4)	1403 ^{hijk} (21)	32 ^f (4)	1370.5 ^{efghijkl} (25.5)	97.7 ^a (0.3)	1.99 ^{cd} (0.13)	1.64 ^{efg} (0.03)
1B150	32.7 ^{jkl} (1.2)	30.9 ^{ij} (1.1)	5.5 ^b (0.1)	42.75 ^m (1.65)	1347 ^k (21)	30 ^f (1)	1317.5 ^{ijklm} (22.5)	97.8 ^a (0.1)	2.55 ^{bc} (0.19)	1.71 ^{efg} (0.05)
1B200	34 ^{ijk} (0.1)	32.35 ^{hij} (0.55)	4.8 ^b (1.9)	44.5 ^{jklm} (1.1)	1400 ^{hijk} (38)	29 ^f (4)	1371.5 ^{efghijkl} (33.5)	97.95 ^a (0.25)	2.01 ^{cd} (0.20)	1.61 ^{efg} (0.05)

Table 1. Continued

TRT	Fermentation Properties									
	Hm (mm)	h (mm)	(Hm-h)/Hm (%)	H'm (mm)	TV (mL)	VL (mL)	VRT (mL)	RC (%)	T1 (hr)	T'1 (hr)
2B0	43.3 ^{bcde} (0.5)	42.75 ^{bcde} (0.05)	1.25 ^b (1.05)	61.15 ^a (4.15)	1911.5 ^a (113.5)	567 ^a (102)	1344.5 ^{ghijkl} (11.5)	70.55 ^f (3.55)	3.40 ^{ab} (0.51)	1.38 ^g (0.13)
2BN	0 ^o (0)	0 ⁿ (0)	0 ^b (0)	5.2 ⁿ (0)	23 ^l (0)	1 ^f (0)	21 ⁿ (0)	94.6 ^a (0)	4 ^a (0)	0.11 ^h (0)
2B50	47.1 ^{ab} (1)	46.85 ^{ab} (0.75)	0.5 ^b (0.5)	51.4 ^{defghij} (0.7)	1509 ^{efghijk} (27)	37 ^f (5)	1472 ^{cdefghij} (32)	97.55 ^a (0.35)	3.95 ^a (0.04)	3.93 ^a (0.02)
2B100	45.25 ^{bc} (0.65)	45 ^{bc} (0.5)	0.55 ^b (0.35)	52.05 ^{defgh} (0.85)	1557.5 ^{cdefghij} (0.5)	35.5 ^f (2.5)	1521.5 ^{cdefgh} (2.5)	97.7 ^a (0.2)	3.94 ^a (0.03)	3.97 ^a (0.03)
2B150	44.3 ^{bcd} (0.3)	44.3 ^{bcd} (0.3)	0 ^b (0)	50.75 ^{defghijkl} (1.95)	1501 ^{efghijk} (62)	37.5 ^f (3.5)	1463.5 ^{defghijk} (58.5)	97.5 ^a (0.1)	4 ^a (0)	3.97 ^a (0.03)
2B200	44.2 ^{bcd} (0.7)	44.1 ^{bcd} (0.8)	0.25 ^b (0.25)	51.1 ^{defghijkl} (1)	1514.5 ^{efghijk} (13.5)	33.5 ^f (6.5)	1481 ^{cdefghij} (7)	97.8 ^a (0.4)	3.98 ^a (0.02)	3.97 ^a (0.03)
3B0	36.85 ^{ghij} (0.75)	36.85 ^{fgh} (0.75)	0 ^b (0)	54.2 ^{bcdef} (1.1)	1737 ^{abcd} (46)	462.5 ^b (20.5)	1275 ^{klm} (25)	73.4 ^{ef} (0.5)	4 ^a (0)	2.09 ^{def} (1.03)
3BN	0 ^o (0)	0 ⁿ (0)	0 ^b (0)	4.9 ⁿ (0.6)	14 ^l (1)	2.5 ^f (0.5)	12 ⁿ (1)	84 ^b (1.2)	2.10 ^{cd} (1.89)	0.11 ^h (0)
3B50	46.85 ^{abc} (2.35)	46.6 ^{abc} (2.1)	0.5 ^b (0.5)	59.05 ^{abc} (2.85)	1698.5 ^{bcde} (114.5)	35.5 ^f (2.5)	1663.5 ^{abc} (111.5)	97.9 ^a (0)	3.95 ^a (0.04)	3.97 ^a (0.03)
3B100	42.35 ^{cdef} (2.45)	42.1 ^{cde} (2.2)	0.55 ^b (0.55)	55.6 ^{abcd} (0.9)	1579.5 ^{cdefgh} (35.5)	29 ^f (3)	1550.5 ^{bcde} (38.5)	98.15 ^a (0.25)	3.98 ^a (0.02)	3.98 ^a (0.02)
3B150	40.05 ^{defg} (1.75)	39.9 ^{def} (1.9)	0.4 ^b (0.4)	53.55 ^{cdef} (2.15)	1522 ^{efghijk} (51)	40 ^f (4)	1482 ^{cdefghij} (56)	97.35 ^a (0.35)	3.95 ^a (0.04)	3.97 ^a (0.03)
3B200	50.8 ^a (4.6)	50.55 ^a (4.65)	0.5 ^b (0.1)	53.15 ^{cdefg} (0.95)	1489.5 ^{fghijk} (11.5)	33.5 ^f (3.5)	1455.5 ^{defghijk} (7.5)	97.75 ^a (0.25)	3.96 ^a (0)	4 ^a (0)

Table 2. Change (percent) of fermentation properties of six commercial wheat flours treated with ascorbic acid levels. Values with * are significantly different (P<0.05) from control samples. Percentage calculated values are from Table 1 and % change = (Sample treated with additive - control sample)/control sample * 100.

TRT	Fermentation properties									
	Hm (%)	h (%)	(Hm-h)/Hm (%)	H'm (%)	TV (%)	VL (%)	VRt (%)	RC (%)	T1 (%)	T'1 (%)
1A50	-5.6	-4.1	-88.9	9.1	3.2	-6.0*	29.6*	25.6*	1.0	25.6*
1A100	-13.2	-12.6	-41.7	28.1*	22.6*	-5.1*	54.6*	26.0*	0.5	16.5
1A150	-1.6	-0.4	-69.4	15.8*	8.4	-8.4*	35.7*	25.0*	-1.5	23.4*
1A200	-6.6	-5.9	-47.2	6.2	3.8	-7.6*	30.1*	25.1*	1.0	23.4*
2A50	-9.8	-9.5	-11.4	-17.3*	-19.2*	-8.0*	2.5	26.8*	8.2	163.3*
2A100	-10.0	-8.9	-52.3	-17.3*	-19.9*	-5.6*	2.2	27.7*	1.1	163.3*
2A150	-3.1	-1.1	-93.2	-8.7	-9.5	-7.1*	15.4*	27.4*	8.4	166.7*
2A200	-3.1	0.2	-100.0	-12.7	-15.7*	-6.0*	7.7	27.6*	9.0	166.7*
3A50	-11.7	-11.1	-100.0	-14.6*	-13.9*	-6.6*	8.1	25.6*	0.5	31.5
3A100	-19.2*	-18.8*	-76.9	-18.2*	-17.3*	-8.8*	3.3	24.8*	0.0	24.2
3A150	-2.7	-2.0	-100.0	3.0	4.7	-7.4*	31.7*	25.8*	0.5	59.4*
3A200	-15.6*	-15.5*	-30.8	-16.3*	-15.2*	-7.3*	2.4	25.3*	-0.3	32.1
1B50	-3.2	52.1*	-84.8*	-23.2*	1.2	-11.4*	23.2*	21.7*	20.9	22.1
1B100	-5.4	47.6*	-82.9*	-24.4*	-0.6	-11.6*	20.7*	21.5*	25.9	17.1
1B150	-1.5	55.7*	-85.9*	-28.2*	-4.6	-10.8*	16.1	21.6*	61.4*	22.1
1B200	2.4	63.0*	-87.7*	-25.3*	-0.8	-10.5*	20.8*	21.8*	27.2	15.0
2B50	8.8	9.6	-60.0	-15.9*	-21.1*	-6.5*	9.5	38.3*	16.2	184.8*
2B100	4.5	5.3	-56.0	-14.9*	-18.5*	-6.3*	13.2	38.5*	15.9	187.7*
2B150	2.3	3.6	-100.0	-17.0*	-21.5*	-6.6*	8.9	38.2*	17.6	187.7*
2B200	2.1	3.2	-80.0	-16.4*	-20.8*	-5.9*	10.2	38.6*	17.1	187.7*
3B50	27.1*	26.5*	0.0	8.9	-2.2	-7.7*	30.5	33.4*	-1.3	90.0*
3B100	14.9*	14.2*	0.0	2.6	-9.1	-6.3*	21.6	33.7*	-0.5	90.4*
3B150	8.7	8.3	0.0	-1.2	-12.4*	-8.6*	16.2	32.6*	-1.3	90.0*
3B200	37.9*	37.2*	0.0	-1.9	-14.2*	-7.2*	14.2	33.2*	-1.0	91.4*

Table 3. Explained variance (%) in PCA of fermentation variables with negative control in flours treated with ascorbic acid.

Ascorbic acid	AXES	PC1	PC2	1+2
	PC (%)	54.7%	20.8%	75.5%
Fermentation	Hm	87	2	89
	h	87	0	87
	(Hm-h)/Hm	0	37	37
	H'm	92	5	97
	TV	92	4	95
	VL	3	71	74
	VRT	95	0	95
	RC	21	52	73
	T1	1	25	26
	T'1	69	14	83

Table 4. Explained variance (%) in PCA of fermentation variables with flour protein in flours treated with ascorbic acid.

Ascorbic acid	AXES	PC1	PC2	1+2
	PC (%)	49.7%	18.9%	68.6%
Fermentation	Hm	87	2	89
	h	87	0	87
	(Hm-h)/Hm	0	37	37
	H'm	92	5	97
	TV	92	3	95
	VL	3	70	74
	VRT	95	0	95
	RC	22	51	73
	T1	1	25	26
	T'1	69	14	83
Flour Protein	FP	0	0	0

Table 5. Explained variance (%) in PCA of fermentation variables without negative control in flours treated with ascorbic acid.

Ascorbic acid	AXES	PC1	PC2	1+2
	PC (%)	32.7%	29%	61.7%
Fermentation	Hm	6	23	29
	h	19	23	41
	(Hm-h)/Hm	41	1	42
	H'm	0	68	69
	TV	2	76	78
	VL	45	45	89
	VRT	57	6	63
	RC	51	39	89
	T1	42	8	50
	T'1	65	0	66

Table 6. Explained variance (%) in PCA of fermentation variables with flour protein and without negative control in flours treated with ascorbic acid.

Ascorbic acid	AXES	PC1	PC2	1+2
	PC (%)	29.8%	26.5%	56.3%
Fermentation	Hm	7	28	35
	h	20	28	48
	(Hm-h)/Hm	41	1	43
	H'm	0	63	63
	TV	2	70	72
	VL	44	46	89
	VRT	56	4	61
	RC	49	40	89
	T1	43	8	51
	T'1	65	1	65
Flour Protein	FP	0	3	3

Table 7. Explained variance (%) in PCA of fermentation variables when compared with visco-elastic, mixing and baking variables in flours treated with ascorbic acid. Definitions of fermentation, visco-elastic, mixing and baking variables explained in Table 2 (Chapter III).

Ascorbic Acid	AXES	PC1	PC2	1+2
	PC (%)	27.2%	24.3%	51.5%
Fermentation	Hm	44	0	45
	H	46	6	51
	(Hm-h)/Hm	3	47	50
	H'm	0	0	1
	TV	0	2	2
	VL	0	29	29
	VRT	1	40	41
	RC	0	34	34
	T1	2	58	60
	T'1	4	52	56
Visco-elastic	SeP	0	80	80
	J-J _r	0	58	58
	RCY	1	61	62
	TCR	2	31	33
	TCC	0	25	25
Mixing	WA	65	0	65
	DT	76	4	80
	ST	66	3	68
	BT	85	2	87
Baking	PH	58	19	77
	LH	73	2	74
	SV	4	1	4
	OSP	3	51	54
	LV	64	7	71
Flour Protein	FP	83	0	83

Table 8. Explained variance (%) in PCA of fermentation variables when compared with baking variables in flours treated with ascorbic acid.

Ascorbic Acid	Axes	PC1	PC2	1+2
	PC (%)	27.13	24.97	52.1
Fermentation	Hm	52	6	58
	h	66	3	67
	(Hm-h)/Hm	23	1	43
	H'm	3	19	7
	TV	6	10	8
	VL	0	46	67
	VRT	9	60	47
	RC	1	49	71
	T1	29	17	45
	T'1	5	50	63
Baking	PH	27	54	82
	LH	71	5	76
	SV	3	8	11
	OSP	21	40	61
	LV	72	0	72
Flour Protein	FP	46	11	57

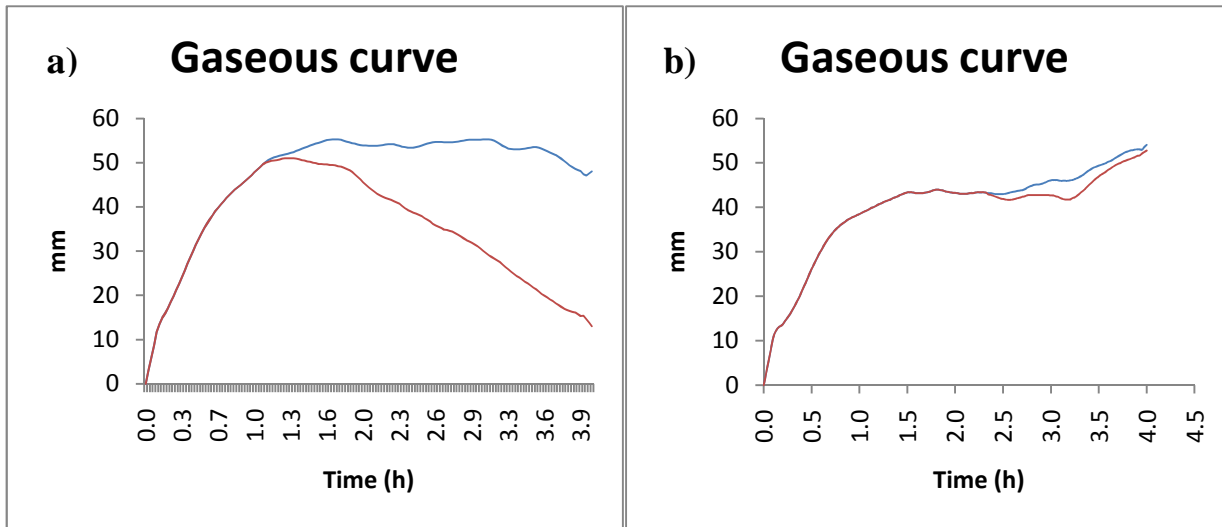


Figure 1. A graphical representation of gaseous curve of a) control sample from flour 3B and b) sample containing 200 ppm of ascorbic acid (3B200). Blue tracings are the total volume and the red is the volume retained.

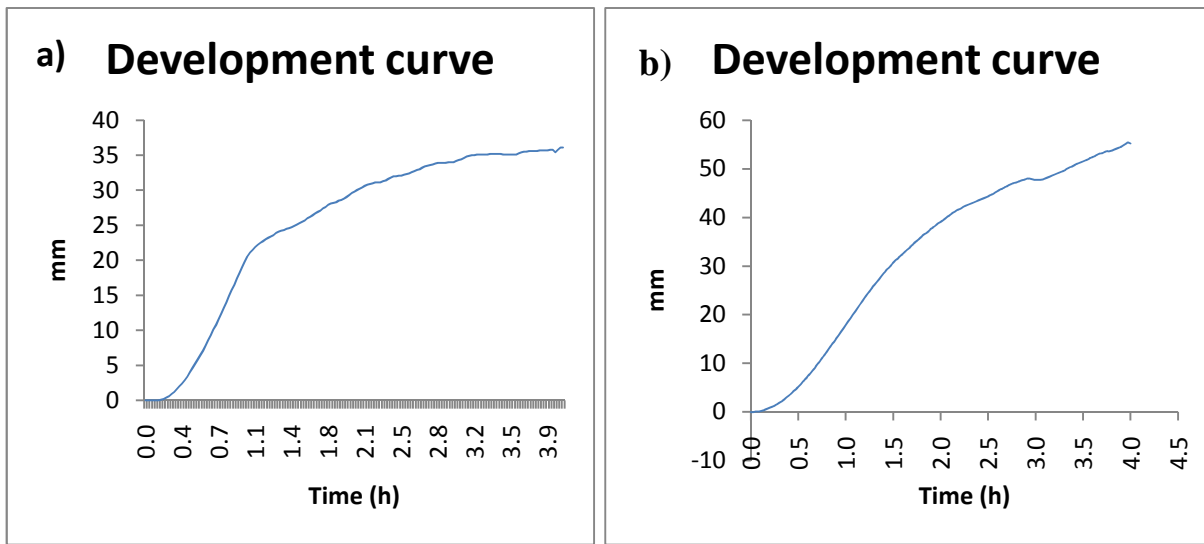


Figure 2. A graphical representation of dough development of a) control sample from flour 3B and b) sample containing 200 ppm of ascorbic acid (3B200).

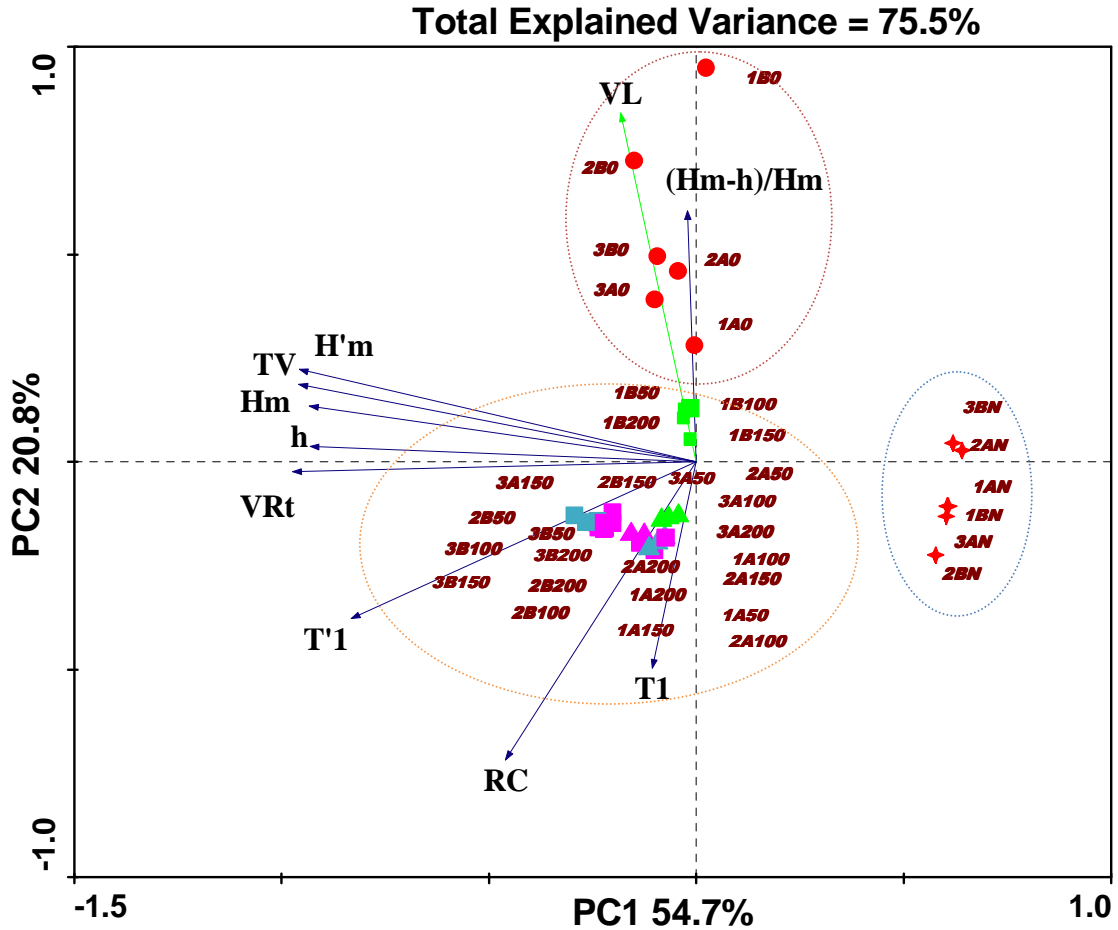


Figure 3. Loading plot of first two principal components based on fermentation properties with negative control of six commercial wheat flours, added with five levels of ascorbic acid. Definitions of fermentation, visco-elastic, mixing and baking variables explained in Table 2 and 3. Flour protein content (%), 1A = 7.95, 2A = 11.19, 3A = 13.68, 1B = 10.4, 2B = 10.59 and 3B = 11.38, respectively. Symbols and definitions: ● -Control samples, ★ -Negative controls ▲ - Low protein A flours, ▲ -Medium protein A flours, ▲ - High protein A flours. ■ - Low protein B flours, ■ - Medium protein B flours, ■ - High protein B flours.

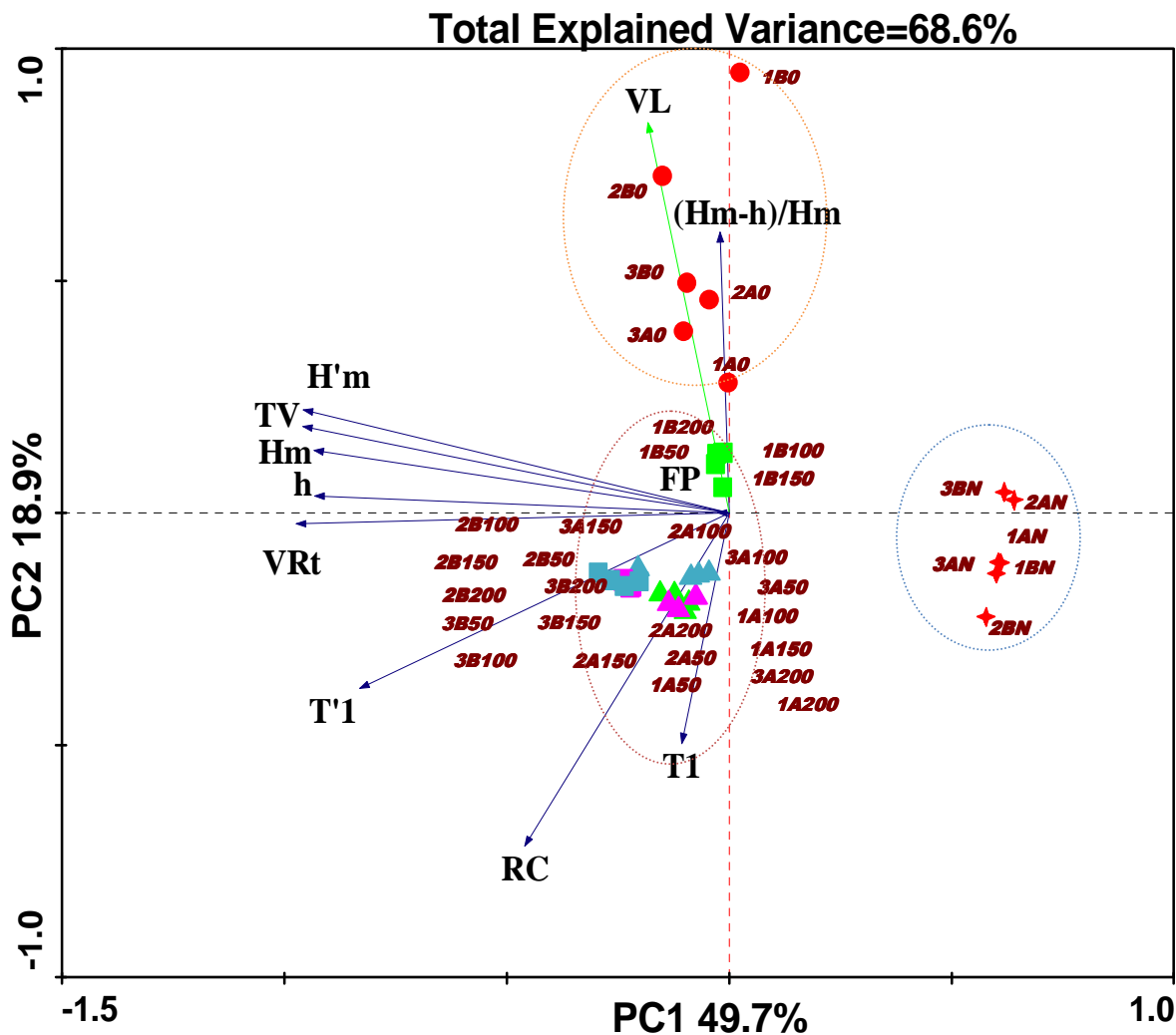


Figure 4. Loading plot of first two principal components based on fermentation properties with flour protein of six commercial wheat flours added with five levels of ascorbic acid. Symbols and definitions: ● -Control samples, ★ - Negative controls, ▲ - Low protein A flours, ▲ -Medium protein A flours, ▲ - High protein A flours. ■ - Low protein B flours, ■ - Medium protein B flours, ■ - High protein B flours.

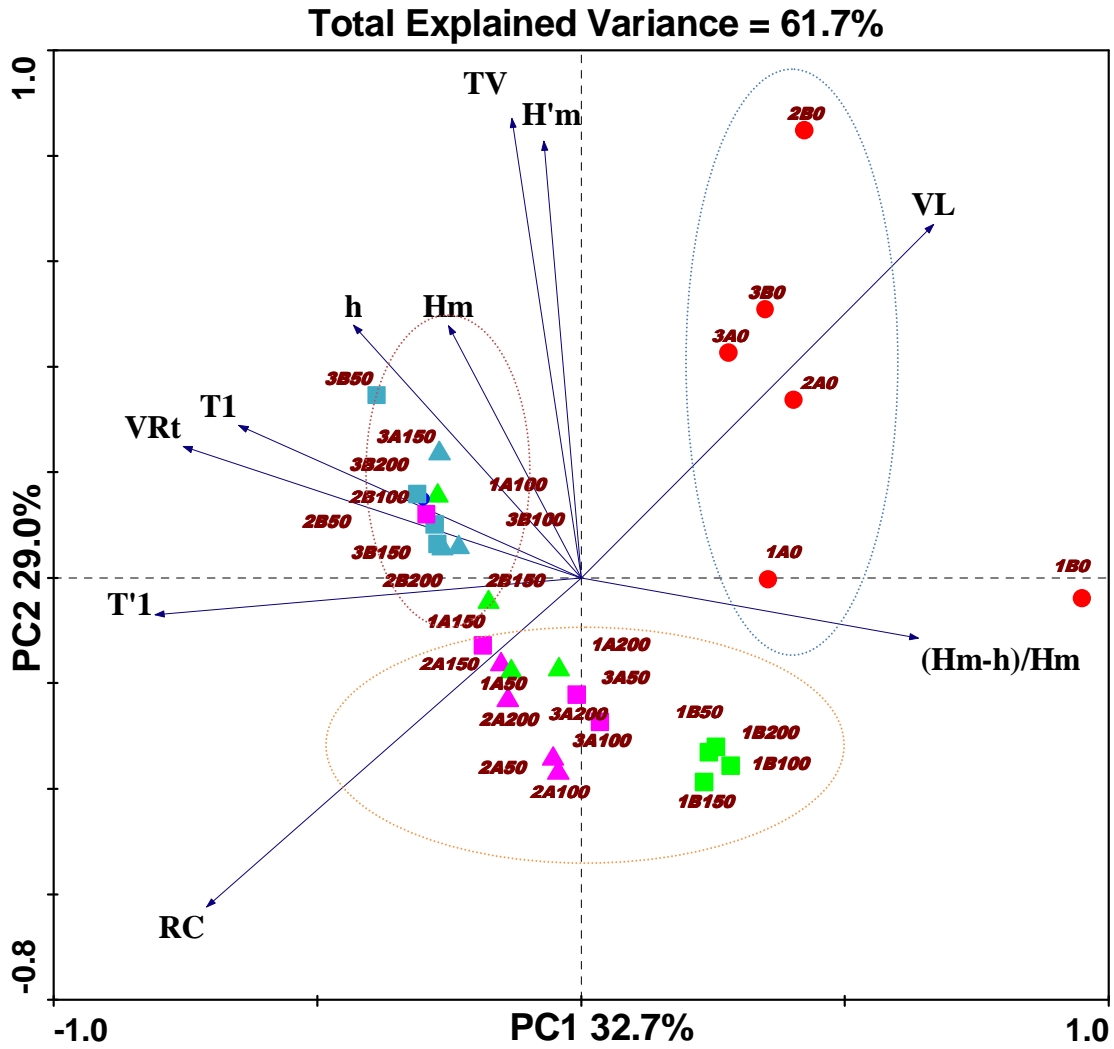


Figure 5. Loading plot of first two principal components based on fermentation properties without negative control of six commercial wheat flours added with five levels of ascorbic acid. Symbols and definitions: ● -Control samples, ▲ – Low protein A flours, ▲ -Medium protein A flours, ▲ - High protein A flours. ■ – Low protein B flours, ■ - Medium protein B flours, ■ - High protein B flours.

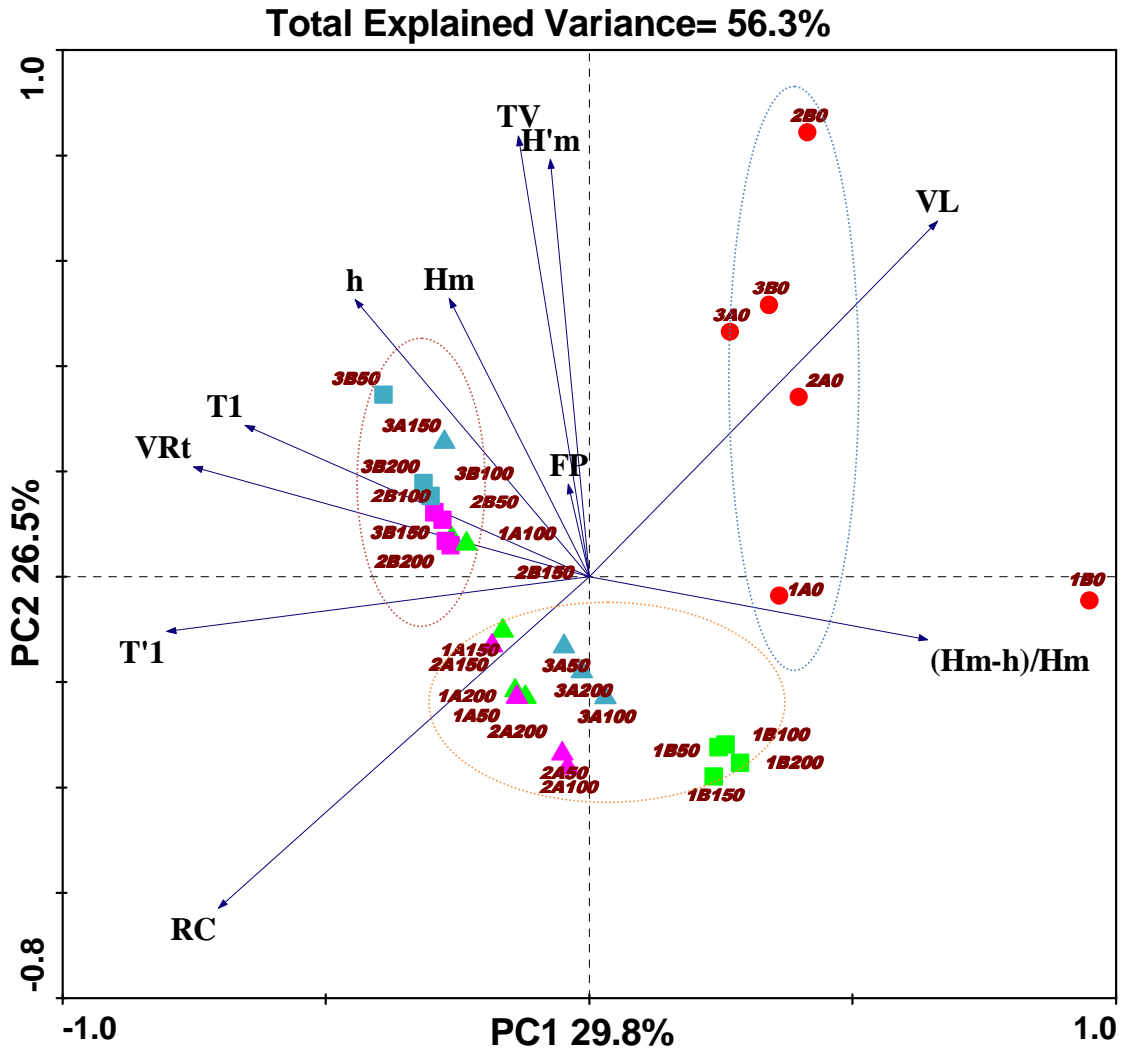


Figure 6. Loading plot of first two principal components based on fermentation properties with flour protein of six commercial wheat flours containing five levels of ascorbic acid. Negative control samples were removed. Symbols and definitions: ● -Control samples, ▲ – Low protein A flours, ▲ -Medium protein A flours, ▲ - High protein A flours. ■- Low protein B flours, ■ - Medium protein B flours, ■ - High protein B flours.

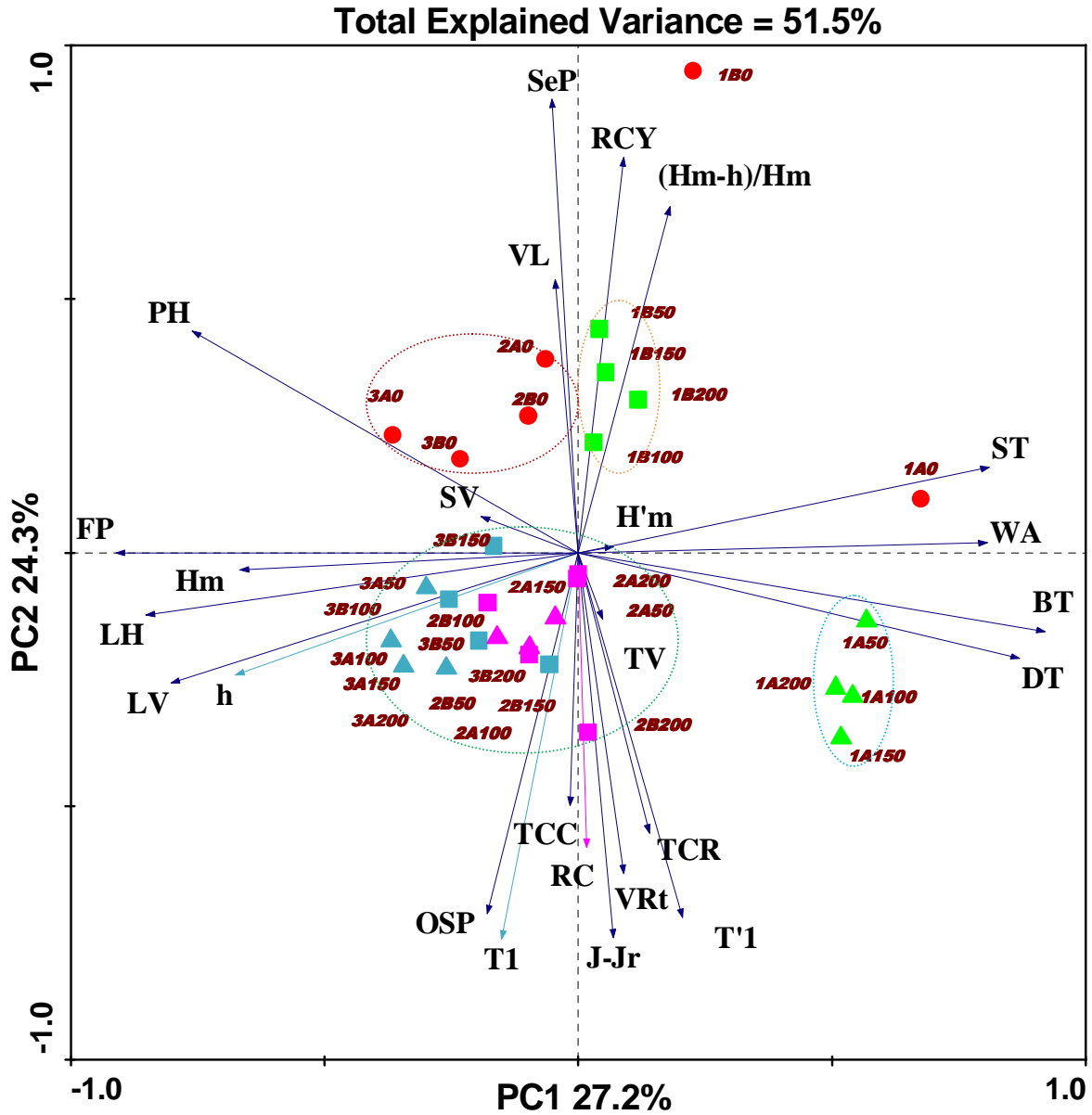


Figure 7. Loading plot of first two principal components based on fermentation, baking, viscoelastic and dough properties of six commercial wheat flours added with five levels of ascorbic acid. Symbols and definitions: ● -Control samples, ▲ - Low protein A flours, ▲ -Medium protein A flours, ▲ - High protein A flours. ■ - Low protein B flours, ■ - Medium protein B flours, ■ - High protein B flours.

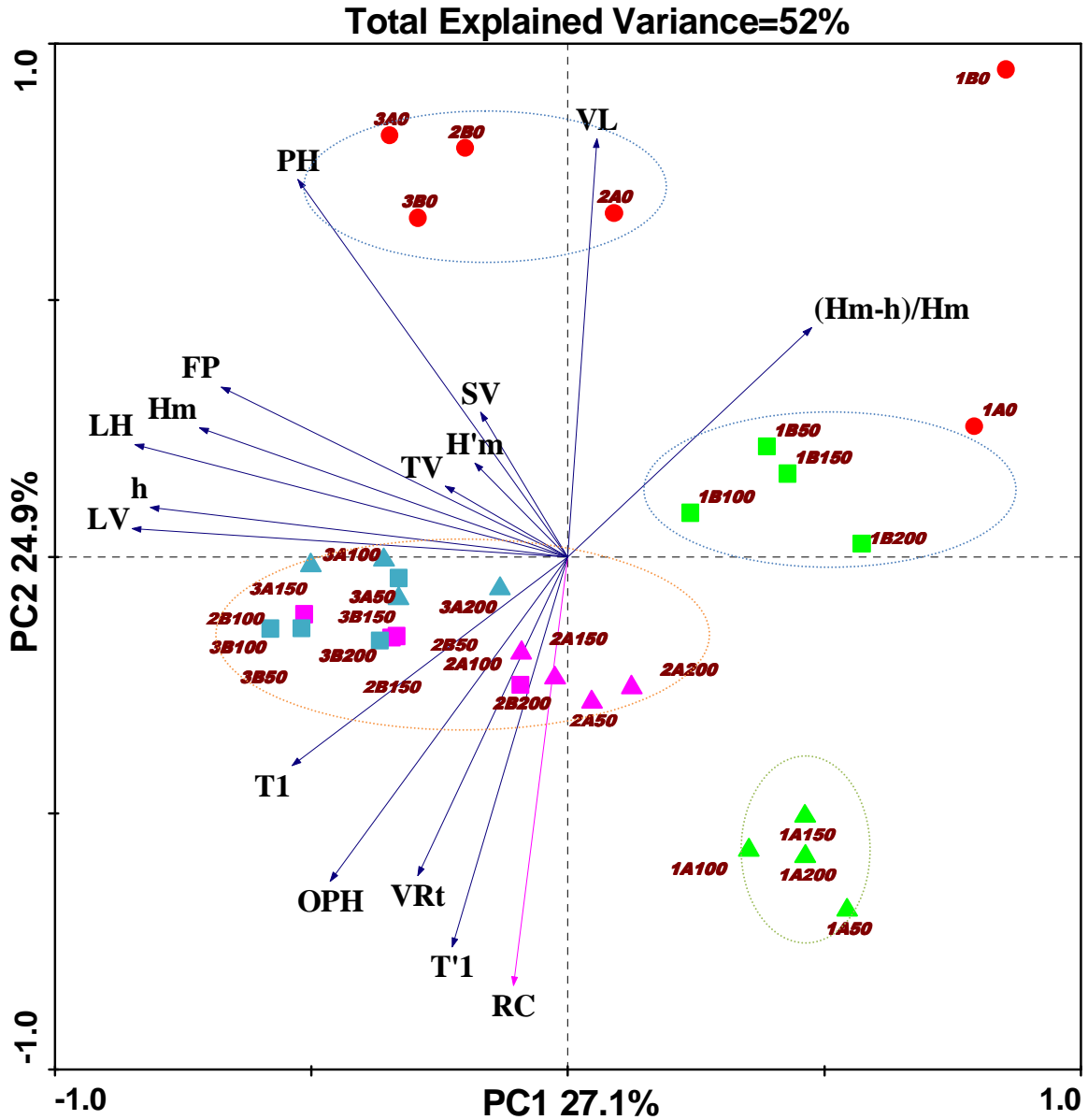


Figure 8. Loading plot of first two principal components based on fermentation and baking properties of six commercial wheat flours added with five levels of ascorbic acid. Symbols and definitions: ● -Control samples,▲ – Low protein A flours, ▲ -Medium protein A flours, ▲ - High protein A flours. ■ – Low protein B flours, ■ - Medium protein B flours, ■ - High protein B flours.

CHAPTER V
EFFECT OF DISRUPTION OF HYDROGEN AND HYDROPHOBIC BONDS ON
FERMENTATION PROPERTIES OF DOUGH

ABSTRACT

The objective of the study is to investigate the effect of the disruption of hydrogen and hydrophobic bonds on fermentation properties of dough and to analyze possible correlation of fermentation and visco-elastic, mixing and baking properties of dough. Disruption of hydrogen and hydrophobic bonds were produced by the addition of four levels of urea (0, 0.5, 1 and 1.5 M). Six commercial hard red winter wheat flours with different protein quantity and quality were used. Flours with no treatment were used as controls and flours with no yeast and no treatment were used as negative controls. Disruption of hydrogen and hydrophobic bonds decreases the height of dough development, maximum height of gaseous release and total volume of gas. Fermentation variables explained more variance (67.7%) than fermentation variables with visco-elastic, mixing and baking variables (53.1%). The ratio of dough heights $[(H_m-h)/H_m]$ is closely related to gluten elastic properties (Sep and RCY). The time taken to reach maximum height of dough development (T1) and time taken to reach maximum height of gaseous release (T'1) are closely related to gluten viscous (TCC and TCR). Total volume (TV) and maximum height of gaseous release (H'm) are closely related to flour protein. Retention coefficient (RC) is negatively related to baking and mixing properties.

1. INTRODUCTION

Gluten plays a key role in determining the baking quality of wheat. The function of gluten depends on the molecular weight of gluten, formation of covalent and non-covalent bonds between glutenin molecules and interactions between glutenin and other flour constituents (Goesacrt et al., 2005). The extractability of gluten proteins decreases during dough fermentation (Graveland et al., 1980; Veraverbeke et al., 1999). Retention of CO₂ and ethanol during fermentation mainly depends on gluten proteins. Loaf volume and crumb structure of bread depends on the amount of gas retained in the dough. Gliadin/glutenin ratio and quality of glutenin fraction are the two main factors that determine gluten protein quality (Goesacrt et al., 2005). Glutenins provides strength and elasticity to the dough due to their large size and monomeric gliadins act as plasticizers. Gluten proteins provide elasticity and plasticity to dough due to the presence of gliadins and glutenins (Goesacrt et al., 2005). The structure of gluten network depends on non-covalent (hydrogen and hydrophobic) bonds as well as disulfide bonds. Hydrogen bonding with water increases by hydration of gluten. When these bonds are disrupted it will affect the fermentation properties of dough. Only few studies investigated the effect of disruption of hydrogen bonds on the fermentation properties of dough. Urea breaks hydrogen bonds and makes dough less stable.

The objectives of the study were:

- 1) To study the effect of disruption of hydrogen and hydrophobic bonds on the fermentation properties of dough using urea.
- 2) To analyze possible correlation of fermentation and flour protein, visco-elastic, baking and mixing properties of dough.

2. Materials and Methods

a. Materials and Labeling

The procurement of wheat flour samples were explained in the Materials and Methods section of Chapter III. Four levels (0, 0.5, 1 and 1.5 M) of urea (VWR International Inc., West Chester, PA) were used. Flours with no urea were used as control (0) and flours with no urea and no yeast were used as negative control (N). Thus site A flours were labeled as 1A0 (positive control), 1AN (negative control), 1A0.5, 1A1, 1A1.5; 2A0, 2A0.5, 2A1, 2A1.5; 3A0, 3A0.5, 3A1 and 3A1.5. Similarly site B flours were labeled as 1B0, 1BN, 1B0.5, 1B1, 1B1.5; 2B0, 2B0.5, 2B1, 2B1.5; 3B0, 3B0.5, 3B1 and 3B1.5.

b. Methods

Dough Preparation

Dough was prepared as described in the Chopin protocol using Chopin AlveoConsistograph. The ingredients consisted of 250 g of flour, 3 g of dry yeast and 5 g of sodium chloride. Urea was added to the flours at 0, 0.5, 1 and 1.5 M concentrations. For 0.5 M urea, a stock solution of 100 ml was prepared containing 3 g of urea. Then 25 ml of stock solution was added to 250 g of flour. In the same way, stock solutions were prepared for 1 M containing 6 g of urea, 9 g of urea for 1.5 M. From the described stock solution, 25 ml was mixed with water added to the flour to obtain each urea addition. The quantity of deionized water added depended on the moisture content of the flour and it was given by the reference table published by the International Association for Cereal Science and Technology (ICC) as described in the Chopin Protocol. The sodium chloride was dissolved in water prior to the addition to dough. Instant dry yeast and urea were blended with 250 g of flour in the kneader bowl. Salt water was progressively added to the flour at the beginning of the first minute of the

mixing period. After one minute, the mixing was stopped to remove the flour sticking to the walls and ensure a homogeneous hydration. The mixing process was continued for 6 minutes. A sample size of 315 g of dough was used for each treatment.

Fermentation Test

Rheofermentometer was used to study the fermentation properties of dough. The dough (315 g) obtained from AlveoConsistograph was placed in the bottom of the aluminum basket and packed it down with hands. The height of the dough in the basket must be leveled out just below the lowest holes. The piston with a 2000 g weight was placed on top of the dough. The basket placed in the F3 Rheofermentometer bowl. Displacement sensor was placed and the whole system was tightly closed and the test was run for a total of 4 h. This time represents 1 h longer than the Chopin Protocol as it was determined experimentally with the samples and treatments in this study.

The F3 Rheofermentometer analyzes the development of a dough sample placed in the bowl. The piston placed on the dough rises. The piston is directly linked to a displacement sensor which will calculate the dough rising. Rheofermentometer is also linked to a pressure sensor through a pneumatic circuit that measures the pressure increase in the fermenting dough. The three curves are dough development, speed of CO₂ release and quantity produced and volume of CO₂ retained in dough. Fermentation variables are defined in Table 1 and visco-elastic, mixing and baking terms are defined in Table 2 (Chapter III).

3. STATISTICAL ANALYSIS

A factorial design within a randomized block design was implemented. Five levels of urea and 3 levels of flour protein were compared in a 5 X 3 factorial. The significant differences in means were compared using Analysis of Variance (ANOVA) with Tukey's comparisons ($\alpha=0.05$) using SAS (Version 9.1 SAS Institute Inc., Cary, NC). Principal Component Analysis (PCA) is a mathematical algorithm that reduces the dimensionality of the data (Ringer, 2008). PCA is performed using Canoco for windows 4.5 (Biometris, Plant Research International, Wageningen, the Netherlands).

4. Results and Discussion

Protein, moisture and ash content of the flour samples and water added are reported in Table 3 (Chapter III). Typical dough fermentation property curves obtained are illustrated in Figures 1 and 2 showing results for sample 3B control (a) and 3B with containing 1 M urea (b). The volume of CO₂ lost (VL) is decreased in sample in which the hydrogen and hydrophobic bonds are disrupted (Fig. 1b). The volume of retention of gas was improved for sample in which hydrogen and hydrophobic bonds are disrupted when compared with control sample. From the dough development curves we can observe that height of dough is improved when the hydrogen and hydrophobic bonds are disrupted (Fig. 2). A summary of the fermentation properties of all samples is found in Table 1 (Chapter III).

Maximum height of the dough (Hm)

Hm is the maximum height of the dough development. As expected control sample without yeast shows no development (Table 1). The overall trend of disrupting hydrogen and hydrophobic bonds is a decrease on Hm. Within each specific flour group and levels of urea,

3A0 and 3A0.5 are the only two comparisons that were statistically different (Table 1). Flour 3A with higher protein (13.7%) shows 14.7% decrease of Hm with 0.5 M urea treatment (Table 2). Overall highlights are: high value of Hm was shown by 2B0 (43.3 mm) and lowest by 1A1 (24.5 mm) (Table 1). The change of the fermentation properties (%) is reported in Table 2. High percentage (5.2%) increment in maximum height was observed in the highest protein sample with 1.5 M urea (3B1.5). A 14.7% decrease in maximum height was observed in the sample 3A with 0.5 M of urea (3A0.5) (Table 2). This suggests that the effect of disruption of hydrogen and hydrophobic bonds on maximum height of dough development is to decrease it by making the dough more viscous. Although 1A1.5 and 3B1.5 show a modest increase in Hm, these are not significantly different.

Height of the dough development (h)

The height of the dough development (mm) at the end of the test was denoted by h. As expected, negative controls showed no development. Comparing the effect of decreasing hydrogen and hydrophobic bonds within sample reveals only two significantly different observations: 3A0 vs. 3A0.5 (39.2 vs. 33.1 mm) and 2B0 vs. 2B1 (47.8 vs. 36.5 mm) (Table 1). The overall trend of the decrease of hydrogen bonds is to decrease h, which is a similar observation as with Hm (Table 1 and 2). Overall highlights are: lowest value of height was observed in 1B0 (19.85 mm) and highest value was observed in 2B0 (42.75 mm) (Table 1). An apparent increase (12.3%) of h but not significant was observed in 1B0.5 and highest (18.2%) decreased (significant) in 2B1.5 (Table 2). This suggests that the effect of disruption hydrogen and hydrophobic bonds on h is not significant in most samples.

Lowering of development percentage [(Hm-h)/Hm]

(Hm-h)/Hm is the ratio of dough height at the end of the fermentation test in percentage. A large percent means the dough has maintained its height during fermentation. Comparing the ratio of dough height within samples, only in two samples 1B and 2B significant changes in ratio of dough were observed as the hydrogen and hydrophobic bonds are disrupted (Table 1). In sample 1B, all the urea levels lowered significantly the ratio of dough height compared to the control. While in sample 2B, 1.5 M urea increased the ratio of dough height compared to control. This apparently contradicted effect could be explained in part by different hydrophobic domains of the gluten proteins of these samples. In sample 2B, a trend is observed to an increase in ratio of dough height with lower urea levels. This suggests that the nature of the flour is more hydrophobic than 1B in which urea causes a decrease of this ratio. Overall highlights are: high value was observed in 1B0 (39.1%) and lowest value in 1A0.5 (0.4%) (Table 1). Highest percentage increase was observed in 2B1.5 (1044%) and lowest percentage decrease was observed in 3A1 (100%) (Table 2). High protein B flours show no change. Medium protein A and B flours show greater increment whereas others decreased.

Maximum height of the gaseous curve (H'm)

H'm is the maximum height of the gaseous release curve. The effect of decreasing hydrogen and hydrophobic bonds caused significant decrease of H'm (Table 1 and 2). Overall highlights are: high value of H'm was shown by 2B0 (61.2 mm) and lowest value was shown by 1B1.5 (37.15mm) (Table 1). Highest percentage (37.6%) decrease in maximum height was observed in 1B1.5 and 2 % decrease in 1A0.5 (Table 2). Maximum height of gaseous release is decreased for all samples by treating with urea (Table 1 and 2).

Total Volume (TV)

TV is the total volume under the gaseous curve. The effect of decreasing hydrogen and hydrophobic bonds causes a decrease in total volume in all samples ($P < 0.05$) (Table 1 and 2). This has similar effect as the observed on Hm. This suggests that hydrogen and hydrophobic bonds are important in forming the fermented dough structure impermeable to gas loss. Overall highlights are: high value of total volume was observed in 2B0 (1911 mL) and lowest in 1B1.5 (1084.5 mL) (Table 1). Highest decrease in total volume (30.8%) was observed in 2B1 and lowest decrease (3.1%) of total volume in 1A0.5 (Table 2).

Volume lost (VL)

VL is the carbon dioxide volume released by the dough during the fermentation test. The effect of decreasing hydrogen and hydrophobic bonds is to decrease the volume lost (Table 1 and 2). Volume lost has to be related to the total volume produced which was lowered by 11.7 to 32.6%. From this lowered volume produced, decreasing hydrogen and hydrophobic bonds lowered the volume lost significantly from 5 to 10%. Overall highlights are: high value was observed in 2B0 (567 mL) and lowest in 1A1.5 (22 mL) (Table 1). Highest percentage (10.5%) of volume lost (less desirable) was observed in 1B0.5 and lowest percentage (5.2) in 3B1 (Table 2).

Volume retained (VRt)

VRt is the carbon dioxide remaining in the dough at the end of the test. Only two samples showed significant differences on volume retention compared to the control. Flour 1A with 0.5 and 1.0 M urea increase volume retention significantly compared to control. Flour 2A with 1.5 M urea decreases volume retention significantly compared to the control (Table 1 and 2). Overall highlights are: high value was observed in 1A0.5 (1437 mL) and lowest in 1B1.5 (1058 mL)

(Table 1). Highest percentage (21.3%) of volume retained was observed in 1A0.5 and VRt is decreased to 12.9% in 2A1.5 (Table 2). Half of the samples decreased the retention of volume gas by disruption of hydrogen and hydrophobic bonds whereas the other half increased the retention volume of gas. This suggests that the samples and treatments have produced matrices with different retention volume gas characteristics.

Retention Coefficient (RC)

Retention coefficient (RC) is the retention volume divided by the total gaseous release. Retention coefficient of all samples and treatments were significantly increased by the decrease in hydrogen and hydrophobic bonds. This suggests that the disruption of these bonds positively contribute to the retention coefficient. One has to be careful interpreting these results without cross referencing the effect on total volume. Total volume decreased significantly all samples and levels of urea. Overall highlights are: high value was observed in 1A0.5, 1A1, 1A1.5, 2A0.5, 2A1, 3A0.5, 3A1 and 3B1 (98%) and lowest value in 2B0 (70%) (Table 1). Highest percentage (38%) of retention coefficient was observed in medium protein B flours (2B0.5, 2B1 and 2B1.5) and lowest percentage (21%) in low protein B flours (1B0.5, 1B1 and 1B1.5) (Table 2). In summary, the effect of disruption of hydrogen and hydrophobic bonds on retention coefficient is to increase.

Time of maximum rise (T1)

T1 is the time taken by the dough to reach maximum height during dough development. Even though the effect of disruption of hydrogen and hydrophobic bonds on T1 appears to be flour specific, they were not significantly different (Table 1 and 2). Overall highlights are: high value was observed in 3A1, 1B1, 3B0 and 3B1 (4 h) and lowest in 1B0 (1.6 h) (Table 1). Highest percentage (16.5%) of time taken to reach maximum height of dough development was observed

in 1B1.5 and time decreased 27.1% in 2B1.5 (Table 2). Time of maximum rise is increased only with low protein A and B flours whereas it decreased with other flours.

Time of maximum rise (T'1)

T'1 is the time spent to reach maximum rise during gaseous release. Observations that significantly increased T'1 were 2B with all levels of urea and 3B with 0.5 M of urea. Only one observation showed significantly decrease on T'1 is 1A with 1.5 M of urea. Overall highlights are: high value was observed in 1A0.5, 2B0.5, 2B1 and 3B0.5 (3.9) and lowest (1.3) in 2A0.5, 1B1 and 2B0 (Table 1). Highest increase (200%) of time taken to reach maximum height of gaseous curve was observed in 2B0.5 and 2B1 and time decreased to 51.6% in 1A1.5 (Table 2). Overall, the effect of disruption of hydrogen and hydrophobic bonds on time of maximum rise shows mostly an increase.

Inda et al., (1991) reported that elasticity of dough decreased when it is treated with 0 to 3 M concentration of urea. McGrane et al., (2004) reported that urea reduces the gel strength by decreasing the intermolecular network formation between water and amylase. Our study shows that dough treated with 0 to 1.5 M of urea reduces the fermentation properties of dough and confirms the important contribution of hydrogen and hydrophobic bonds in the structure that retains the gas produced during fermentation.

PCA results

Principal component analyses were performed on the data sets obtained from fermentation parameters.

Fermentation variables with and without flour protein

PCA were performed on the data sets to assess the relationship of flour protein and fermentation properties (Fig. 3 and 4). Figure 3 represents the fermentation properties alone and

all the samples and urea treatments. Principal component axis 1 (PC1) explained 55.2% variance and principal component axis 2 (PC2) explained 19.8% variance. Total explained variance is 77% (Table 3). Among fermentation properties, the highest contribution of variance was total volume (TV) (95.8%) and volume of retention (VRt) (95%) in PC1 whereas in PC2 highest contribution of variance (64.6%) was retention coefficient (RC) (Table 3). Figure 4 displays the fermentation properties plus flour protein. Principal component axis 1 (PC1) explained 50.2% variance and principal component axis 2 (PC2) explained 18.1% variance. Total explained variance is 68.3% (Table 4). Among fermentation properties with flour protein, the highest contribution of variance (95.7%) was total volume (TV) on PC1 whereas on PC2 the highest contribution of variance (61.5%) was retention coefficient (RC) (Table 4). Only 0.3% of explained variance was contributed by flour protein on PC1 and 3.7% on PC2 (Table 4). In both graphs (Fig. 3 and 4), most of the fermentation variables are on PC1. Flours treated with urea are very close to PC1 when compared with control and negative samples. All control samples are closely related among themselves and to volume lost. They are well separated from the treated flours. Negative controls are also closely related among themselves and well separated from the samples with changes due to urea. So negative controls are removed from the data sets and PCA was compared. The results suggest that the urea samples are closely related to volume of the gas retained by the dough during fermentation in the first component. These samples are negatively related to volume lost.

Fermentation variables without negative control Vs fermentation variables with flour protein and without negative control

PCA were performed to assess the relationship of fermentation variables and protein without the negative controls (Fig. 5 and 6). From the fermentation properties on Figure 5,

principal component axis 1 (PC1) explained 43.3% variance and principal component axis 2 (PC2) explained 24.4% variance (Fig. 5). Total explained variance is 67.7% (Table 5). Among fermentation properties, the highest contribution of variance (73.8%) was volume lost (VL) on PC1 whereas on PC2 the highest contribution of variance (58.3%) was lowering development percentage ($[Hm-h]/Hm$) (Table 5). In comparison, when flour protein was included (Figure 6), principal component axis 1(PC1) explained 39.8% variance and principal component axis 2 (PC2) explained 22.5% variance. Total explained variance is 62.3% (Table 6). Among fermentation properties with flour protein, the highest contribution of variance (89.9%) was total volume (TV) on PC1 whereas on PC2 the highest contribution of variance (52.5%) was lowering development percentage ($[Hm-h]/Hm$) (Table 6). Only 6.8% of explained variance was contributed by flour protein on PC1 and 8.6% on PC2 (Table 6). As the total explained variance of fermentation variables (67.7%) is 5.4 units of percentage higher than fermentation variables with flour protein (62.3%), we can say that compared to changes in hydrogen and hydrophobic bonds in this set, flour protein appears to have a small effect and is marginally correlated to other fermentation variables. Controls are closely related to volume lost (VL) and are separated from the flours. Low protein B flours which are treated with urea are separated and closely related to lowering development percentage ($[Hm-h]/Hm$). They are negatively correlated to flour protein (FP) and volume of gas retained (VRt). By disruption of hydrogen bonds, this group of samples have high values of ($[Hm-h]/Hm$) but they have lower volume retained to begin with.

Fermentation properties Vs Fermentation properties with visco-elastic, mixing, baking properties

The relationship of fermentation variables with visco-elastic, mixing and baking properties was investigated (Fig. 7). From Figure 6, principal component axis 1 (PC1) explained

39.8% variance and principal component axis 2 (PC2) explained 22.5% variance. Total explained variance is 62.3% (Table 6). From Figure 7, principal component axis 1(PC1) explained 30.5% variance and principal component axis 2 (PC2) explained 22.6% variance. Total explained variance is 53.1% (Table 7). Among all properties, the highest contribution of variance (81.5%) was flour protein (FP) and second major component that contributes high variance (78.3%) is specific volume (SV) on PC1 whereas on PC2 the highest contribution of variance (82.9%) was separation time (SeP) (Table 7). As the total explained variance of fermentation variables (62.3%) is higher than fermentation variables with visco-elastic, mixing and baking variables (53.1%), we can say that there are more differences in fermentation variables compared to the combination of all the variables. In other words, fermentation properties separated the properties of these samples and treatment more efficiently. In Fig 7, all variables are closely associated. Low protein B flours are separated from other flours. The ratio of dough heights $[(H_m-h)/H_m]$ is closely related to elastic properties (Sep and RCY). Time taken to reach maximum height of dough development (T1) and time taken to reach maximum height of gaseous release (T'1) are closely related to gluten viscous (TCC and TCR). Total volume (TV) and maximum height of gaseous release (H'm) are closely related to flour protein. Retention coefficient (RC) is negatively related to baking and mixing properties. All control samples are well separated and are negatively correlated to lowering development percentage $[(H_m-h)/H_m]$. PCA analysis is performed on flour protein, fermentation properties and baking properties.

Relationship of flour protein, fermentation and baking properties

PCA were performed on the data sets of flour protein, fermentation variables and baking properties (Fig. 8). PCA analyses of fermentation properties with flour protein were already

performed (Fig. 6 and Table 6). From Figure 6, principal component axis 1 (PC1) explained 39.8% variance and principal component axis 2 (PC2) explained 22.5% variance. Total explained variance is 62.3% (Table 6). From Figure 8, principal component axis 1 (PC1) explained 40.8% variance and principal component axis 2 (PC2) explained 15.4% variance. Total explained variance is 56.3% (Table 8). The highest contribution of explained variance (81.5%) was loaf volume (LV) and second highest variance (81%) was explained by specific volume (SV) on PC1 whereas on PC2 the highest contribution of variance (60.4%) was lowering development percentage ($[(Hm-h)/Hm]$) (Table 8). Flour protein explained 18.2% variance on PC1 and 3% on PC2 (Table 8). As the total explained variance of fermentation variables (62.3%) is higher than fermentation variables with baking variables (56.3%), we can say fermentation properties explain more variance than baking properties. All control samples were negatively associated with retention coefficient (RC) and positively related with volume lost (VL) and loaf volume (LH). Low protein flours are separated from the rest of the flours.

5. Conclusions

Null Hypothesis is rejected as there is significant effect of disruption of hydrogen and hydrophobic bonds (addition of urea) on fermentation properties when compared to the control samples. The effect of disruption of hydrogen and hydrophobic bonds decreased maximum height of gaseous release, total volume of gas and volume lost.

Fermentation variables explained more variance (67.7%) than fermentation variables with visco-elastic, mixing and baking variables (53.1%). The ratio of dough heights $[(Hm-h)/Hm]$ is closely related to gluten elastic properties (Sep and RCY). Time taken to reach maximum height of dough development (T1) and time taken to reach maximum height of gaseous release (T'1) are closely related to viscous properties (TCC and TCR). Total volume (TV) and maximum

height of gaseous release (H'm) are closely related to flour protein. Retention coefficient (RC) is negatively related to baking and mixing properties.

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Table 1. Fermentation properties in six commercial wheat flours treated with urea levels. Means (n=2) with same superscripts in a column are not significantly different (P > 0.05). The standard deviations of means are shown in parentheses.

TRT	Fermentation Properties									
	Hm (mm)	h (mm)	(Hm-h) /Hm (%)	H'm (mm)	TV (mL)	VL (mL)	VRt (mL)	RC (%)	T1 (h)	T'1 (h)
1A0	25 ^{klm} (1.3)	24.55 ^{klmno} (1.35)	1.8 ^{ghi} (0.3)	47.5 ^{defghi} (2.5)	1507.5 ^{defgh} (76.5)	323 ^{de} (37)	1184.5 ^{hijk} (39.5)	78.65 ^{cdef} (1.35)	3.93 ^a (0.0)	3.1 ^{abcd} (0.2)
1AN	0 ⁿ (0)	0 ^p (0)	0 ⁱ (0)	5 ^q (0.1)	12.5 ^r (0.5)	2 ^s (0)	11 ^l (0)	83.25 ^{bcd} (2.05)	4 ^a (0.0)	0.1 ^j (0.0)
1A0.5	24.9 ^{lm} (0.2)	24.8 ^{klmno} (0.1)	0.4 ⁱ (0.4)	46.55 ^{defghij} (0.05)	1461.5 ^{efghij} (1.5)	25.5 ^{fg} (0.5)	1437 ^a (1)	98.3 ^a (0)	3.97 ^a (0.0)	3.9 ^a (0.0)
1A1	24.5 ^m (1.1)	24.2 ^{klmno} (1.2)	1.25 ^{ghi} (0.45)	43.7 ^{ijklmn} (1.5)	1382.5 ^{ghijklm} (58.5)	23 ^{fg} (7)	1360 ^{abcde} (52)	98.35 ^a (0.45)	3.96 ^a (0.0)	2.8 ^{bcdef} (1.2)
1A1.5	25.8 ^{ijklm} (0.2)	25.2 ^{ijklmn} (0.6)	2.35 ^{ghi} (1.55)	40.9 ^{mnop} (1)	1274.5 ^{lmnop} (22.5)	22 ^{fg} (5)	1252 ^{efghij} (17)	98.3 ^a (0.4)	3.95 ^a (0.0)	1.5 ⁱ (0.1)
2A0	32.15 ^{efghi} (0.55)	31.45 ^{fghi} (1.15)	2.2 ^{ghi} (1.9)	53.1 ^c (1)	1695.5 ^c (27.5)	389.5 ^c (11.5)	1306 ^{abcdefgh} (16)	77.05 ^{ef} (0.35)	3.67 ^{abc} (0.3)	1.5 ⁱ (0.2)
2AN	0 ⁿ (0)	0 ^p (0)	0 ⁱ (0)	4.9 ^q (1.3)	12 ^f (3)	3.5 ^g (0.5)	9 ^l (3)	70.2 ^g (6)	4 ^a (0.0)	0.1 ^j (0.0)
2A0.5	30.35 ^{fghij} (0.75)	28.95 ^{hijk} (0.65)	4.5 ^{fghi} (4.5)	46.25 ^{efghijk} (0.15)	1449.5 ^{efghij} (28.5)	29 ^{fg} (5)	1421 ^{abc} (23)	98 ^a (0.3)	2.88 ^{abcdef} (1.1)	1.3 ⁱ (0.1)
2A1	30.4 ^{fghij} (1)	29.25 ^{ghijk} (0.35)	3.7 ^{ghi} (2)	43.95 ^{hijklmn} (0.05)	1322.5 ^{ijklmno} (3.5)	23.5 ^{fg} (4.5)	1299 ^{bcdefgh} (1)	98.25 ^a (0.35)	2.98 ^{abcde} (0.5)	1.5 ⁱ (0.0)
2A1.5	29.75 ^{ghijkl} (0.25)	27.55 ^{ijk} (0.25)	7.4 ^{efghi} (1.6)	38.7 ^{op} (2.1)	1162 ^{pq} (60)	24 ^{fg} (3)	1138 ^{ijk} (57)	97.95 ^a (0.15)	2.69 ^{bcdefg} (0.0)	1.5 ⁱ (0.1)
3A0	39.4 ^{abcd} (2.2)	39.15 ^{abcd} (2.45)	0.65 ⁱ (0.65)	54.3 ^{bc} (0.1)	1669 ^c (13)	362.5 ^{cd} (13.5)	1307 ^{abcdefgh} (0)	78.3 ^{def} (0.6)	3.98 ^a (0.0)	1.6 ^{hi} (0.0)
3AN	0 ⁿ (0)	0 ^p (0)	0 ⁱ (0)	4.35 ^q (0.75)	17.5 ^r (3.5)	2.5 ^g (0.5)	15.5 ^l (4.5)	85.5 ^b (6.5)	4 ^a (0.0)	0.1 ^j (0.0)
3A0.5	33.6 ^{efgh} (1.3)	33.1 ^{efgh} (1.3)	1.45 ^{ghi} (0.05)	44.25 ^{fghijklmn} (1.25)	1361.5 ^{ijklmn} (43.5)	24.5 ^{fg} (5.5)	1336.5 ^{abcdefg} (37.5)	98.2 ^a (0.4)	3.83 ^{ab} (0.1)	1.6 ^{ghi} (0.1)
3A1	35.75 ^{cde} (0.65)	35.75 ^{bcdef} (0.65)	0 ⁱ (0)	44.3 ^{fghijklmn} (0)	1327.5 ^{ijklmno} (9.5)	24 ^{fg} (3)	1303.5 ^{bcdefgh} (6.5)	98.15 ^a (0.25)	4 ^a (0.0)	1.7 ^{ghi} (0.1)
3A1.5	37.1 ^{bcde} (3)	36.7 ^{bcde} (3)	1.1 ^{hi} (0.1)	41.15 ^{lmnop} (0.45)	1237 ^{nop} (4)	26.5 ^{fg} (0.5)	1210.5 ^{ghij} (4.5)	97.85 ^a (0.05)	3.56 ^{abc} (0.4)	1.8 ^{fghi} (0.0)

Table 1. Continued

TRT	Fermentation Properties									
	Hm (mm)	h (mm)	(Hm-h)/ Hm (%)	H'm (mm)	TV (mL)	VL (mL)	VRt (mL)	RC (%)	T1 (h)	T'1 (h)
1B0	33.2 ^{efghi} (2.3)	19.85 ^o (3.75)	39.1 ^a (15.5)	59.55 ^a (1.75)	1412 ^{fghijkl} (32)	277 ^e (15)	1135 ^{jk} (17)	80.4 ^{bcde} (0.6)	1.58 ^g (0.2)	1.4 ⁱ (0.0)
1BN	0 ⁿ (0)	0 ^p (0)	0 ⁱ (0)	5.5 ^q (1.8)	18.5 ^r (6.5)	3 ^g (2)	15.5 ^l (4.5)	83.35 ^{bcd} (4.65)	4 ^a (0.0)	0.1 ^j (0.0)
1B0.5	28.35 ^{ijklm} (1.35)	22.3 ^{lmno} (0.2)	21.15 ^{bc} (3.05)	42.2 ^{ijklmno} (1.2)	1247.5 ^{mnpq} (29.5)	29.5 ^{fg} (8.5)	1217.5 ^{fghij} (20.5)	97.65 ^a (0.65)	1.72 ^{fg} (0.1)	1.4 ⁱ (0.0)
1B1	30 ^{fghijk} (0)	21.85 ^{mno} (0.45)	27.2 ^b (1.5)	39.8 ^{nop} (0.1)	1163 ^{pq} (2)	27 ^{fg} (5)	1136 ^{jk} (7)	97.65 ^a (0.45)	1.82 ^{efg} (0.0)	1.3 ⁱ (0.0)
1B1.5	28.55 ^{hijklm} (0.35)	20.25 ^{no} (0.15)	29.05 ^b (1.35)	37.15 ^p (0.55)	1084.5 ^q (13.5)	26.5 ^{fg} (0.5)	1058 ^k (14)	97.55 ^a (0.05)	1.84 ^{efg} (0.0)	1.5 ⁱ (0.0)
2B0	43.3 ^a (0.5)	42.75 ^a (0.05)	1.25 ^{ghi} (1.05)	61.15 ^a (4.15)	1911.5 ^a (113.5)	567 ^a (102)	1344.5 ^{abcdef} (11.5)	70.55 ^g (3.55)	3.40 ^{abc} (0.5)	1.3 ⁱ (0.1)
2BN	0 ⁿ (0)	0 ^p (0)	0 ⁱ (0)	5.2 ^q (0)	23 ^r (0)	1 ^g (0)	21 ^l (0)	94.6 ^a (0)	4 ^a (0.0)	0.1 ^j (0.0)
2B0.5	39.6 ^{abcd} (0.9)	37.9 ^{abcde} (0.4)	4.25 ^{fghi} (1.15)	42.7 ^{ijklmno} (0.2)	1345.5 ^{ijklmno} (4.5)	35.5 ^{fg} (4.5)	1309.5 ^{abcdefgh} (0.5)	97.35 ^a (0.35)	2.50 ^{cdefg} (0.2)	3.9 ^a (0.1)
2B1	40.85 ^{abc} (0.55)	36.55 ^{bcde} (0.75)	10.55 ^{defg} (0.65)	41.75 ^{klmno} (0.35)	1323.5 ^{ijklmno} (21.5)	34 ^{fg} (2)	1289 ^{cdefgh} (20)	97.4 ^a (0.1)	2.69 ^{bcdefg} (0.0)	3.9 ^a (0.0)
2B1.5	40.8 ^{abc} (2)	34.95 ^{cdef} (1.15)	14.3 ^{cde} (1.4)	40.9 ^{mnpq} (1.3)	1288 ^{klmnop} (40)	32.5 ^{fg} (6.5)	1255.5 ^{efghij} (33.5)	97.5 ^a (0.4)	2.48 ^{cdefg} (0.0)	2.7 ^{cdefg} (1.3)
3B0	36.85 ^{bcde} (0.75)	36.85 ^{bcde} (0.75)	0 ⁱ (0)	54.2 ^{bc} (1.1)	1737 ^{bc} (46)	462.5 ^b (20.5)	1275 ^{defgh} (25)	73.4 ^{fg} (0.5)	4 ^a (0.0)	2.0 ^{efghi} (1.0)
3BN	0 ⁿ (0)	0 ^p (0)	0 ⁱ (0)	4.9 ^q (0.6)	14 ^r (1)	2.5 ^g (0.5)	12 ^l (1)	84 ^{bc} (1.2)	2.10 ^{defg} (1.9)	0.1 ^j (0.0)
3B0.5	36.2 ^{cde} (3.9)	34.7 ^{cdef} (2.7)	3.8 ^{ghi} (2.9)	44.05 ^{ghijklmn} (1.95)	1367 ^{hijklmn} (50)	32 ^{fg} (9)	1335 ^{abcdefg} (41)	97.7 ^a (0.6)	3.11 ^{abcd} (0.7)	3.9 ^a (0.0)
3B1	34.9 ^{def} (2.3)	34.1 ^{defg} (2.6)	2.35 ^{ghi} (1.05)	41 ^{mnpq} (0.5)	1280 ^{lmnop} (4)	24 ^{fg} (4)	1255.5 ^{efghij} (0.5)	98.1 ^a (0.3)	3.88 ^{ab} (0.1)	2.6 ^{defgh} (1.3)
3B1.5	38.75 ^{abcd} (0.25)	38.1 ^{abcde} (0)	1.65 ^{ghi} (0.65)	38.75 ^{op} (1.95)	1209.5 ^{opq} (47.5)	24.5 ^{fg} (2.5)	1185 ^{hijk} (50)	97.95 ^a (1)	3.22 ^{abcd} (0.0)	1.4 ⁱ (0.1)

Table 2. Change (percent) of fermentation properties of six commercial wheat flours treated with urea levels. Values with * are significantly different (P<0.05) when compared to control samples.

TRT	Fermentation properties									
	Hm (%)	h (%)	(Hm-h)/Hm (%)	H'm (%)	TV (%)	VL (%)	VRt (%)	RC (%)	T1 (%)	T'1 (%)
1A0.5	-0.4	1.0	-77.8	-2.0	-3.1	-7.9*	21.3*	25.0*	1.0	25.8
1A1	-2.0	-1.4	-30.6	-8.0	-8.3	-7.1*	14.8*	25.0*	0.8	-9.7
1A1.5	3.2	2.6	30.6	-13.9*	-15.5*	-6.88	5.7	25.0*	0.5	-51.6*
2A0.5	-5.6	-7.9	104.5	-12.9*	-14.5*	-7.4*	8.8	27.2*	-21.5	-13.3
2A1	-5.1	-7.0	68.2	-17.2*	-22.0*	-6.0*	-0.5	27.5*	-18.8	0.0
2A1.5	-7.5	-12.4	236.4	-27.1*	-31.5*	-6.2*	-12.9*	27.1*	-26.7	0.0
3A0.5	-14.7*	-15.5*	123.1	-18.5*	-18.4*	-6.8*	2.3	25.4*	-3.8	0.0
3A1	-9.3	-8.7	-100.0	-18.4*	-20.5*	-6.6*	-0.3	25.4*	0.5	6.2
3A1.5	-5.8	-6.3	69.2	-24.2*	-25.9*	-7.3*	-7.4	25.0*	-10.6	12.5
1B0.5	-14.6	12.3	-45.9*	-29.1*	-11.7*	-10.6*	7.27	21.5*	8.9	0.0
1B1	-9.6	10.1	-30.4*	-33.2*	-17.6*	-9.7*	0.09	21.5*	15.2	-7.1
1B1.5	-14.0	2.0	-25.7*	-37.6*	-23.2*	-9.6*	-6.78	21.3*	16.5	7.1
2B0.5	-8.5	-11.3	240.0	-30.2*	-29.6*	-6.3*	-2.60	38.0*	-26.5	200.0*
2B1	-5.7	-14.5*	744.0	-31.7*	-30.8*	-6.0*	-4.13	38.1*	-20.9	200.0*
2B1.5	-5.8	-18.2	1044*	-33.1*	-32.6*	-5.7*	-6.62	38.2*	-27.1	107.7*
3B0.5	-1.8	-5.8	0.0	-18.7*	-21.3*	-6.9*	4.71	33.1*	-22.3	95.0*
3B1	-5.3	-7.5	0.0	-24.4*	-26.3*	-5.2*	-1.53	33.7*	-3.0	30.0
3B1.5	5.2	3.4	0.0	-28.5*	-30.4*	-5.3*	-7.06	33.4*	-19.5	-30.0

Percentage calculated from values in Table 1 and % change = (Sample treated with additive-Control sample)/control sample * 100.

Table 3. Explained variance (%) in PCA of fermentation variables with negative control in flours treated with urea.

UREA	AXES	PC1	PC2	1+2
	PC (%)	55.2%	19.8%	75%
Fermentation	Hm	94	0	94
	h	89	1	90
	(Hm-h)/Hm	5	30	35
	H'm	94	1	95
	TV	96	2	98
	VL	19	59	78
	VRT	95	1	96
	RC	5	65	70
	T1	4	38	42
	T'1	51	2	53

Table 4. Explained variance (%) in PCA of fermentation variables and flour protein in flours treated with urea.

UREA	AXES	PC1	PC2	1+2
	PC (%)	50.2%	18.1%	68.31%
Fermentation	Hm	94	0	94
	h	89	1	90
	(Hm-h)/Hm	5	32	38
	H'm	94	1	95
	TV	96	2	98
	VL	19	56	74
	VRT	95	1	96
	RC	5	61	67
	T1	4	39	43
	T'1	50	3	53
Flour Protein	FP	0	4	4

Table 5. Explained variance (%) in PCA of fermentation variables without negative control in flours treated with urea.

UREA	AXES	PC1	PC2	1+2
	PC (%)	43.3%	24.4%	67.7%
Fermentation	Hm	20	1	21
	h	37	22	60
	(Hm-h)/Hm	23	58	81
	H'm	70	18	88
	TV	93	1	94
	VL	74	22	96
	VRT	25	35	60
	RC	68	26	94
	T1	23	34	57
	T'1	0	26	27

Table 6. Explained variance (%) in PCA of fermentation variables with flour protein and without negative control in flours treated with urea.

UREA	AXES	PC1	PC2	1+2
	PC (%)	39.8%	22.5%	62.3%
Fermentation	Hm	24	4	29
	h	42	30	72
	(Hm-h)/Hm	24	52	76
	H'm	67	21	89
	TV	90	3	93
	VL	71	25	96
	VRT	24	28	52
	RC	65	29	95
	T1	23	27	50
	T'1	0	19	19
Flour Protein	FP	7	9	15

Table 7. Explained variance (%) in PCA of fermentation variables when compared with visco-elastic, mixing and baking variables in flours treated with urea. Definitions of fermentation, visco-elastic, mixing and baking variables explained in Table 2 (Chapter III).

UREA	AXES	PC1	PC2	1+2
	PC (%)	30.5%	22.5%	53%
Fermentation	Hm	26	5	30
	h	33	30	63
	(Hm-h)/Hm	8	54	62
	H'm	38	1	39
	TV	39	3	42
	VL	31	0	31
	VRT	10	18	28
	RC	29	0	29
	T1	0	27	27
	T'1	6	24	31
Visco-elastic	SeP	0	83	83
	J-J _r	3	76	79
	RCY	4	48	52
	TCR	8	53	62
	TCC	7	57	63
Mixing	WA	30	0	30
	DT	30	16	46
	ST	50	11	61
	BT	41	16	56
Baking	PH	45	15	60
	LH	71	11	82
	SV	78	7	86
	OSP	40	1	41
	LV	52	2	54
Flour Protein	FP	81	7	88

Table 8. Explained variance (%) in PCA of fermentation variables when compared with baking variables in flours treated with urea.

UREA	Axes	PC1	PC2	1+2
	PC (%)	40.8	15.4	56.3
Fermentation	Hm	14	19	33
	h	20	56	76
	(Hm-h)/Hm	7	60	67
	H'm	66	1	67
	TV	68	6	74
	VL	57	0	57
	VRT	15	29	45
	RC	54	1	55
	T1	1	25	25
	T'1	4	19	24
Baking	PH	50	10	60
	LH	75	9	85
	SV	81	5	86
	OSP	39	2	41
	LV	81	4	85
Flour Protein	FP	18	3	21

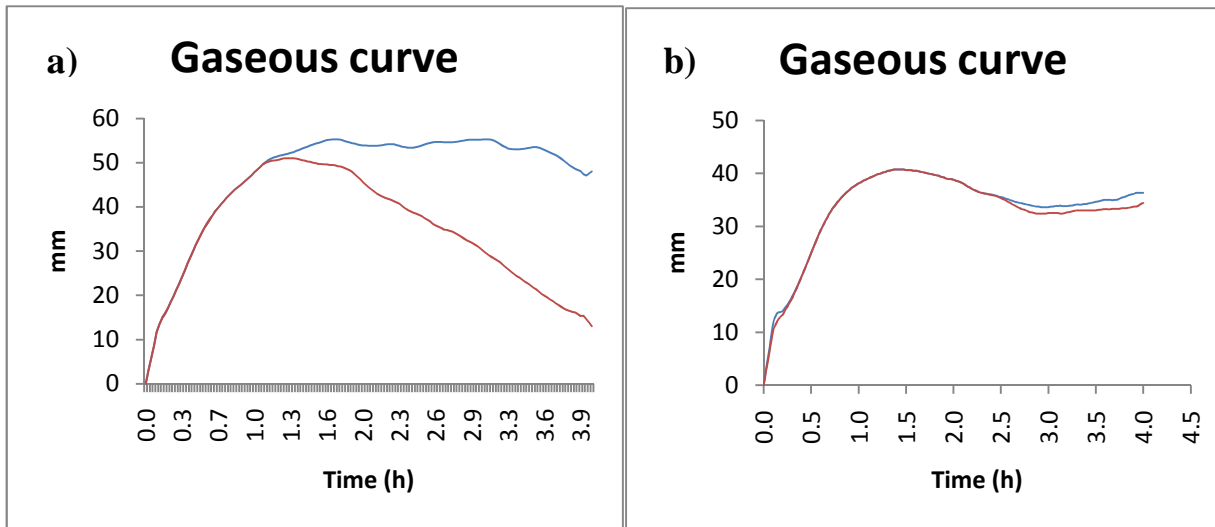


Figure 1. A graphical representation of gaseous curve of a) control sample from flour 3B and b) 3B flour containing 1 M of urea (3B1). Blue tracings are the total volume and the red is the volume retained.

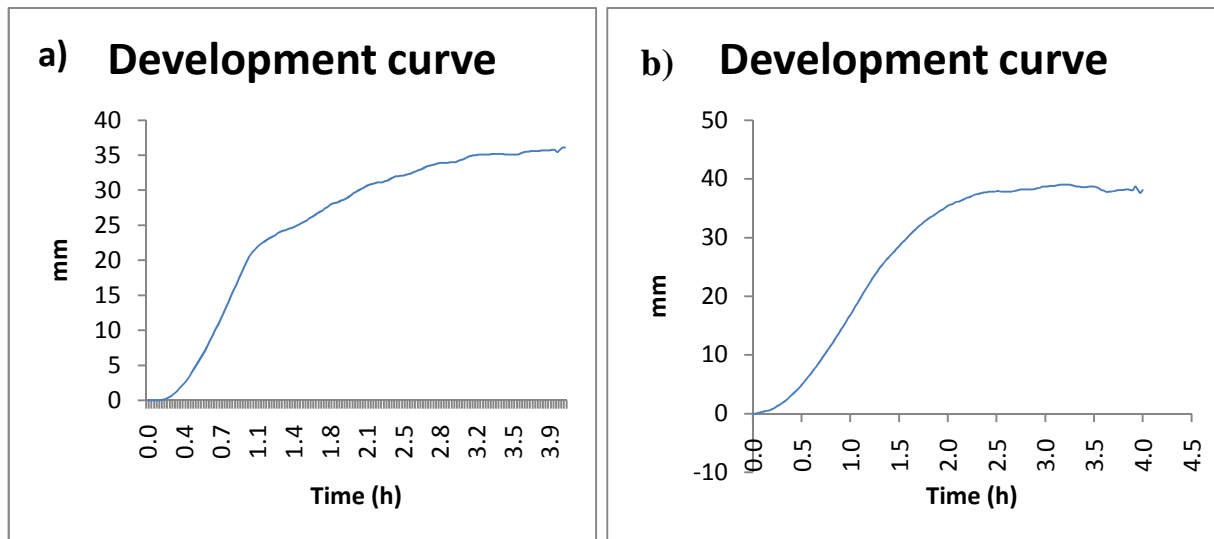


Figure 2. A graphical representation of dough development of a) control sample from flour 3B and b) 3B flour containing 1 M of urea (3B1).

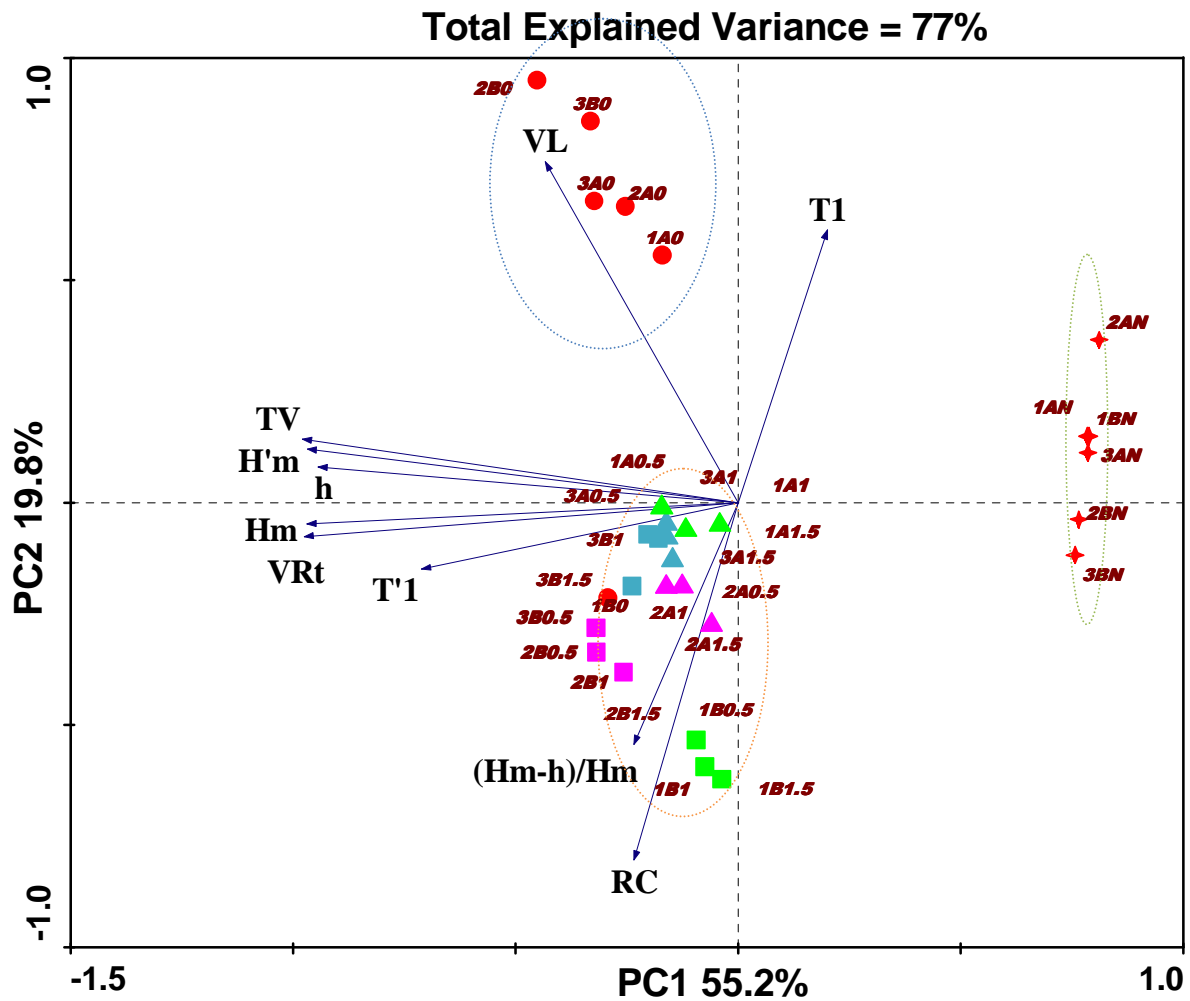


Figure. 3. Loading plot of first two principal components based on fermentation properties with negative control of six commercial wheat flours, added with four levels of urea. Definitions of fermentation, visco-elastic, mixing and baking variables explained in Table 2 and 3. Flour protein content (%), 1A = 7.95, 2A = 11.19, 3A = 13.68, 1B = 10.4, 2B = 10.59 and 3B = 11.38, respectively. Symbols and definitions: ● -Control samples, ★ - Negative controls ▲ – Low protein A flours, ▲ -Medium protein A flours, ▲ - High protein A flours. ■ – Low protein B flours, ■ - Medium protein B flours, ■ - High protein B flours.

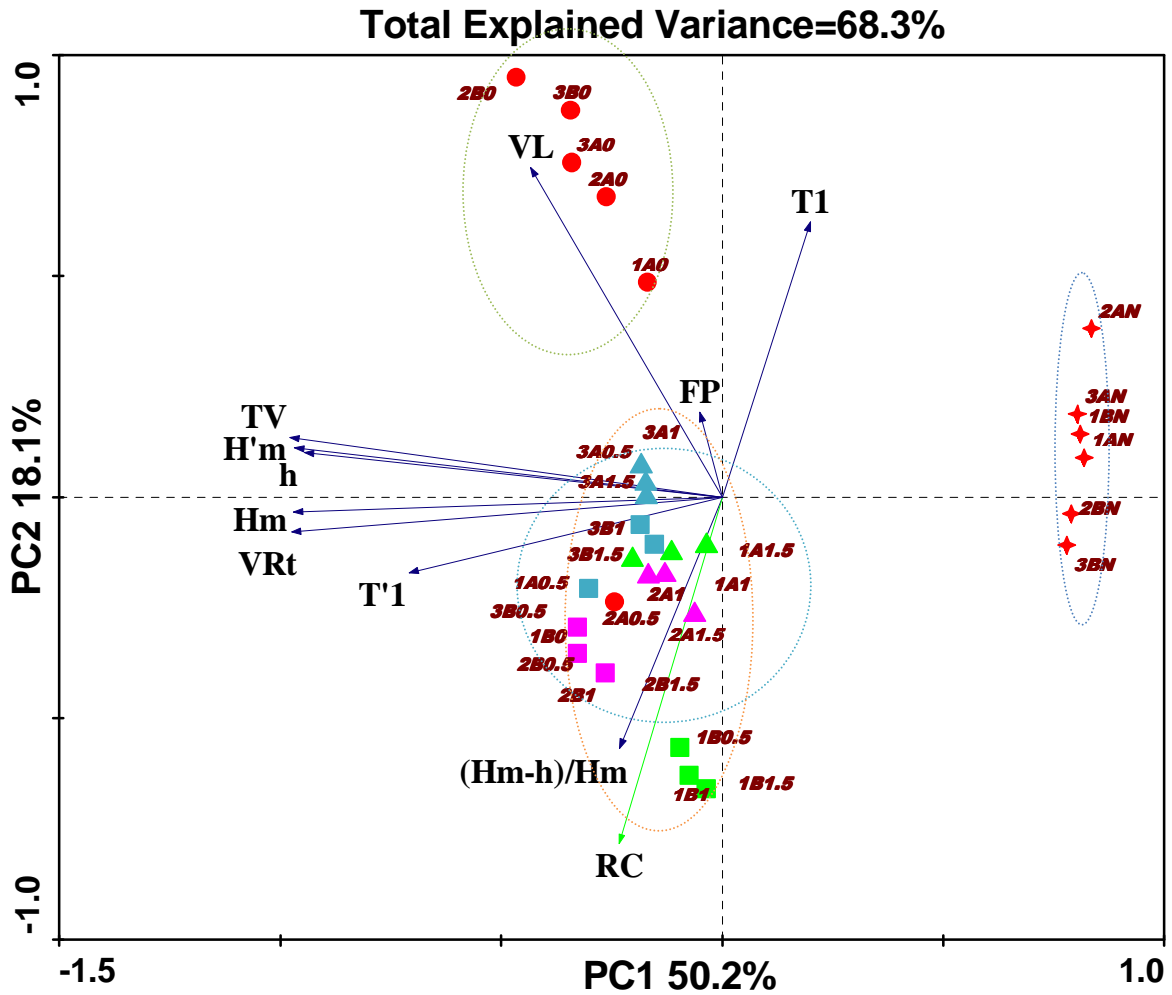


Figure. 4. Loading plot of first two principal components based on fermentation properties with flour protein of six commercial wheat flours added with four levels of urea. Symbols and definitions: ● -Control samples,★ - Negative controls▲ – Low protein A flours, ▲ -Medium protein A flours, ▲ - High protein A flours, ■ – Low protein B flours, ■ - Medium protein B flours, ■ - High protein B flours.

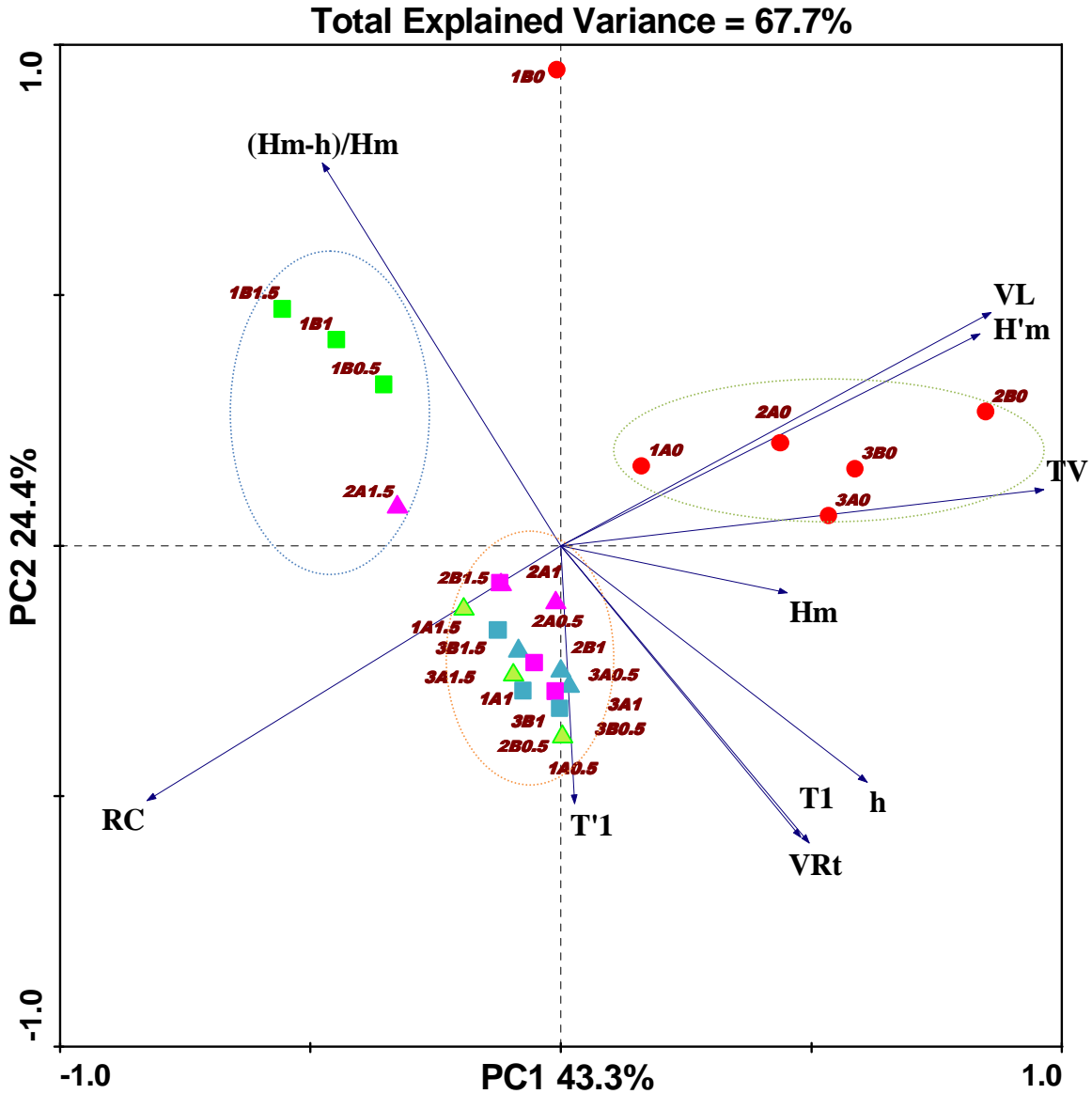


Figure. 5. Loading plot of first two principal components based on fermentation properties without negative control of six commercial wheat flours added with four levels of urea. Symbols and definitions: ● -Control samples, ▲ – Low protein A flours, ▲ -Medium protein A flours, ▲ - High protein A flours. ■ – Low protein B flours, ■ - Medium protein B flours, ■ - High protein B flours.

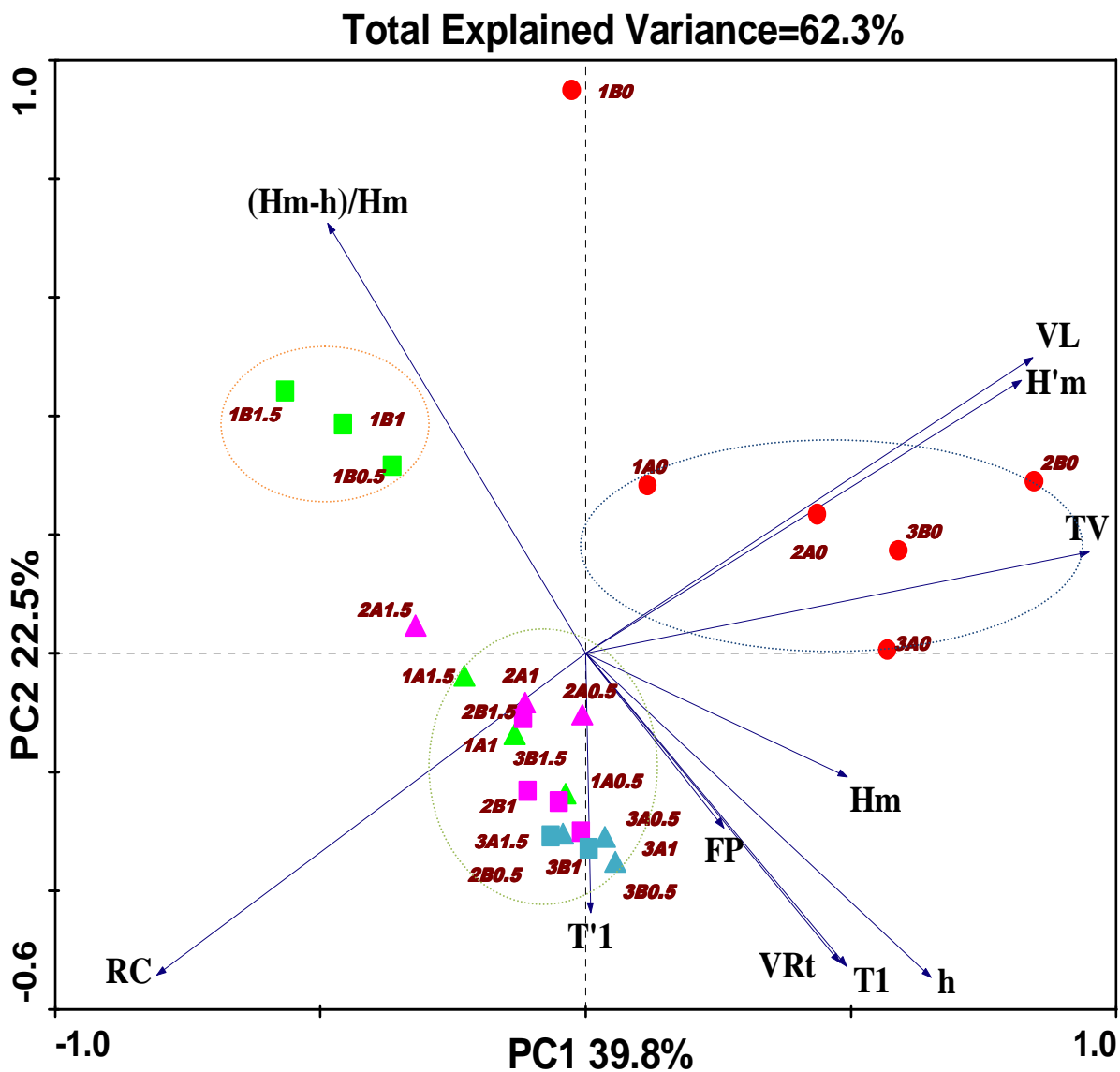


Figure. 6. Loading plot of first two principal components based on fermentation properties with flour protein of six commercial wheat flours containing four levels of urea. Negative control samples were removed. Symbols and definitions: ● -Control samples, ▲ - Low protein A flours, ▲ -Medium protein A flours, ▲ - High protein A flours, ■ - Low protein B flours, ■ - Medium protein B flours, ■ - High protein B flours.

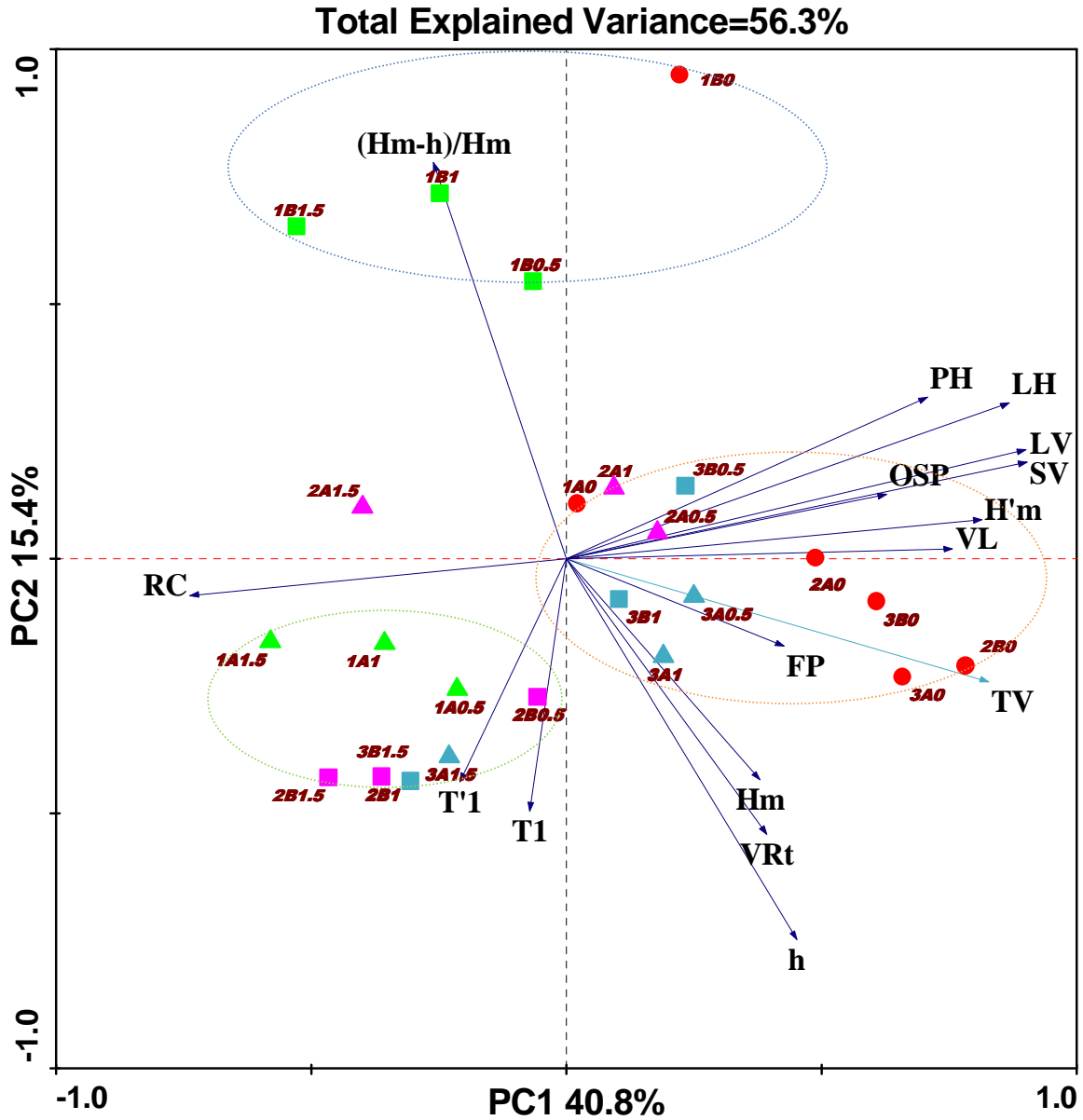


Figure. 8. Loading plot of first two principal components based on fermentation and baking properties of six commercial wheat flours added with four levels of urea. Symbols and definitions: ● -Control samples, ▲ - Low protein A flours, ▲ -Medium protein A flours, ▲ - High protein A flours. ■ - Low protein B flours, ■ - Medium protein B flours, ■ - High protein B flours.

CHAPTER VI
EFFECT OF DISRUPTION OF DISULFIDE BONDS ON FERMENTATION PROPERTIES
OF DOUGH

ABSTRACT

The objective of the study is to quantify the effect of disruption of disulfide bonds on fermentation properties of dough and to analyze possible correlation of fermentation and visco-elastic, mixing and baking properties of dough. Four levels of reduced states were obtained by the addition of dithiothreitol (DTT) (0, 0.1, 0.25 and 0.5 mM). Five commercial hard red winter wheat flours with different protein content were used. Flours with no treatment were used as controls and flours with no yeast and no treatment were used as negative controls. Fermentation properties of dough were measured using a Rheofermentometer F₃. Addition of DTT decreases the height of the dough development, maximum height of gaseous release and total volume of gas. Fermentation variables explained more variance (66.2%) than the fermentation variables combined with visco-elastic, mixing and baking variables (57.2%). The ratio of dough heights $[(H_m-h)/H_m]$ is closely related to gluten elastic properties (Sep and RCY). Retention coefficient (RC) is closely related to viscous properties (TCC and TCR). Maximum height of the dough (H_m) and height of the dough at the end of the test (h) are closely related to flour protein (FP). Volume lost (VL) is closely related to baking properties (LH and SV).

1. INTRODUCTION

Disulfide bonds play a major role in gluten strength. Gluten consists of gliadins and glutenins. Monomeric gliadins can form only intrachain disulfide bonds whereas glutenin can form both intra- and interchain disulfide bonds (Shewry and Tatham, 1997). Dough quality depends on molecular weight (MW) distribution of glutenins which is governed by the state of disulfide structure which depends on genetic factors, environmental factors and the redox state (Wieser, 2007). Disulfide bonds hold the gluten subunits and form large polymer size matrices. High molecular weight glutenin subunits (HMW) and low molecular weight glutenin subunits (LMW) are the two major groups. By reducing the interchain disulfide bonds, HMW and LMW subunits are separated (Shewry and Tatham, 1997). Dough structure and loaf quality depends on HMW sub fraction of glutenin. Humphris et al., (2000) reported that the ability of reduced and disulfide linkage free high molecular weight glutenin fractions to form branched hydrogen bonding structures can be estimated using atomic force microscopy. Gao et al., (1992) reported that disruption of disulfide bonds starts at 0.08 mM of DTT and dough stickiness started increasing at 3 mM of DTT. Kim and Bushuk (1995) reported that two Canadian hard red winter wheat flours with protein contents of 6.8 and 9.6% showed decrease in elasticity by 79 and 97%, respectively with 0.1 mM of DTT. Strong and weak gluten flours when treated with DTT at 500 ppm showed high decrease in elasticity in strong gluten compared to weak gluten flours (Khatkar et al., 2005).

The objectives of the study were:

- 1) To study the effect of disruption of disulfide bonds on the fermentation properties of dough using DTT.
- 2) To analyze possible correlation of fermentation and visco-elastic, baking and mixing properties of dough.

2. Materials and Methods

a. Materials and Labeling

Five commercial hard red wheat flours were obtained from two different milling supplies A and B. They differ in protein content. Four levels (0, 0.1, 0.25 and 0.5 mM) of DTT (VWR International, West Chester, PA) were used. Flours with no DTT were used as control and flours with no DTT and no yeast were used as negative control. Thus site A flours were labeled as 1A0 (positive control), 1AN (negative control), 1A0.1, 1A0.25 and 1A0.5; 2A0, 2A0.1, 2A0.25, 2A0.5; 3A0, 3A0.1, 3A0.2 and, 3A0.5. Similarly site B flours were labeled as 1B0, 1BN, 1B0.1, 1B0.25, 1B0.5; 3B0, 3B0.1, 3B0.25 and 3B0.5. The protein, moisture and ash contents were determined using the NIR system (FOSS NIR Systems Inc, Laurel, MD) as shown in Table 1 (Chapter III).

b. Methods

Dough Preparation

Dough was prepared as described in the Chopin protocol using Chopin Alveo - Consistograph. The ingredients consisted of 250 g of flour, 3 g of dry yeast and 5 g of sodium chloride. DTT was added to the flours at 0, 0.1, 0.25 and 0.5 mM. A stock solution of 100 ml was prepared containing 1.54 g of DTT. For 0.1 mM of DTT, a working solution of 1000 ml was prepared by adding 0.1 ml of stock solution. In the same way, working solutions were prepared

for 0.25 mM containing 2.5 ml of stock solution in 1000 ml and 5 ml for 0.5 mM. From the described working solutions, 125 ml was mixed with water added to the flour to obtain each DTT addition. The quantity of deionized water added depended on the moisture content of the flour and it was given by the reference table published by the International Association for Cereal Science and Technology (ICC) as described in the Chopin Protocol. The sodium chloride was dissolved in water prior to the addition to dough. Instant dry yeast and DTT are blended with 250 g of flour in the kneader bowl. Salt water was progressively added to the flour at the beginning of the first minute mixing period. After one minute, the mixing was stopped to remove the flour sticking to the walls and ensure a homogeneous hydration. The mixing process was continued for 6 minutes. A sample size of 315 g of dough was used for each treatment.

Fermentation Test

Rheofermentometer was used to study the fermentation properties of dough. The dough (315 g) obtained from AlveoConsistograph was placed in the bottom of the aluminum basket and packed it down with hands. The height of the dough in the basket must be leveled out just below the lowest holes. The piston with a 2000 g weight was placed on top of the dough. The basket placed in the F3 Rheofermentometer bowl. Displacement sensor was placed and the whole system was tightly closed and the test was run for a total of 4 h. This time represents 1 h longer than the Chopin Protocol as it was determined experimentally with the samples and treatments in this study.

The F3 Rheofermentometer analyzes the development of a dough sample placed in the bowl. The piston placed on the dough rises. The piston is directly linked to a displacement sensor which will calculate the dough rising. Rheofermentometer is also linked to a pressure sensor through a pneumatic circuit that measures the pressure increase in the fermenting dough. The

three curves are dough development, speed of CO₂ release and quantity produced and volume of CO₂ retained in dough. Fermentation variables are defined in Table 1 and visco-elastic, mixing and baking properties are defined in Table 2 (Chapter III).

3. STATISTICAL ANALYSIS

A factorial design within a randomized block design was implemented. Five levels of DTT and 3 levels of flour protein were compared in a 5 X 3 factorial. The significant differences in means were compared using Analysis of Variance (ANOVA) with Tukey's comparisons ($\alpha=0.05$) using SAS (Version 9.1 SAS Institute Inc., Cary, NC). Principal Component Analysis (PCA) is a mathematical algorithm that reduces the dimensionality of the data (Ringer, 2008). PCA is performed using Canoco for windows 4.5 (Biometris, Plant Research International, Wageningen, the Netherlands).

4. Results and Discussion

Protein, moisture and ash content of the flour samples and water added are reported in Table 3 (Chapter III). Typical dough fermentation property curves are illustrated in Figures 1 and 2 showing results for sample 3B control (a) and 3B containing 0.5 mM DTT (b). The volume of CO₂ lost (VL) is decreased in the sample in which disulfide bonds are disrupted (Fig. 1). Volume of retention of gas was improved for sample with disruption of disulfide bonds when compared with control sample. From the dough development curves we can observe that the height of dough is improved when disulfide bonds are disrupted (Fig. 2). A summary of the definition of fermentation properties of all samples is found in Table 1 (Chapter III).

Maximum height of the dough (Hm)

Hm is the maximum height of the dough development. As expected control sample without yeast shows no development (Table 1). The overall trend of disrupting disulfide bonds is a decrease on Hm. Observations that significantly decreased Hm were 3A and 1B flours with all levels of DTT. Only one comparison of 3B flour with 0.25 mM of DTT significantly increased Hm (Table 1). Overall highlights are: high value of Hm was shown by 3B0.25 (43.2 mm) and lowest by 1A0.5 (24.4 mm) (Table 1 and 2). The change of the fermentation properties (%) is reported in Table 2. A trend of high percentage (17.1%) increment in maximum height was observed in 3B0.25 (Table 2). A trend of 25.5% decrease in maximum height was observed in the sample 1B0.5 (Table 2). Overall maximum height of dough development decreased except for most of the 1A and 3B flours.

Height of the dough development (h)

The height of the dough development (mm) at the end of the test was denoted by h. As expected, negative controls showed no development. Observations that significantly decreased height of the dough development are 2A flour with 0.1 Mm and 3A flours with all levels of DTT (Table 1). Only one comparison of 3B flour with 0.25 mM of DTT significantly increased Hm compared to the control (Table 1 and 2). Overall highlights are: lowest value of height was observed in 1B0 (19.85 mm) and highest value was observed in 3B0.25 (43.1 mm) (Table 1). A trend to high increase (16.9%) of h was observed in 1B0.1 and to high decrease (23.4%) is observed in 3A0.5 (Table 2). Dough height at the end of the test is decreased for 2A and 3A flours whereas it increased for other flours.

Lowering of development percentage [(Hm-h)/Hm]

(Hm-h)/Hm is the ratio of dough height at the end of the fermentation test in percentage. A large percent means the dough has maintained its height during fermentation. Comparing the ratio of dough height within samples, only in two samples 2A and 1B significant changes in ratio of dough were observed as the disulfide bonds are disrupted (Table 1 and 2). In sample 1B, all the urea levels lowered significantly the ratio of dough height compared to the control. While in sample 2A, 0.5 mM DTT increased the ratio of dough height compared to control. Overall highlights are: high value was observed in 1B0 (39.1%) and lowest value in 3B0 (0) (Table 1). A trend to high percentage increase was observed in 3A0.5 (576.9%) and low percentage decrease in 1A0.5 (77.8%) (Table 2). Overall lowering of development percentage decreased for low protein flours 1A and 1B.

Maximum height of the gaseous curve (H'm)

H'm is the maximum height of the gaseous release curve. Overall trend of decrease of disulfide bonds is to decrease H'm. Observations that are significantly decreased H'm by decreasing the disulfide bonds were 2A, 3A and 1B flours with all levels and 3B flour with 0.1 mM of DTT. Only one observation significantly increased H'm is 1A with 0.5 mM of DTT (Table 1 and 2). Overall highlights are: high value of H'm was shown by 1B0 (59.55 mm) and lowest value was shown by 1B0.25 (40.65 mm) (Table 1). A trend to an increase (10%) in maximum height was observed in 1A0.5 and 31.7% decreased in 1B0.25 (Table 2). Maximum height of gaseous release is increased for low protein flours (1A and 1B) whereas it decreased with other flours (2A, 3A and 1B).

Total Volume (TV)

TV is the total volume of gaseous curve. The effect of decreasing disulfide bonds causes a decrease in total volume in all samples except 1A flours with all levels ($P < 0.05$) (Table 1 and 2). This has similar effect as the observed on Hm. This suggests that disulfide bonds are important in forming the fermented dough structure impermeable to gas loss. Overall highlights are: high value of total volume was observed in 3B0 (1737 mL) and lowest in 1B0.25 (1268.5 mL) (Table 1). A highest percentage increase in total volume (9.7%) was observed in 1A0.5 and lowest decrease (25.2%) is observed in 3A0.1 (Table 2). Total volume is decreased with all flours except 1A.

Volume lost (VL)

VL is the carbon dioxide volume released by the dough during the fermentation test. The effect of decreasing disulfide bonds is to decrease the volume lost (Table 1 and 2). Volume lost has to be related to the total volume produced which was lowered by 9 to 25.2%. From this lowered volume produced, decreasing disulfide bonds lowered the volume lost significantly from 6 to 15%. Overall highlights are: high value was observed in 3B0 (462.5 mL) and lowest in 1B0.5 (26.5 mL) (Table 1). Highest percentage (13.5%) of volume lost was observed in 1A0.5 and lowest percentage (6.05%) in 3B0.1 (Table 2).

Volume retained (VRt)

VRt is the carbon dioxide remained in the dough at the end of the test. The effect of disruption of disulfide bonds on volume retention is to increase. Observations that are significantly increased volume retention by decreasing the disulfide bonds were 1A flours with all levels, 1B with 0.1 and 0.5 mM of DTT and 3A and 3B flours with 0.25 and 0.5 mM of DTT. Overall highlights and trends are: high value was observed in 3B0.5 (1618 mL) and lowest in

1B0 (1135 mL) (Table 1). Highest percentage (35.88%) of volume retained was observed in 1A0.5 and lowest percentage decrease (0.3%) was observed in 3B0.1 (Table 2).

Retention Coefficient (RC)

Retention coefficient (RC) is the retention volume divided by the total gaseous release. Retention coefficient of all samples and treatment were significantly increased by the decrease in sulfide bonds. This suggests that the disruption of these bonds positively affected retention coefficient (Table 1 and 2). Overall highlights are: high value was observed in 2A0.5 and 1B0.5 (97.9%) and lowest value in 3B0 (73.4%) (Table 1). Highest percentage (33%) of retention coefficient was observed in all low protein B flours (3B) and lowest percentage (21%) in all 1B flours (Table 2).

Time of maximum rise (T1)

T1 is the time taken by the dough to reach maximum height during dough development. The effect of decreasing disulfide bonds on T1 is to decrease. Observations that significantly decreased T1 were 2A, 3A and 3B flour treated with 0.5 mM of DTT. Overall highlights are: high value was observed in 3B0 (4 h) and lowest in 1B0 (1.5 h) (Table 1). Highest percentage (13.3%) of time taken to reach maximum height of dough development was observed in 1B0.1 and time decreased to 47.2% in 2A0.5 (Table 2).

Time of maximum rise (T'1)

T'1 is the time spent to reach maximum rise during gaseous release. The effect of decreasing disulfide bonds on T'1 is to increase. Observations that are significantly increased T'1 were 2A and 1B flours with 0.5 mM of DTT and 3B flours with all levels. Overall highlights are: high value was observed in all high protein B flours (3.9) and lowest in 1B0 (1.4) (Table 1).

Highest increase (95%) of time taken to reach maximum height of gaseous curve was observed in all high protein B flours and time decreased 17.7% in 1A0.1 (Table 2).

Jones et al., (1974) reported that small amount of DTT decreases the consistency of the dough which in turn affect the rate of dough development. Our study shows that effect of DTT on height of dough during fermentation is sample specific. Khatkar et al., (2005) proved that addition of DTT showed highest percentage decrease of elasticity in strong gluten and lowest percentage of decrease in weak gluten. Our study suggests that high protein flours are positively affected by the disruption of disulfide bonds and this effect is higher compared to that on low protein flours. The proposition is that high protein samples also have more disulfide bonds in their structure and therefore the effect is higher compared to low protein flours with perhaps lower potential of forming disulfide bonds.

PCA results

Principal component analyses were performed on the data sets obtained from fermentation parameters.

Fermentation variables Vs fermentation variables with flour protein

PCA were performed on the data sets, to assess the relationship of flour protein and fermentation properties (Fig. 3 and 4). Figure 3 represents the fermentation properties alone and all the samples. Principal component axis 1 (PC1) explained 56.9% variance and principal component axis 2 (PC2) explained 19.3% variance. Total explained variance is 76.2% (Table 3). Among fermentation properties, the highest contribution of variance (95%) was maximum height of gaseous release (H'm), total volume (TV) and volume of retention (VRt) in PC1 whereas in PC2 the highest contribution of variance (56.6%) was time to reach maximum height of dough development (T1) (Table 3). Figure 4 displays the fermentation properties plus flour protein.

Principal component axis 1 (PC1) explained 51.8% variance and principal component axis 2 (PC2) explained 17.7% variance. Total explained variance is 69.5% (Table 4). Among fermentation properties with flour protein, the highest contribution of variance (95.6%) was maximum height of gaseous release (H'm) and second highest contribution of variance (95.1%) was volume of retention (VRt) on PC1 whereas on PC2 the highest contribution of variance (54.8%) was time to reach maximum height of dough development (T1) (Table 4). Only 0.13% of explained variance was contributed by flour protein on PC1 and 2.5% on PC2 (Table 4). This suggests that the variation of protein is weakly related to the volume lost and its contribution to the variance is very small when compared with samples with changes in their disulfide bonds. In both the graphs (Fig. 3 and 4), most of the fermentation variables are on PC1. Flours treated with DTT are very close to PC1 when compared with control and negative samples. All control samples are closely related among themselves and to volume lost. They are well separated from the flours treated with DTT. Negative controls are also closely related among themselves and well separated from the samples with reduced disulfide bonds. So negative controls are removed from the data sets and PCA was compared. It also suggests that the samples treated with DTT are closely related to volume of the gas retained by the dough during fermentation in the first component. These samples are negatively related to volume lost.

Fermentation variables without negative control Vs fermentation variables with flour protein and without negative control

PCA were performed to assess the relationship of fermentation variables and protein without the negative controls (Fig. 5 and 6). From the fermentation properties on Figure 5, principal component axis 1 (PC1) explained 40.3% variance and principal component axis 2 (PC2) explained 25.9% variance (Fig. 5). Total explained variance is 66.2% (Table 5). Among

fermentation properties, the highest contribution of variance (76.3%) was total volume (TV) on PC1 whereas on PC2 the highest contribution of variance (76.9%) was retention coefficient (RC) (Table 5). In comparison, when flour protein was included (Fig. 6), principal component axis 1 (PC1) explained 37% variance and principal component axis 2 (PC2) explained 23.6% variance. Total explained variance is 60.6% (Table 6). Among fermentation properties with flour protein, the highest contribution of variance (73.3%) was total volume (TV) on PC1 whereas on PC2 the highest contribution of variance (74.9%) was retention coefficient (RC) (Table 6). Only 5.4% of explained variance was contributed by flour protein on PC1 and 0.9% on PC2 (Table 6). As the total explained variance of fermentation variables (66.2%) is 5.6 units of percentage higher than fermentation variables with flour protein (60.6%), we can say that compared to changes in disulfide bonds in this set, flour protein appears to have a small effect and is marginally correlated to other fermentation variables. Controls are closely related to volume lost (VL) and are separated from the flours treated with DTT. All flours except low protein B flours treated with DTT are closely related to volume of gas retained (VRt) and negatively correlated to volume lost (VL) and lowering development percentage ($[Hm-h]/Hm$). Low protein B flours are separated from other flours treated with DTT. By disruption of disulfide bonds, the fermented dough retained more volume and improved retention coefficient.

Fermentation properties Vs Fermentation properties with visco-elastic, mixing, baking properties

The relationship of fermentation variables with visco-elastic, mixing and baking properties was investigated (Fig. 7). From Figure 6, principal component axis 1 (PC1) explained 37% variance and principal component axis 2 (PC2) explained 23.6% variance. Total explained variance is 60.6% (Table 6). From Figure 7, principal component axis 1(PC1) explained 35.9%

variance and principal component axis 2 (PC2) explained 21.3% variance. Total explained variance is 57.2% (Table 7). Among all properties, the highest contribution of variance (91.8%) was flour protein (FP) and second major component that contributes high variance (91.6%) is specific volume (SV) on PC1 whereas on PC2 the highest contribution of variance (64.2%) was lowering of development percentage ($[(Hm-h)/Hm]$) (Table 7). As the total explained variance of fermentation variables (60.6%) is 3.5 units of percentage higher than fermentation variables with visco-elastic, mixing and baking variables (57.2%), we can say that the samples are better separated based on fermentation properties differences. From Figure 7, we can deduce that some variables are closely associated meaning they give redundant information. The ratio of dough heights $[(Hm-h)/Hm]$ is closely related to gluten elastic properties (Sep and RCY). Retention coefficient (RC) is closely related to gluten viscous (TCC and TCR). The maximum height of the dough (Hm) and height of the dough at the end of the test (h) are closely related to flour protein (FP). Volume lost (VL) is closely related to baking properties (LH and SV). Samples 1A and 1A representing low protein flours appeared to be in different groups and separated from other flours. This suggests that they have different properties. All baking properties show greatest contribution of explained variance on PC2. PCA analysis is performed on flour protein, fermentation properties and baking properties.

Relationship of flour protein, Fermentation and baking properties

PCA were performed on the data sets of flour protein, fermentation variables and baking properties (Fig. 8). PCA analyses of fermentation properties with flour protein were already performed (Fig. 6 and Table 6). From Figure 6, principal component axis 1 (PC1) explained 37% variance and principal component axis 2 (PC2) explained 23.6% variance. Total explained variance is 60.6% (Table 6). From Figure 8, principal component axis 1 (PC1) explained 42.8%

variance and principal component axis 2 (PC2) explained 22.3% variance. Total explained variance is 65.1% (Table 8). The highest contribution of explained variance (90.9%) was height of loaf volume (LH) on PC1 whereas on PC2 the highest contribution of variance (73.5%) was volume of gas retained (VRt) (Table 8). Flour protein explained 26.5% variance on PC1 and 0.1% on PC2 (Table 8). As the total explained variance of fermentation variables (60.6%) is less than fermentation variables with baking variables (65.1%), we can say the combination of baking and fermentation properties explain more variance than fermentation properties by themselves. Although a 4.5% increase in the explained variance is good, it will be occasions in which using fermentation parameters alone will be sufficient when taking into account the time consuming test of baking. In other words, a good approximation of the performance of flours can be estimated by analyzing the fermentation properties. It appears that including visco-elastic and mixing, baking properties is not as effective in separating the effect of the disruption of disulfide in flour samples as it is the comparison of the fermentation properties. All control samples were negatively associated with gas retained (VRt) and positively related with volume lost (VL). Volume lost is the variable most closely related to loaf height and loaf volume.

5. Conclusions

Null Hypothesis is rejected as there is significant effect of disruption of disulfide bonds of the dough (addition of DTT) on fermentation properties when compared to the control samples. Disruption of disulfide bonds decreases maximum height of the dough and maximum height of the gaseous release for most flours. Total volume and volume lost is also decreased.

Fermentation variables explained more variance (66.2%) than the fermentation variables combined with visco-elastic, mixing and baking variables (57.2%). The ratio of dough heights $[(H_m-h)/H_m]$ is closely related to gluten elastic properties (Sep and RCY). Retention coefficient (RC) is closely related to viscous properties (TCC and TCR). Maximum height of the dough (H_m) and height of the dough at the end of the test (h) are closely related to flour protein (FP). Volume lost (VL) is closely related to baking properties (LH and SV).

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Table 1. Fermentation properties in five commercial wheat flours treated with DTT levels. Means (n=2) with same superscripts in a column are not significantly different (P <0.05). The standard deviations of means are shown in parenthesis.

TRT	Fermentation Properties									
	Hm (mm)	H (mm)	(Hm-h)/Hm (%)	H'm (mm)	TV (mL)	VL (mL)	VRt (mL)	RC (%)	T1 (h)	T'1 (h)
1A0	25 ^{klm} (1.3)	24.55 ^{ijklm} (1.4)	1.8 ^{ghi} (0.3)	47.5 ^{fgh} (2.5)	1507.5 ^{defg} (76.5)	323 ^{cd} (37.0)	1184.5 ^{kl} (39.5)	78.65 ^{cdef} (1.4)	3.9 ^{ab} (0.0)	3.16 ^{abcd} (0.2)
1AN	0 ⁿ (0.0)	0 ^o (0.0)	0 ⁱ (0.0)	5 ^l (0.1)	12.5 ^k (0.5)	2 ^f (0.0)	11 ^m (0.0)	83.25 ^{bcd} (2.1)	4 ^a (0.0)	0.1 ^k (0.0)
1A0.1	26.75 ^{ijklm} (2.6)	26.45 ^{ghijkl} (2.3)	1.45 ^{ghi} (1.1)	50.55 ^{def} (2.4)	1591.5 ^{bcde} (98.5)	41.5 ^{ef} (6.5)	1550.5 ^{ab} (104.5)	97.35 ^a (0.6)	3.9 ^{ab} (0.1)	2.6 ^{defghi} (1.2)
1A0.25	26.3 ^{ijklm} (0.8)	26.2 ^{hijkl} (0.8)	1 ^{hi} (1.0)	50.45 ^{def} (0.4)	1578 ^{bcde} (27.0)	33.5 ^{ef} (1.5)	1544.5 ^{ab} (25.5)	97.85 ^a (0.1)	3.8 ^{ab} (0.1)	3.8 ^{ab} (0.1)
1A0.5	24.35 ^m (4.0)	24.05 ^{klmn} (4.2)	0.4 ⁱ (0.0)	52.25 ^{cde} (3.6)	1653 ^{abc} (121.0)	43.5 ^{ef} (12.5)	1609.5 ^a (133.5)	97.3 ^a (1.0)	3.9 ^a (0.0)	2.8 ^{bcdefg} (1.2)
2A0	32.15 ^{cdefgh} (0.6)	31.45 ^{defg} (1.2)	2.2 ^{ghi} (1.9)	53.1 ^{cde} (1.0)	1695.5 ^{ab} (27.5)	389.5 ^b (11.5)	1306 ^{fghijk} (16.0)	77.05 ^{ef} (0.4)	3.6 ^{abc} (0.3)	1.5 ^j (0.2)
2AN	0 ⁿ (0.0)	0 ^o (0.0)	0 ⁱ (0.0)	4.9 ^l (1.3)	12 ^k (3.0)	3.5 ^f (0.5)	9 ^m (3.0)	70.2 ^g (6.0)	4 ^a (0.0)	0.1 ^k (0.0)
2A0.1	27.2 ^{hijklm} (1.4)	27 ^{hijkl} (1.4)	10.55 ^{efg} (8.5)	43.65 ^{hijk} (3.4)	1372 ^{ghij} (102.0)	47 ^{ef} (9.0)	1324.5 ^{defghij} (111.5)	96.5 ^a (0.9)	2.73 ^{bcdefgh} (0.9)	1.9 ^{efghij} (0.1)
2A0.25	29.3 ^{fghijklm} (0.5)	28.9 ^{ghij} (0.3)	4.8 ^{fghi} (0.1)	45.65 ^{ghij} (1.1)	1425 ^{fghi} (37.0)	31 ^{ef} (6.0)	1394 ^{cdefghi} (31.0)	97.85 ^a (0.4)	2.5 ^{cdefgh} (0.1)	1.8 ^{efghij} (0.0)
2A0.5	29.65 ^{efghijkl} (0.8)	29.2 ^{efghij} (0.6)	13.6 ^{def} (1.5)	46.55 ^{fgh} (1.7)	1434 ^{fghi} (60.0)	29.5 ^{ef} (1.5)	1404.5 ^{cdefg} (61.5)	97.9 ^a (0.2)	1.9 ^{fgh} (0.1)	2.9 ^{abcde} (1.1)
3A0	39.4 ^{ab} (2.2)	39.15 ^{ab} (2.5)	0.65 ⁱ (0.7)	54.3 ^{bcd} (0.1)	1669 ^{ab} (13.0)	362.5 ^{bc} (13.5)	1307 ^{fghijk} (0.0)	78.3 ^{def} (0.6)	3.9 ^a (0.0)	1.6 ^{ij} (0.0)
3AN	0 ⁿ (0.0)	0 ^o (0.0)	0 ⁱ (0.0)	4.35 ^l (0.8)	17.5 ^k (3.5)	2.5 ^f (0.5)	15.5 ^m (4.5)	85.5 ^b (6.5)	4 ^a (0.0)	0.1 ^k (0.0)
3A0.1	31.35 ^{defghij} (0.6)	31.05 ^{defgh} (0.5)	0.95 ^{hi} (0.4)	43.5 ^{hijk} (1.0)	1338.5 ^{hij} (21.5)	27.5 ^{ef} (4.5)	1311.5 ^{efghijk} (17.5)	97.95 ^a (0.3)	3.9 ^{ab} (0.0)	2.0 ^{efghij} (0.0)
3A0.25	32.5 ^{cdefg} (0.2)	32.35 ^{cdef} (0.1)	0.45 ⁱ (0.5)	49.6 ^{efg} (0.2)	1518.5 ^{cdef} (1.5)	56 ^{ef} (24.0)	1462.5 ^{bc} (25.5)	96.3 ^a (1.6)	3.6 ^{abc} (0.4)	1.8 ^{fghij} (0.0)
3A0.5	31.45 ^{defghi} (3.6)	30 ^{efghi} (2.8)	4.4 ^{fghi} (1.9)	49.05 ^{efg} (1.2)	1479 ^{efgh} (25.0)	34.5 ^{ef} (0.5)	1444.5 ^{bcd} (24.5)	97.7 ^a (0.0)	2.7 ^{bcdefgh} (0.0)	1.8 ^{fghij} (0.0)

Table 1. Continued

TRT	Fermentation Properties									
	Hm (mm)	h (mm)	(Hm-h)/Hm (%)	H'm (mm)	TV (mL)	VL (mL)	VRt (mL)	RC (%)	T1 (h)	T'1 (h)
1B0	33.2 ^{cdef} (2.3)	19.85 ⁿ (3.8)	39.1 ^a (15.5)	59.55 ^a (1.8)	1412 ^{fghi} (32.0)	277 ^d (15.0)	1135 ^l (17.0)	80.4 ^{bcde} (0.6)	1.5 ^h (0.2)	1.4 ^j (0.0)
1BN	0 ⁿ (0.0)	0 ^o (0.0)	0 ⁱ (0.0)	5.5 ^l (1.8)	18.5 ^k (6.5)	3 ^f (2.0)	15.5 ^m (4.5)	83.35 ^{bcd} (4.7)	4 ^a (0.0)	0.1 ^k (0.0)
1B0.1	28.1 ^{ghijklm} (0.5)	23.2 ^{lmn} (0.2)	17.4 ^{cde} (2.2)	41.95 ^{ijk} (0.7)	1339 ^{hij} (18.0)	32 ^{ef} (4.0)	1307.5 ^{efghijk} (21.5)	97.6 ^a (0.3)	1.7 ^{gh} (0.0)	1.7 ^{ghij} (0.1)
1B0.25	25.2 ^{klm} (1.1)	20.7 ^{mn} (0.7)	17.8 ^{bcde} (0.8)	40.65 ^k (0.2)	1268.5 ^j (8.5)	30.5 ^{ef} (0.5)	1238.5 ^{jkl} (7.5)	97.65 ^a (0.1)	1.6 ^h (0.1)	1.7 ^{hij} (0.1)
1B0.5	24.75 ^{lm} (0.2)	19.9 ⁿ (0.1)	19.6 ^{bcde} (0.1)	41.45 ^{jk} (2.7)	1296.5 ^{ij} (83.5)	26.5 ^{ef} (0.5)	1270 ^{ijk} (83.0)	97.95 ^a (0.1)	1.6 ^h (0.1)	2.7 ^{cdefgh} (1.2)
3B0	36.85 ^{bc} (0.8)	36.85 ^{bc} (0.8)	0 ⁱ (0.0)	54.2 ^{bcd} (1.1)	1737 ^a (46.0)	462.5 ^a (20.5)	1275 ^{ghijk} (25.0)	73.4 ^{fg} (0.5)	4 ^a (0.0)	2.0 ^{efghij} (1.0)
3BN	0 ⁿ (0.0)	0 ^o (0.0)	0 ⁱ (0.0)	4.9 ^l (0.6)	14 ^k (1.0)	2.5 ^f (0.5)	12 ^m (1.0)	84 ^{bc} (1.2)	2.1 ^{efgh} (1.9)	0.1 ^k (0.0)
3B0.1	39.8 ^{ab} (7.1)	39.65 ^{ab} (7.0)	0.3 ⁱ (0.3)	45.45 ^{ghijk} (2.0)	1299 ^{ij} (40.0)	28 ^{ef} (0.0)	1271 ^{hijk} (40.0)	97.85 ^a (0.1)	3.8 ^{ab} (0.1)	3.9 ^a (0.0)
3B0.25	43.15 ^a (2.3)	43.05 ^a (2.4)	0.25 ⁱ (0.3)	58.4 ^{ab} (0.0)	1651 ^{abc} (10.0)	36 ^{ef} (2.0)	1614.5 ^a (11.5)	97.8 ^a (0.1)	3.9 ^a (0.0)	3.9 ^a (0.0)
3B0.5	41.05 ^{ab} (1.4)	36.8 ^{bc} (0.2)	10.25 ^{efgh} (3.5)	58.6 ^{ab} (2.3)	1652 ^{abc} (31.0)	33.5 ^{ef} (3.5)	1618.5 ^a (34.5)	97.95 ^a (0.3)	2.2 ^{defgh} (0.1)	3.9 ^a (0.0)

Table 2. Change (percent) of fermentation properties of five commercial wheat flours treated with DTT levels.

TRT	Fermentation properties									
	Hm (%)	h (%)	(Hm-h)/Hm (%)	H'm (%)	TV (%)	VL (%)	VRt (%)	RC (%)	T1 (%)	T'1 (%)
1A0.1	7.0	7.9	-19.4	6.42	5.6	-12.8*	30.90*	23.8*	0.0	-17.7
1A0.25	5.2	6.7	-44.4	6.21	4.7	-10.4*	30.39*	24.4*	-2.6	20.3
1A0.5	-2.6	-2.0	-77.8	10.00*	9.7*	-13.5*	35.88*	23.7*	0.0	-11.4
2A0.1	-15.4	-14.1*	379.5	-17.80*	-19.1*	-12.1*	1.42	25.2*	-24.2	26.7
2A0.25	-8.9	-8.1	118.2	-14.03*	-16.0*	-8.0*	6.74	27.0*	-30.6	20
2A0.5	-7.8	-7.2	518.2*	-12.34*	-15.4*	-7.6*	7.54	27.1*	-47.2*	93.3*
3A0.1	-20.4*	-20.7*	46.2	-19.89*	-19.8*	-7.6*	0.34	25.1*	0.0	25
3A0.25	-17.5*	-17.4*	-30.8	-8.66*	-9.0*	-15.4*	11.90*	23.0*	-7.7	12.5
3A0.5	-20.2*	-23.4*	576.9	-9.67*	-11.4*	-9.5*	10.52*	24.8*	-30.8*	12.5
1B0.1	-15.4*	16.9	-55.5*	-29.55*	-5.2	-11.55*	15.20*	21.4*	13.3	21.4
1B0.25	-24.1*	4.3	-54.5*	-31.74*	-10.2*	-11.01*	9.12	21.5*	6.7	21.4
1B0.5	-25.5*	0.3	-49.9*	-30.39*	-8.2	-9.57*	11.89*	21.8*	6.7	92.9*
3B0.1	8.0	7.6	0.0	-16.14*	-25.2*	-6.05*	-0.31	33.3*	-5.0	95*
3B0.25	17.1*	16.8*	0.0	7.75	-5.0	-7.78*	26.63*	33.2*	-2.5	95*
3B0.5	11.4	-0.1	0.0	8.12	-4.9	-7.24*	26.94*	33.4*	-45*	95*

Values with * are significantly different to control samples (P<0.05). Percentage calculated from values in Table 1 and % change = (Sample treated with additive- Control sample)/Control sample * 100.

Table 3. Explained variance (%) in PCA of fermentation variables with negative control in flours treated with DTT.

DTT	AXES	PC1	PC2	1+2
	PC (%)	56.9%	19.3%	76.2%
Fermentation	Hm	94	1	94
	h	88	4	93
	(Hm-h)/Hm	5	34	39
	H'm	96	1	96
	TV	95	1	96
	VL	10	50	61
	VRT	95	0	96
	RC	22	45	67
	T1	2	57	58
	T'1	62	1	63

Table 4. Explained variance (%) in PCA of fermentation variables and flour protein in flours treated with DTT.

DTT	AXES	PC1	PC2	1+2
	PC (%)	51.8%	17.7%	69.5%
Fermentation	Hm	94	1	95
	h	89	5	94
	(Hm-h)/Hm	5	34	39
	H'm	96	0	96
	TV	95	1	96
	VL	10	50	60
	VRT	95	0	96
	RC	22	45	67
	T1	2	55	57
	T'1	62	1	63
Flour Protein	FP	0	2	3

Table 5. Explained variance (%) in PCA of fermentation variables without negative control in flours treated with DTT.

DTT	AXES	PC1	PC2	1+2
	PC (%)	40.3%	25.9%	66.2%
Fermentation	Hm	47	0	47
	h	69	5	74
	(Hm-h)/Hm	37	16	53
	H'm	58	3	60
	TV	76	1	77
	VL	23	72	95
	VRT	19	53	72
	RC	19	77	96
	T1	54	2	56
	T'1	2	31	33

Table 6. Explained variance (%) in PCA of fermentation variables and flour protein and without negative control in flours treated with DTT.

DTT	AXES	PC1	PC2	1+2
	PC (%)	37%	23.6%	60.6%
Fermentation	Hm	51	0	51
	h	72	5	77
	(Hm-h)/Hm	36	16	53
	H'm	56	2	59
	TV	73	0	74
	VL	23	70	93
	VRT	17	55	72
	RC	19	75	94
	T1	52	3	55
	T'1	2	32	34
Flour Protein	FP	5	1	6

Table 7. Explained variance (%) in PCA of fermentation variables when compared with visco-elastic, mixing and baking variables in gluten and flours treated with DTT. Definitions of fermentation, visco-elastic, mixing and baking variables explained in Table 2 (Chapter III).

DTT	AXES	PC1	PC2	1+2
	PC (%)	35.9%	21.3%	57.2%
Fermentation	Hm	33	28	61
	h	18	64	82
	(Hm-h)/Hm	1	64	65
	H'm	7	13	20
	TV	1	34	35
	VL	40	0	40
	VRT	23	37	61
	RC	39	1	40
	T1	0	55	55
	T'1	18	8	26
Visco-elastic	SeP	40	35	75
	J-J _r	26	33	59
	RCY	32	38	70
	TCR	29	20	50
	TCC	30	32	61
Mixing	WA	46	1	46
	DT	25	18	44
	ST	40	15	55
Baking	BT	19	19	38
	PH	84	1	85
	LH	83	0	83
	SV	92	1	93
	OPH	39	0	39
Flour Protein	LV	42	11	54
	FP	92	3	95

Table 8. Explained variance (%) in PCA of fermentation variables when compared with baking variables in gluten and flours treated with DTT.

DTT	Axes	PC1	PC2	1+2
	PC (%)	42.8	22.3	65.1
Fermentation	Hm	42	21	63
	H	36	47	82
	(Hm-h)/Hm	2	47	50
	H'm	20	30	50
	TV	15	50	65
	VL	61	2	63
	VRT	12	74	86
	RC	59	4	62
	T1	11	42	53
	T'1	8	21	29
Baking	PH	76	3	79
	LH	91	6	97
	SV	84	3	87
	OSP	56	7	63
	LV	85	1	86
Flour Protein	FP	27	0	27

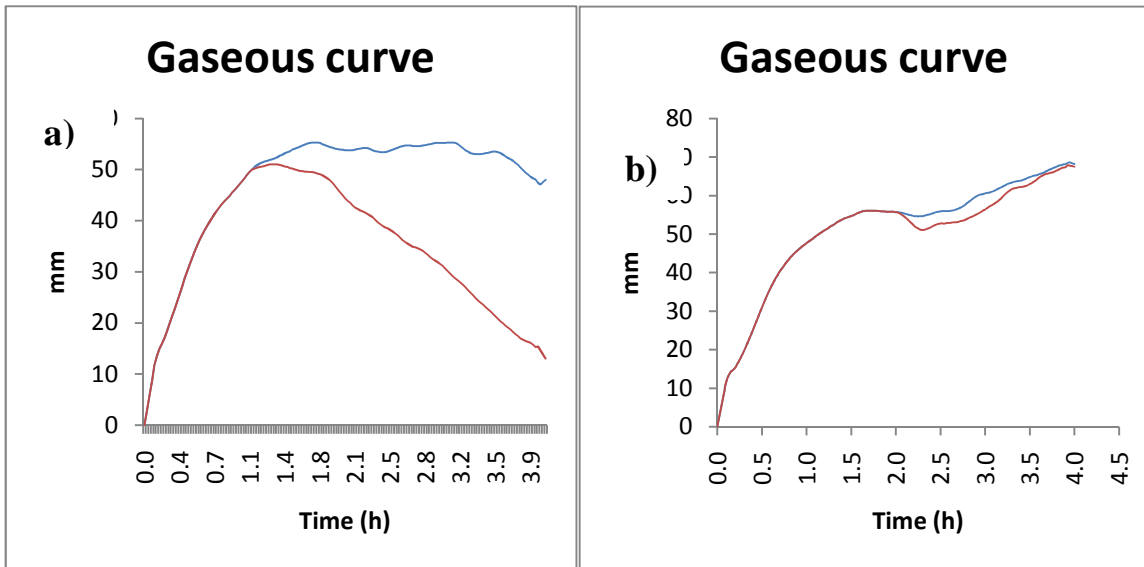


Figure 1. A graphical representation of gaseous curve of a) control sample from flour 3B and b) 3B flour containing 0.5 mM of DTT (3B0.5). Blue tracings are the total volume and the red is the volume retained.

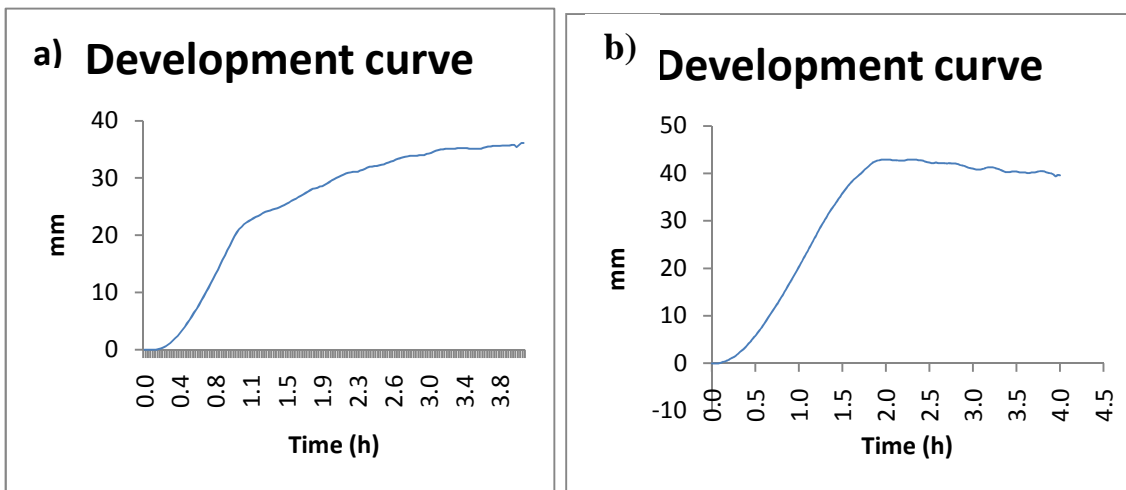


Figure 2. A graphical representation of dough development of a) control sample from flour 3B and b) flour 3B containing 0.5 mM of DTT (3B0.5).

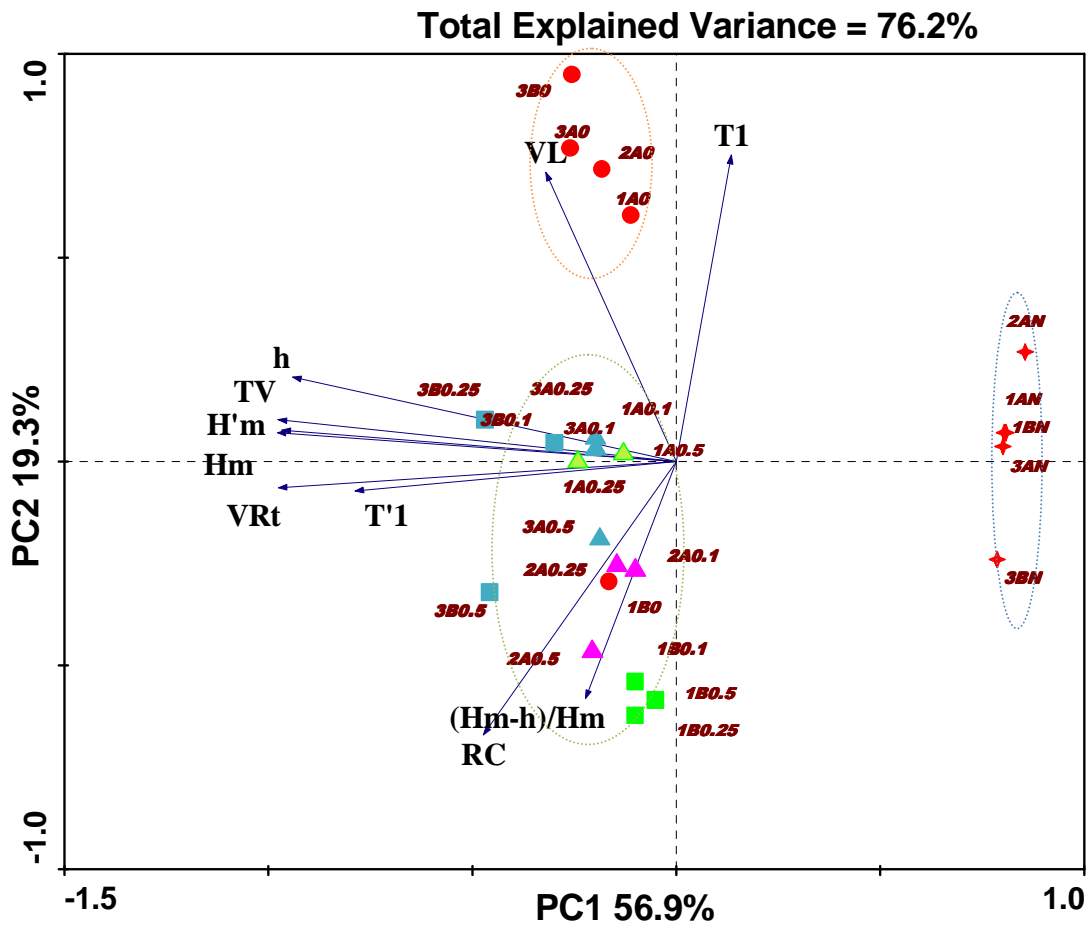


Figure 3. Loading plot of first two principal components based on fermentation properties with negative control of five commercial wheat flours, added with four levels of DTT. Definitions of fermentation, visco-elastic, mixing and baking variables explained in Table 2 and 3 (Chapter III). Flour protein content (%), 1A = 7.95, 2A = 11.19, 3A = 13.68, 1B = 10.4 and 3B = 11.38, respectively. Symbols and definitions: ● -Control samples; ★ -Negative controls. ▲ - Low protein A flours, ▲ -Medium protein A flours, ▲ - High protein A flours. ■ - Low protein B flours, ■ - Medium protein B flours, ■ - High protein B flours.

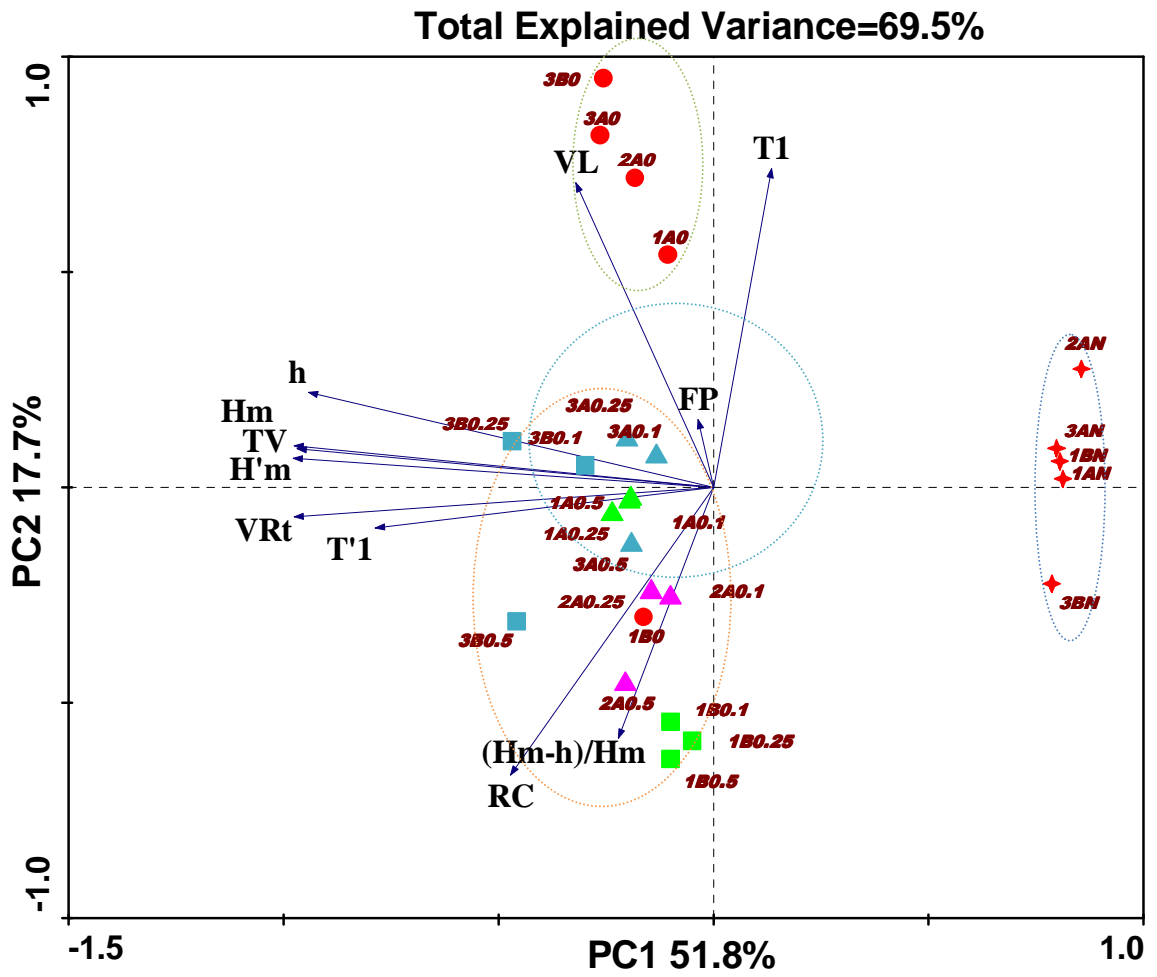


Figure 4. Loading plot of first two principal components based on fermentation properties with flour protein of five commercial wheat flours added with four levels of DTT. Symbols and definitions: ● - Control samples, ★ - Negative controls, ▲ - Low protein A flours, ▲ - Medium protein A flours, ▲ - High protein A flours, ■ - Low protein B flours, ■ - Medium protein B flours, ■ - High protein B flours.

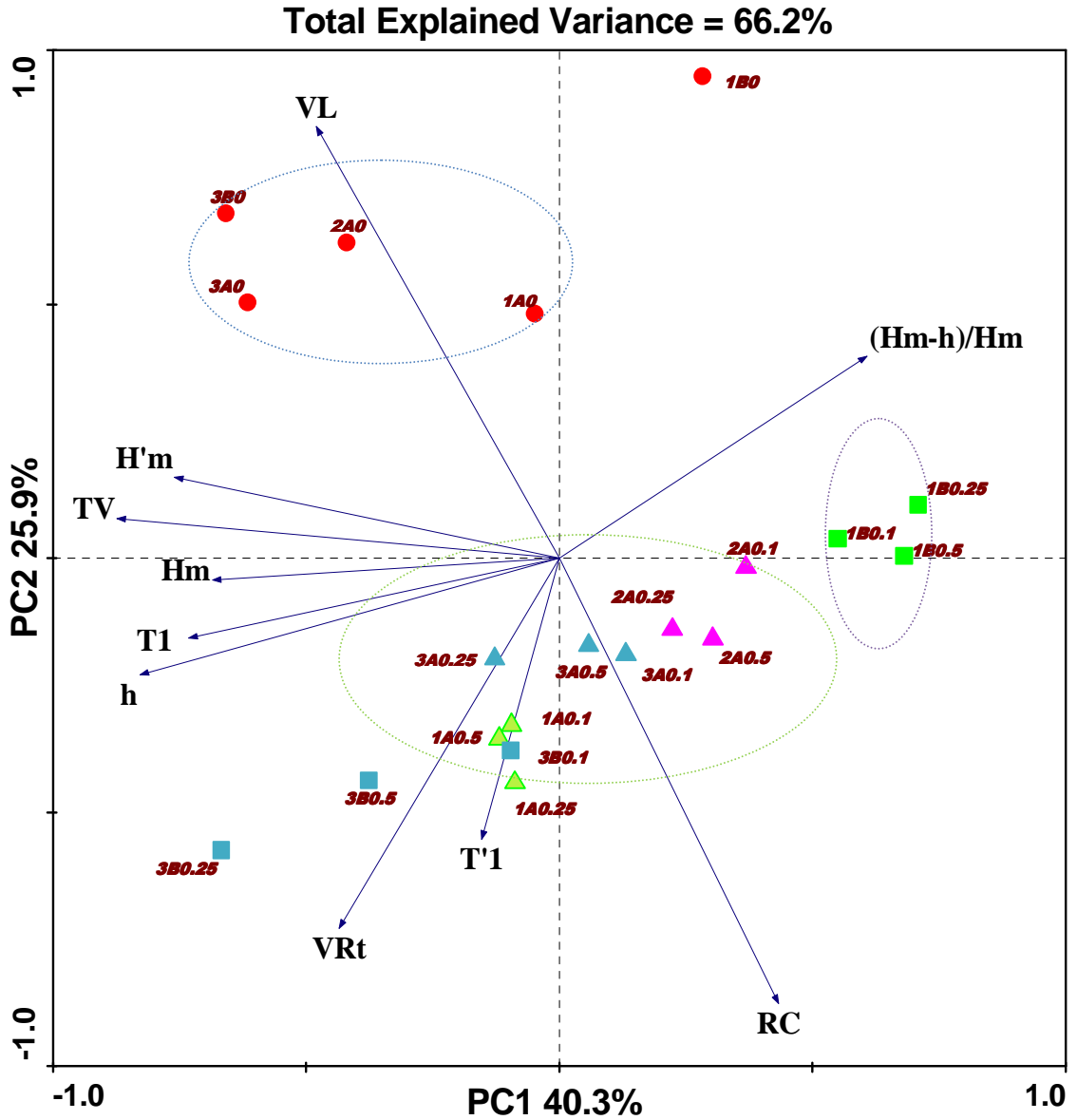


Figure 5. Loading plot of first two principal components based on fermentation properties without negative control of five commercial wheat flours added with four levels of DTT. Symbols and definitions: ● -Control samples;★ - Negative controls,▲- Low protein A flours, ▲ -Medium protein A flours,▲ - High protein A flours. ■ – Low protein B flours, ■ Medium protein B flours, ■ High protein B flours.

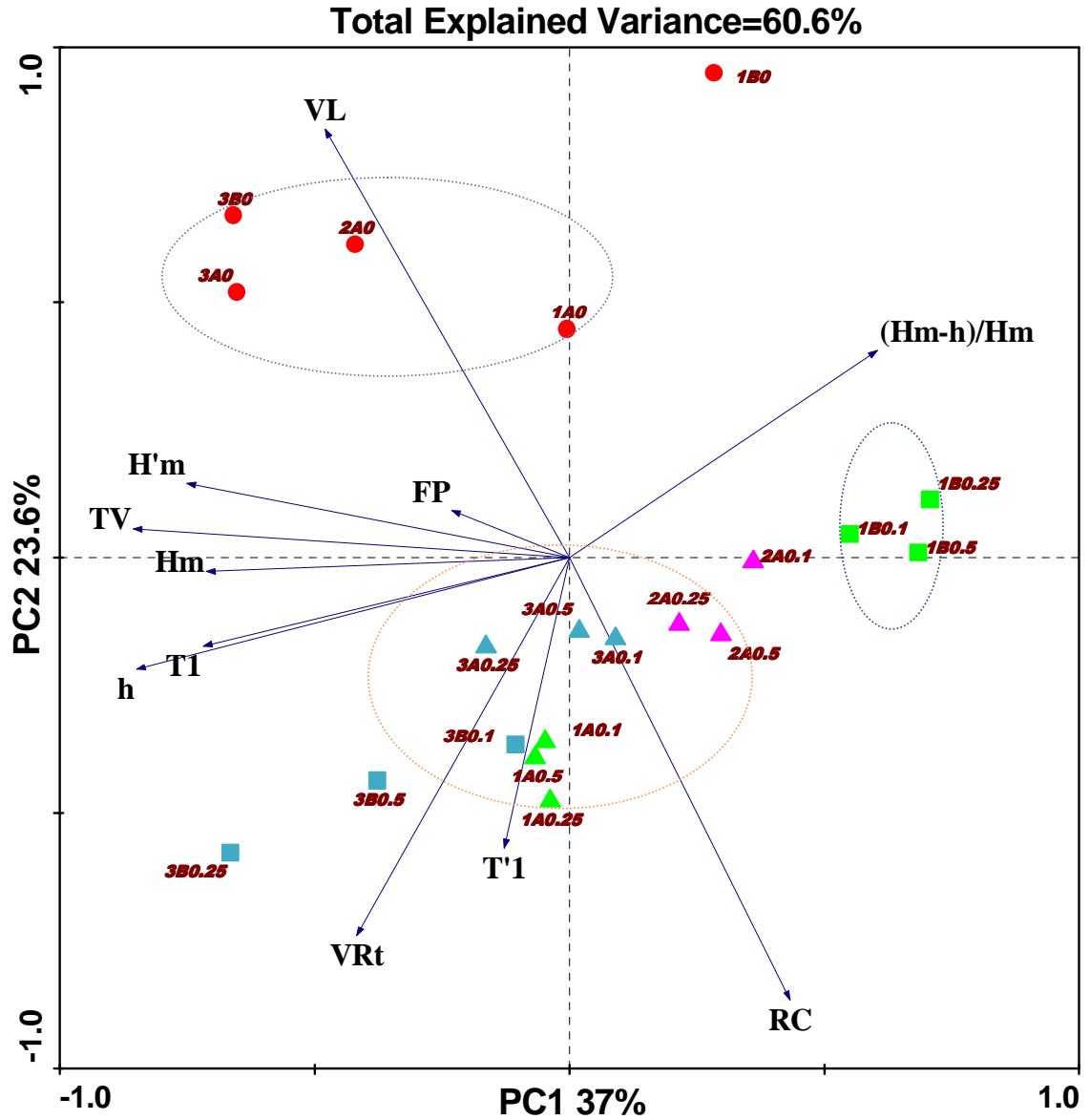


Figure 6. Loading plot of first two principal components based on fermentation properties with flour protein of five commercial wheat flours containing four levels of DTT. Negative control samples were removed. Symbols and definitions: ● -Control samples, ★ - Negative controls, ▲ - Low protein A flours, ▲ - Medium protein A flours, ▲ - High protein A flours. ■ - Low protein B flours, ■ - Medium protein B flours, ■ - High protein B flours.

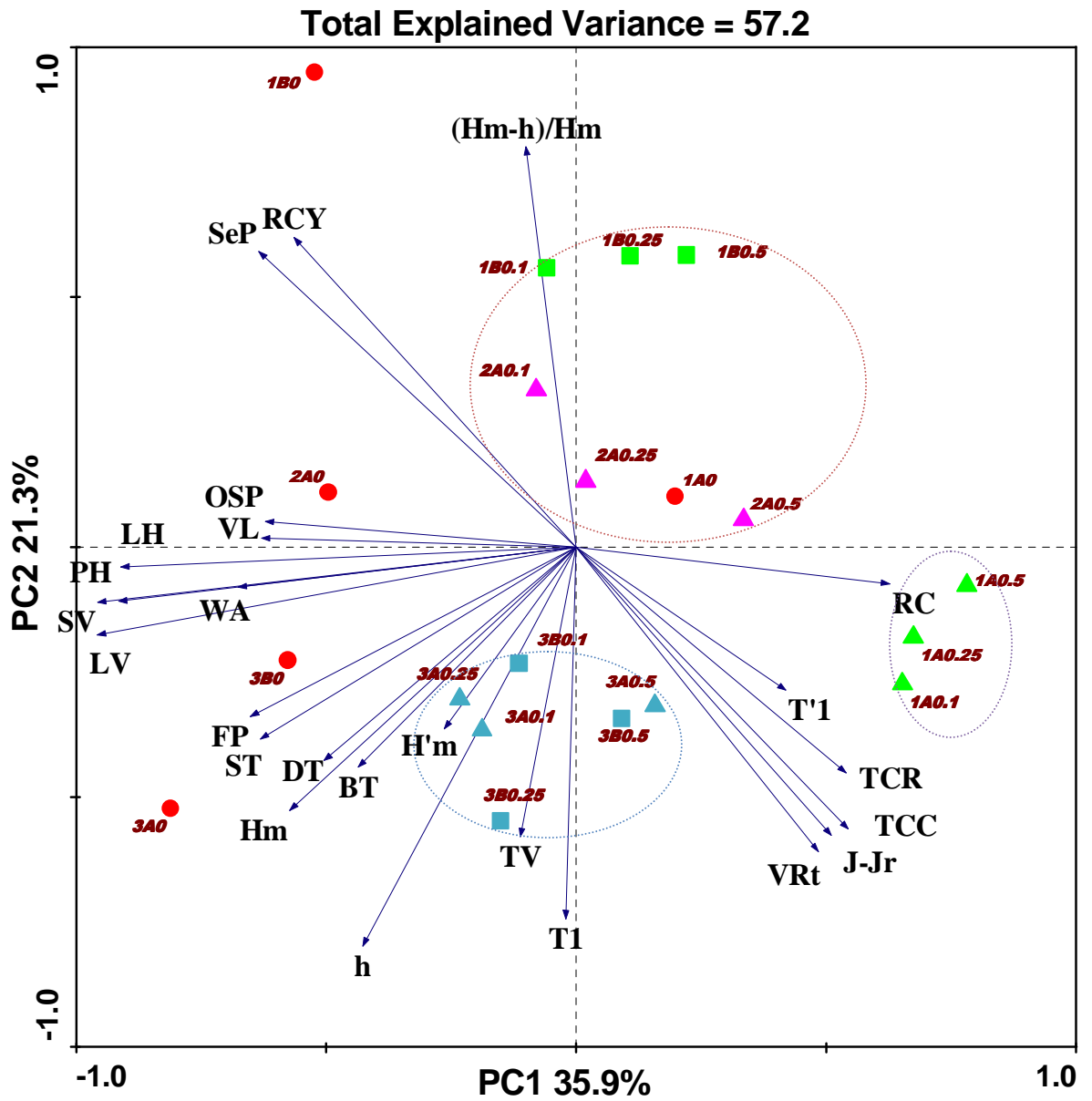


Figure 7. Loading plot of first two principal components based on fermentation, baking, visco-elastic and dough properties of five commercial wheat flours added with four levels of DTT. Symbols and definitions: ● - Control samples, ▲ - Low protein A flours, ▲ - Medium protein A flours, ▲ - High protein A flours, ■ - Low protein B flours, ■ - Medium protein B flours, ■ - High protein B flours.

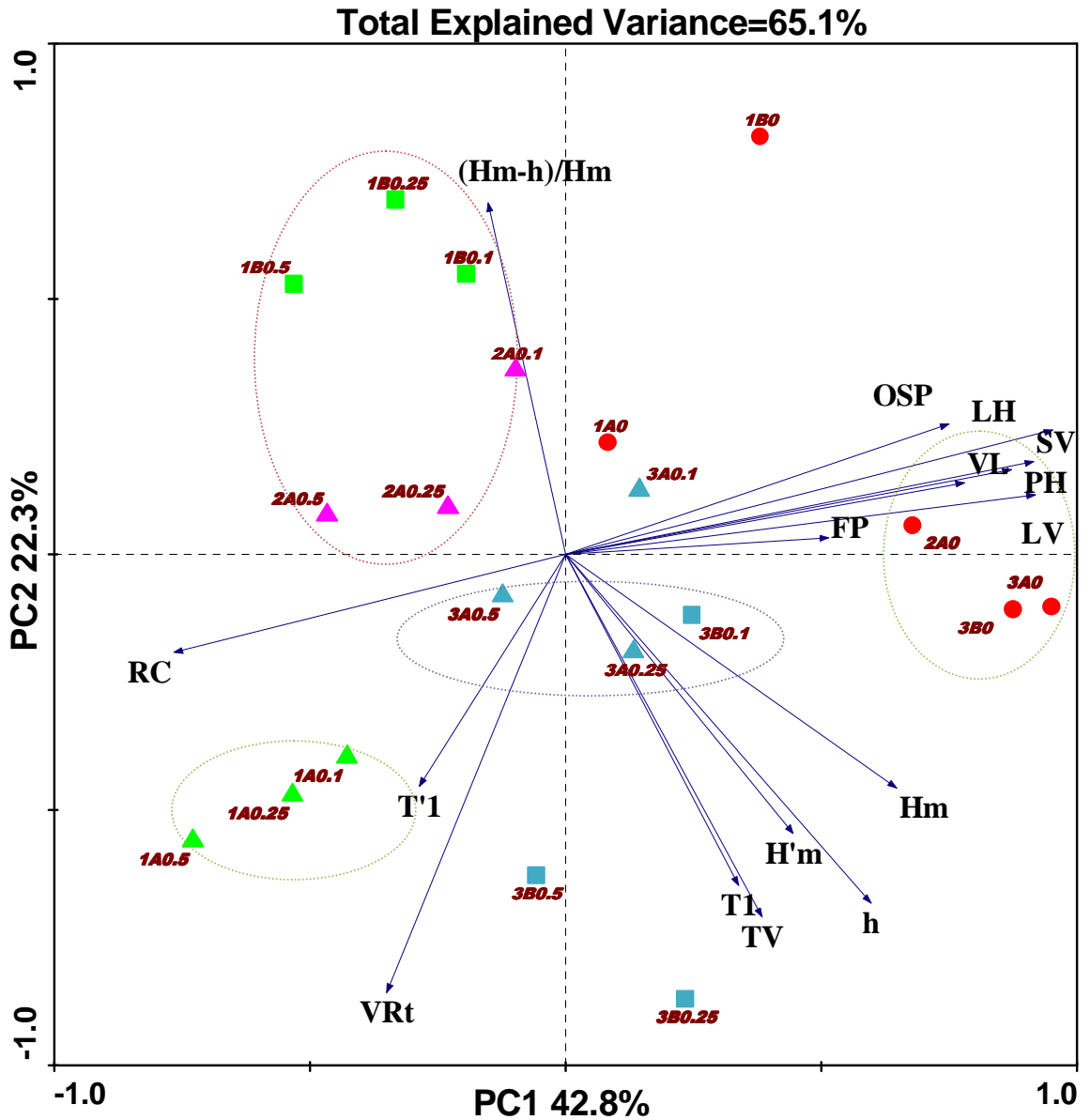


Figure 8. Loading plot of first two principal components based on fermentation and baking properties of five commercial wheat flours added with four levels of DTT. Symbols and definitions: ● -Control samples, ▲ - Low protein A flours, ▲ - Medium protein A flours, ▲ - High protein A flours, ■ - Low protein B flours, ■ - Medium protein B flours, ■ - High protein B flours.

CHAPTER VII CONCLUSIONS

By reducing the surface tension of the dough, height of the dough is significantly improved in 1A and 3B flours, retention volume of the gas is increased (11.3-52.1%). Volume of gas lost is reduced (7.3-16.4%). Retention coefficient is increased (21-38%). Treating the flours with DATEM showed increment in dough development and in percentage of gas retained. DATEM of levels 0.3% and 0.6% showed larger increment when compared to flour treated with 1% of DATEM. Fermentation variables explained more variance (69.2%) than the fermentation variables with visco-elastic, mixing and baking variables (47.9%). The ratio of dough heights $[(H_m-h)/H_m]$ is closely related to gluten elastic properties (Sep and RCY). Volume lost (VL) is closely related to viscous properties (J-Jr, TCR) and negatively related to elastic properties. Maximum height of the dough (H_m) and dough height (h) are closely related to baking properties (LV and SV).

By oxidizing the dough, maximum height of the dough development decreases for A flours and increases for B flours. Maximum height of gaseous release shows an increase with low protein A flours. Highest percentage of retention volume of gas was observed in low protein A flour (1A100). Flours treated with 100 ppm ascorbic acid showed good increment compared to other concentrations. Highest percentage of retention coefficient of gas was observed in medium protein flours. Fermentation variables explained more variance (61.7%) than fermentation variables with visco-elastic, mixing and baking variables (51.5%). The ratio of dough heights $[(H_m-h)/H_m]$ and volume lost (VL) are closely related to gluten elastic properties (Sep and RCY). The time

taken to reach maximum height of the dough (T1) is closely related to gluten viscous (TCC) and baking properties (OSP). Maximum height of the dough (Hm) and dough height (h) are closely related to flour protein (FP) and baking properties (LH and LV).

The effect of disruption of hydrogen and hydrophobic bonds decreased maximum height of gaseous release, total volume of gas and volume lost. Fermentation variables explained more variance (67.7%) than fermentation variables with visco-elastic, mixing and baking variables (53.1%). The ratio of dough heights [(Hm-h)/Hm] is closely related to gluten elastic properties (Sep and RCY). The time taken to reach maximum height of dough development (T1) and time taken to reach maximum height of gaseous release (T'1) are closely related to viscous properties (TCC and TCR). Total volume (TV) and maximum height of gaseous release (H'm) are closely related to flour protein. Retention coefficient (RC) is negatively related to baking and mixing properties.

Disruption of disulfide bonds decreases maximum height of the dough and maximum height of the gaseous release for most flours. Total volume and volume lost is also decreased. Fermentation variables explained more variance (66.2%) than fermentation variables with visco-elastic, mixing and baking variables (57.2%). The ratio of dough heights [(Hm-h)/Hm] is closely related to gluten elastic properties (Sep and RCY). Retention coefficient (RC) is closely related to gluten viscous (TCC and TCR). Maximum height of the dough (Hm) and height of the dough at the end of the test (h) are closely related to flour protein (FP). Volume lost (VL) is closely related to baking properties (LH and SV).

Overall correlation of fermentation properties with visco-elastic, mixing and baking properties: ratio of dough heights $[(H_m-h)/H_m]$ is closely related to gluten elastic properties. In the red-ox state, the time taken to reach maximum height of dough development (T1) is closely related to gluten viscous and height of the dough development (h) is closely related to flour protein. Retention coefficient (RC) is not useful in predicting baking properties as it is negatively related to flour protein and baking. Mixing properties are not related to fermentation.

CHAPTER VIII FUTURE STUDIES

The study focused on effect of fermentation properties of flours with DATEM (surfactant), ascorbic acid (oxidizing agent), urea (non covalent hydrogen bond disruption in glutenin) and DTT (disulfide linkage disruption in glutenin). Correlations were identified among fermentation, visco-elastic, baking, mixing properties in dough to establish the relationship between fermentation, visco-elastic, baking and mixing properties. Fermentation properties were measured by Rheofermentometer.

Although the different levels of DATEM, ascorbic acid, urea and DTT used were based in ranges of literature reports, it will be advisable to optimize the concentrations for each chemical reagent used. This can be achieved in a separate study with appropriate experimental design and statistical modeling using response surface methodology. Another suggestion is to increase the number of replicates (experimental units) to three or four. This will increase the power of the analysis.

APPENDIX 1

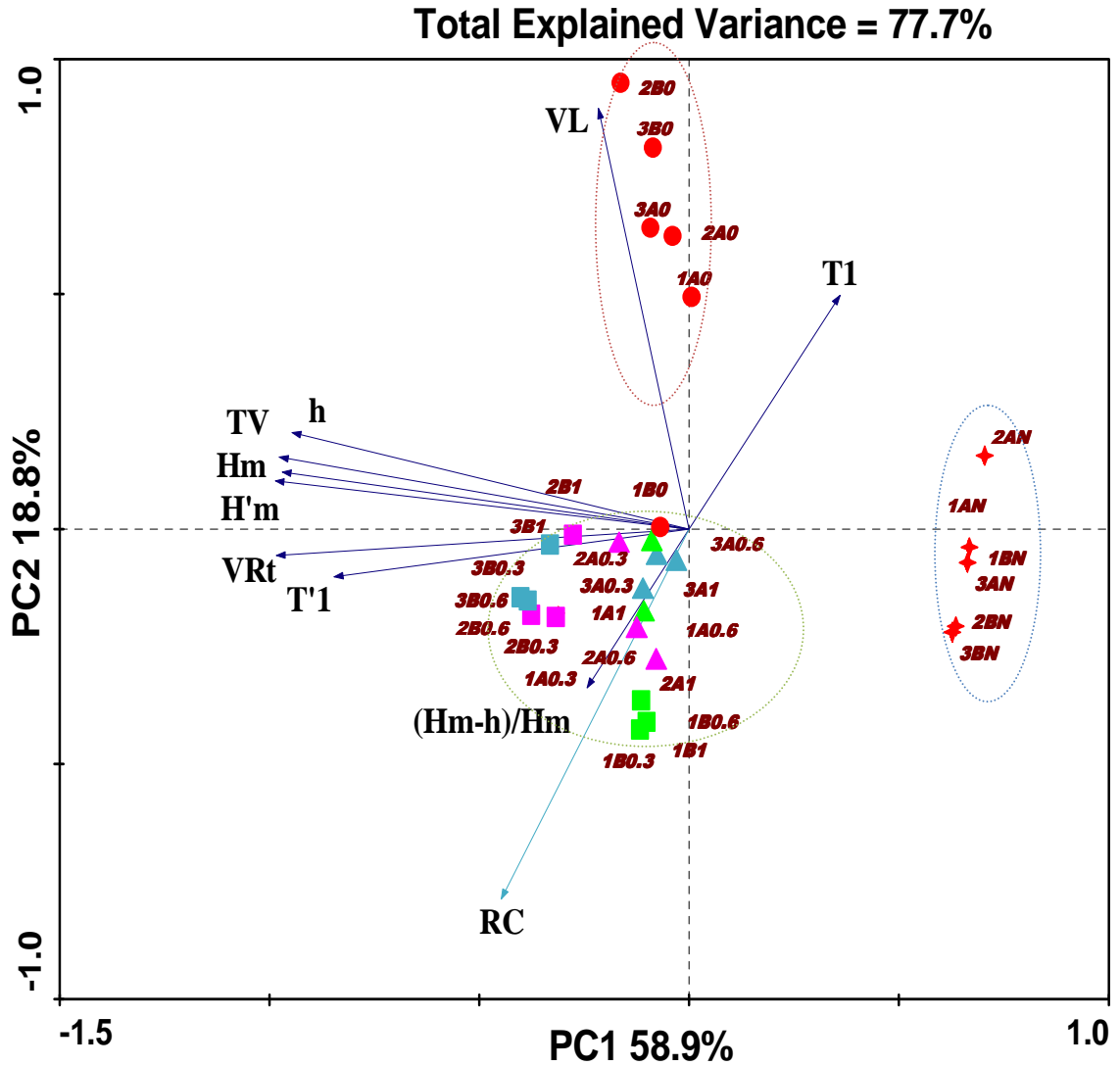


Figure 1. Loading plot of first two principal components based on fermentation properties with negative control of six commercial wheat flours, added with four levels of DATEM. Definitions of fermentation, visco-elastic, mixing and baking variables explained in Table 2 and 3. Flour protein content (%), 1A = 7.95, 2A = 11.19, 3A = 13.68, 1B = 10.4, 2B = 10.59 and 3B = 11.38, respectively. Symbols and definitions: ● -Control samples, ★ - Negative controls. ▲ - Low protein A flours, ▲ - Medium protein A flours, ▲ - High protein A flours. ■ - Low protein B flours, ■ - Medium protein B flours, ■ - High protein B flours.

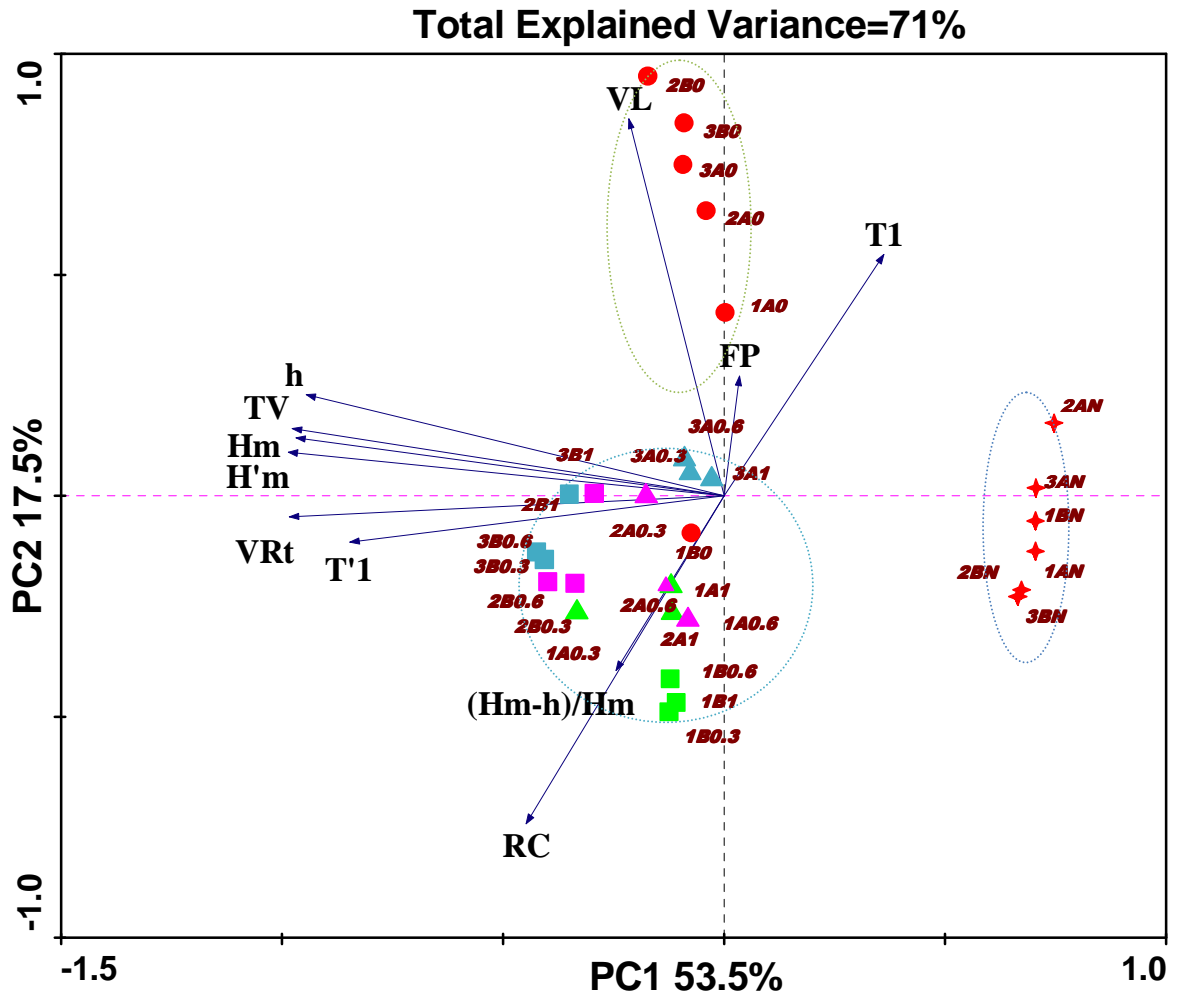


Figure 2. Loading plot of first two principal components based on fermentation properties with flour protein of six commercial wheat flours added with four levels of DATEM. Symbols and definitions: ● -Control samples, ★ - Negative controls, ▲ - Low protein A flours, ▲ -Medium protein A flours, ▲ - High protein A flours. ■ - Low protein B flours, ■ - Medium protein B flours, ■ - High protein B flours.

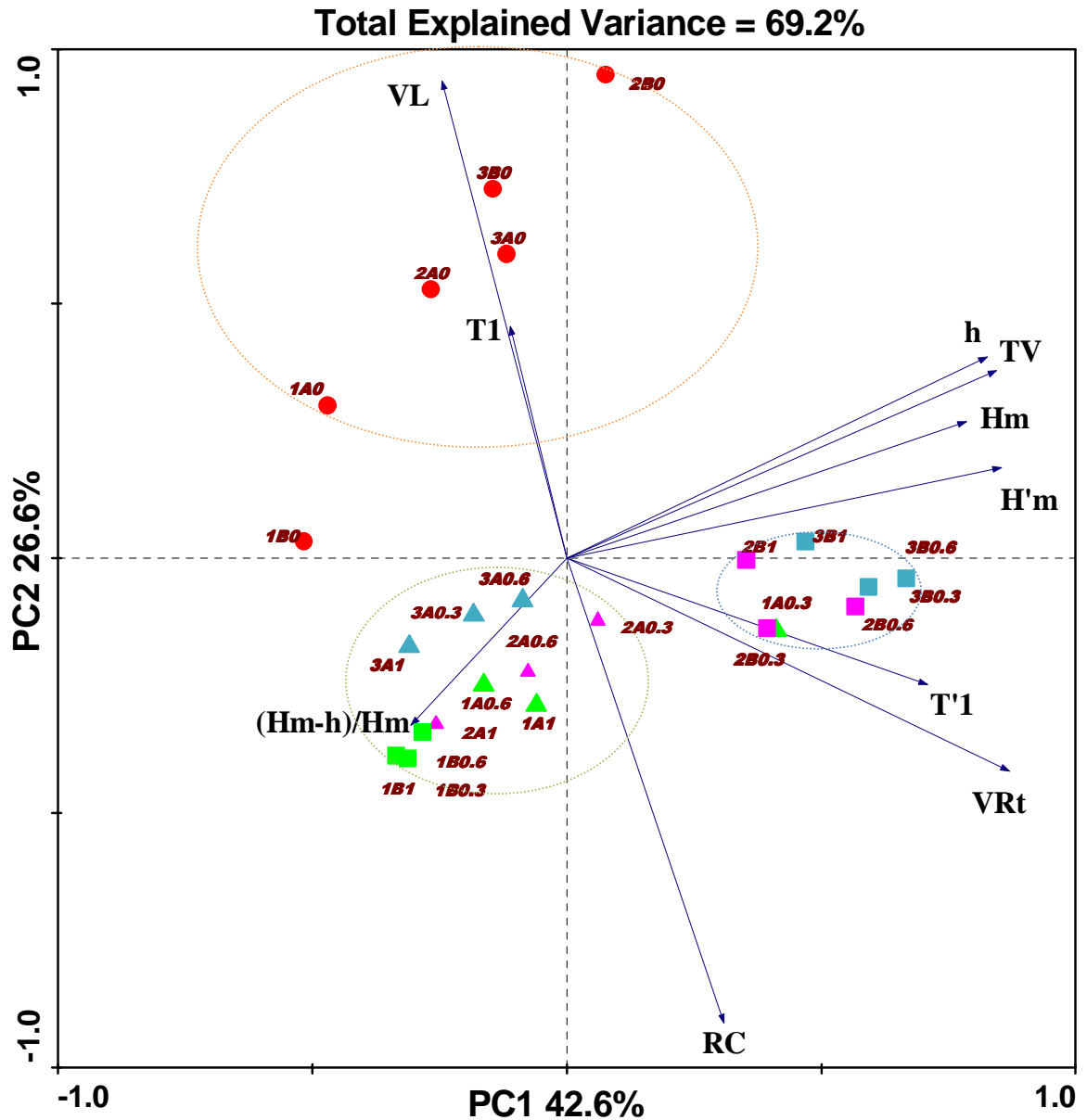


Figure 3. Loading plot of first two principal components based on fermentation properties without negative control of six commercial wheat flours added with four levels of DATEM. Symbols and definitions: ● -Control samples, ▲ - Low protein A flours, ▲ - Medium protein A flours, ▲ - High protein A flours. ■ - Low protein B flours, ■ - Medium protein B flours, ■ - High protein B flours.

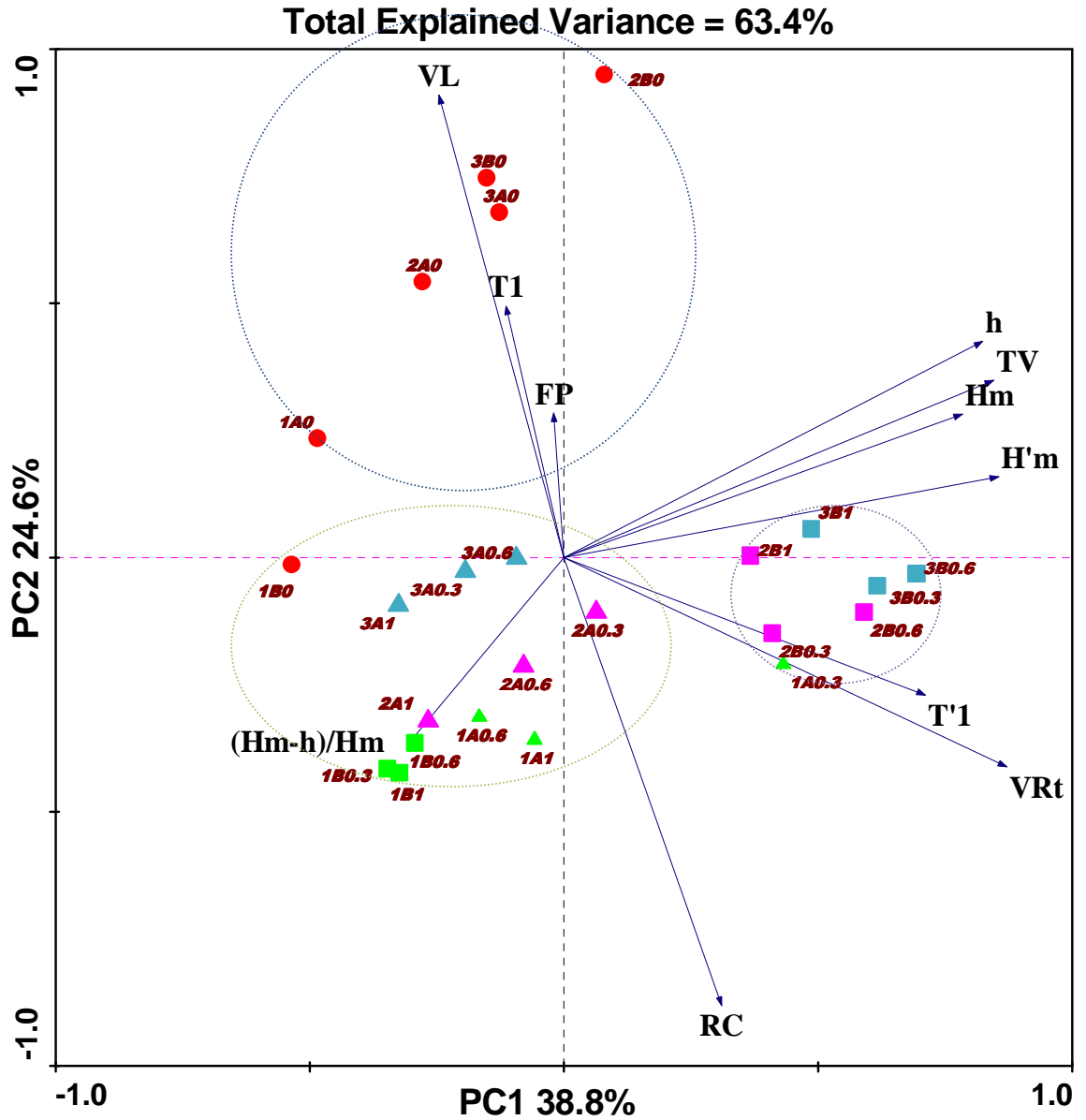


Figure 4. Loading plot of first two principal components based on fermentation properties with flour protein of six commercial wheat flours containing four levels of DATEM. Negative control samples were removed. Symbols and definitions: ● -Control samples, ▲ -Low protein A flours, ▲ -Medium protein A flours, ▲ - High protein A flours, ■ - Low protein B flours, ■ - Medium protein B flours, ■ - High protein B flours.

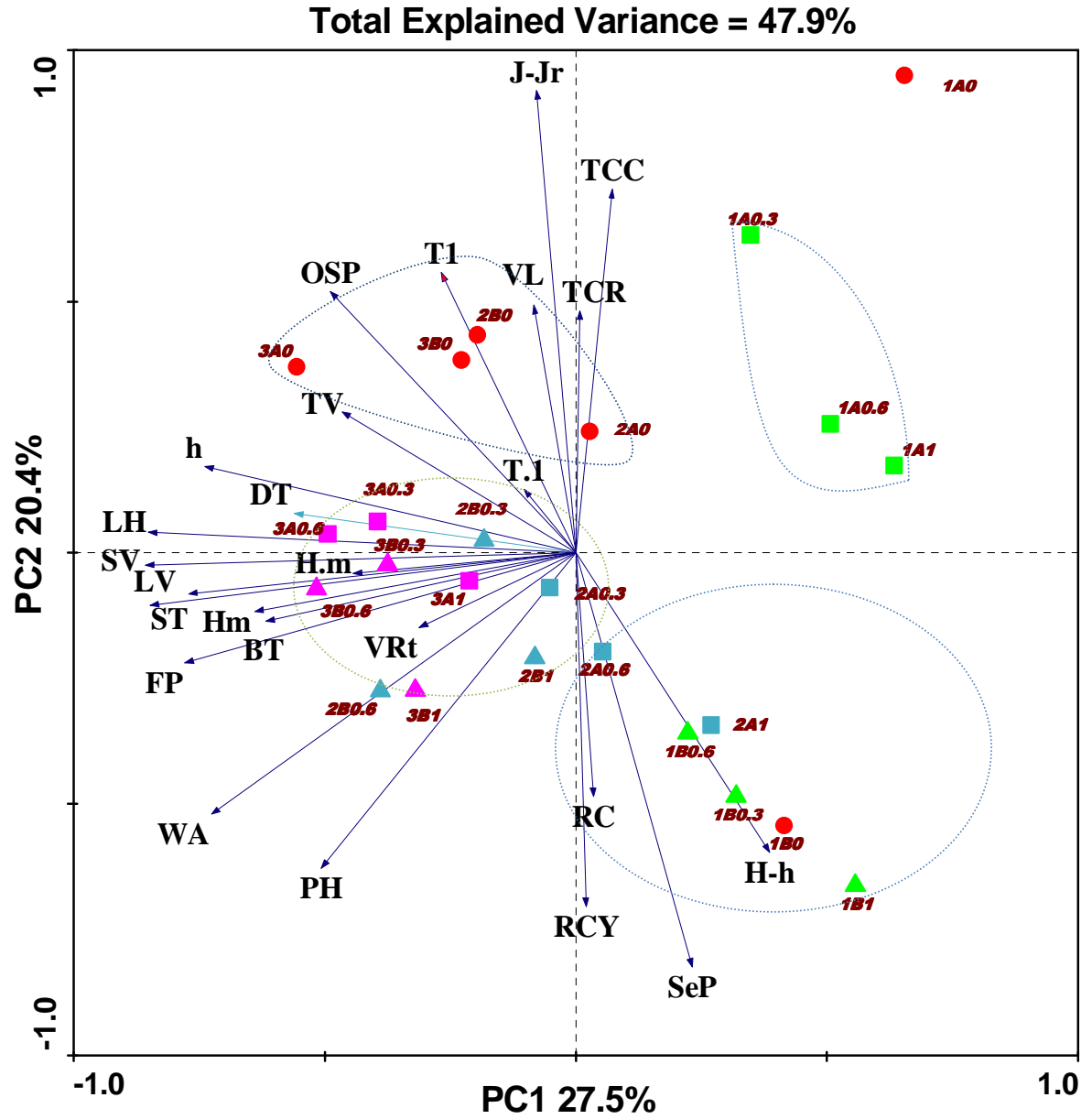


Figure 5. Loading plot of first two principal components based on fermentation, baking, visco-elastic and dough properties of six commercial wheat flours added with four levels of DATEM. Symbols and definitions: ●-Control samples, ▲- Low protein A flours, ▲- Medium protein A flours, ▲- High protein A flours. ■- Low protein B flours, ■- Medium protein B flours, ■- High protein B flours.

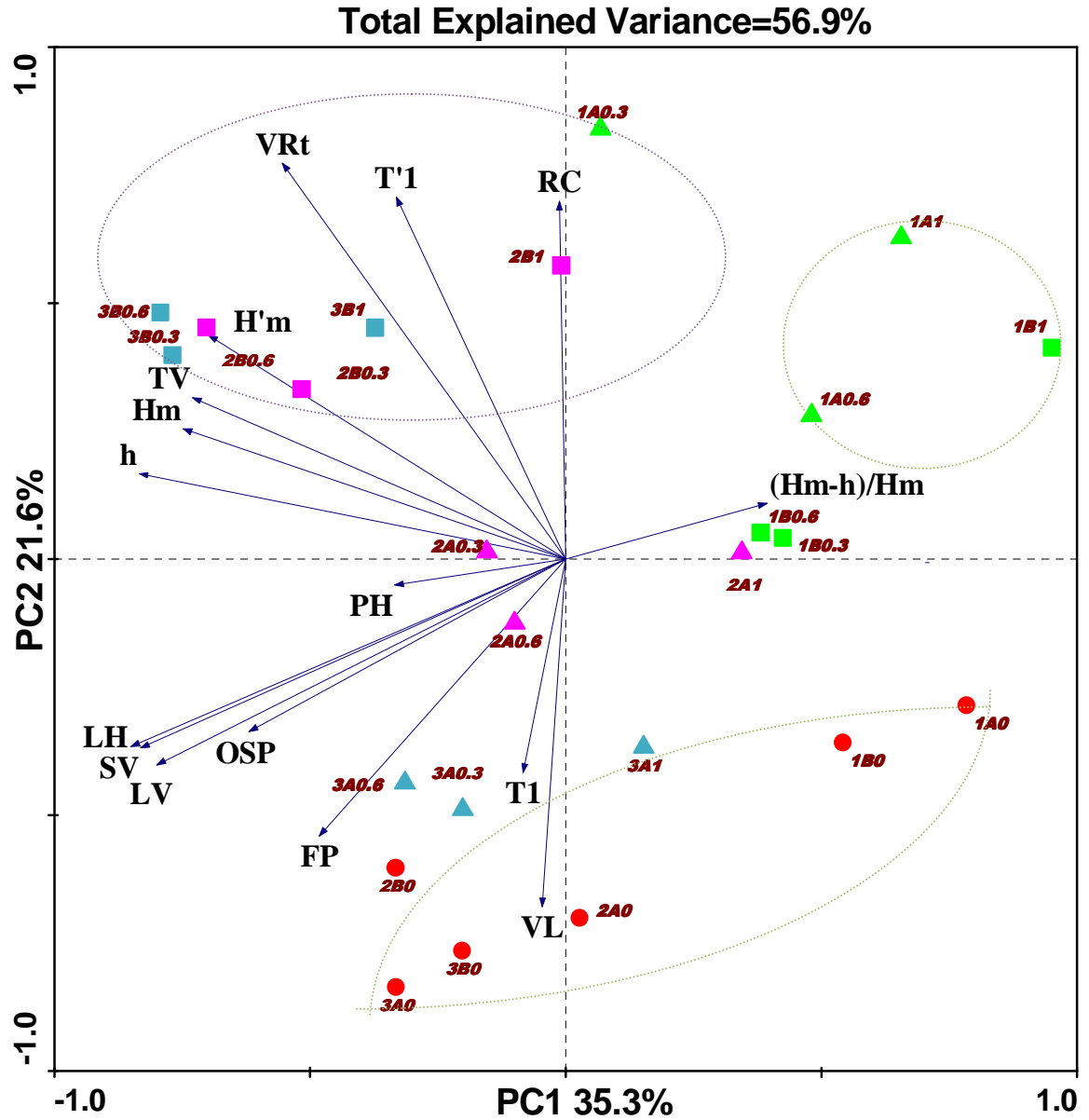


Figure 6. Loading plot of first two principal components based on fermentation and baking properties of six commercial wheat flours added with four levels of DATEM. Symbols and definitions: ● -Control samples, ▲ - Low protein A flours, ▲ - Medium protein A flours, ▲ - High protein A flours. ■ - Low protein B flours, ■ - Medium protein B flours, ■ - High protein B flours.

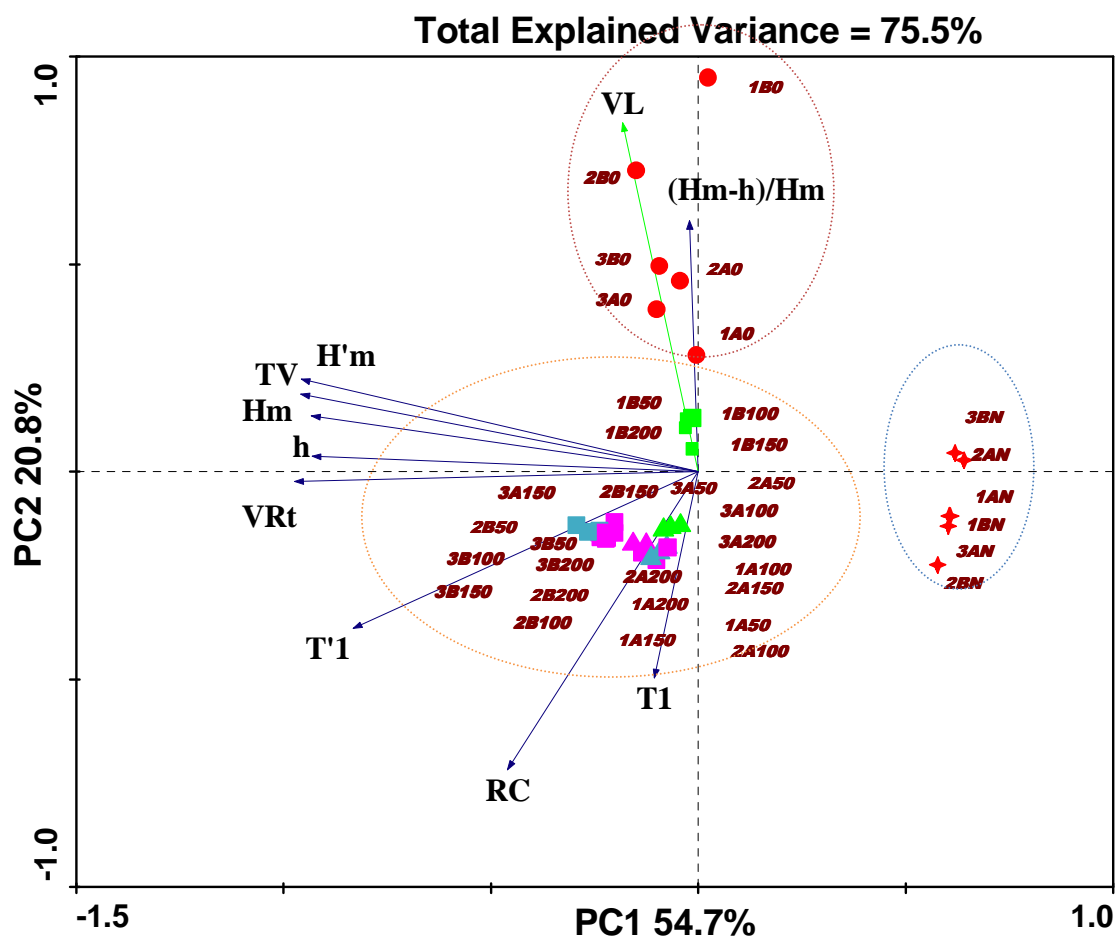


Figure 7. Loading plot of first two principal components based on fermentation properties with negative control of six commercial wheat flours, added with five levels of ascorbic acid. Definitions of fermentation, visco-elastic, mixing and baking variables explained in Table 2 and 3. Flour protein content (%), 1A = 7.95, 2A = 11.19, 3A = 13.68, 1B = 10.4, 2B = 10.59 and 3B = 11.38, respectively. Symbols and definitions: ● -Control samples, ★ - Negative controls, ▲ - Low protein A flours, ▲ - Medium protein A flours, ▲ - High protein A flours. ■ - Low protein B flours, ■ - Medium protein B flours, ■ - High protein B flours.

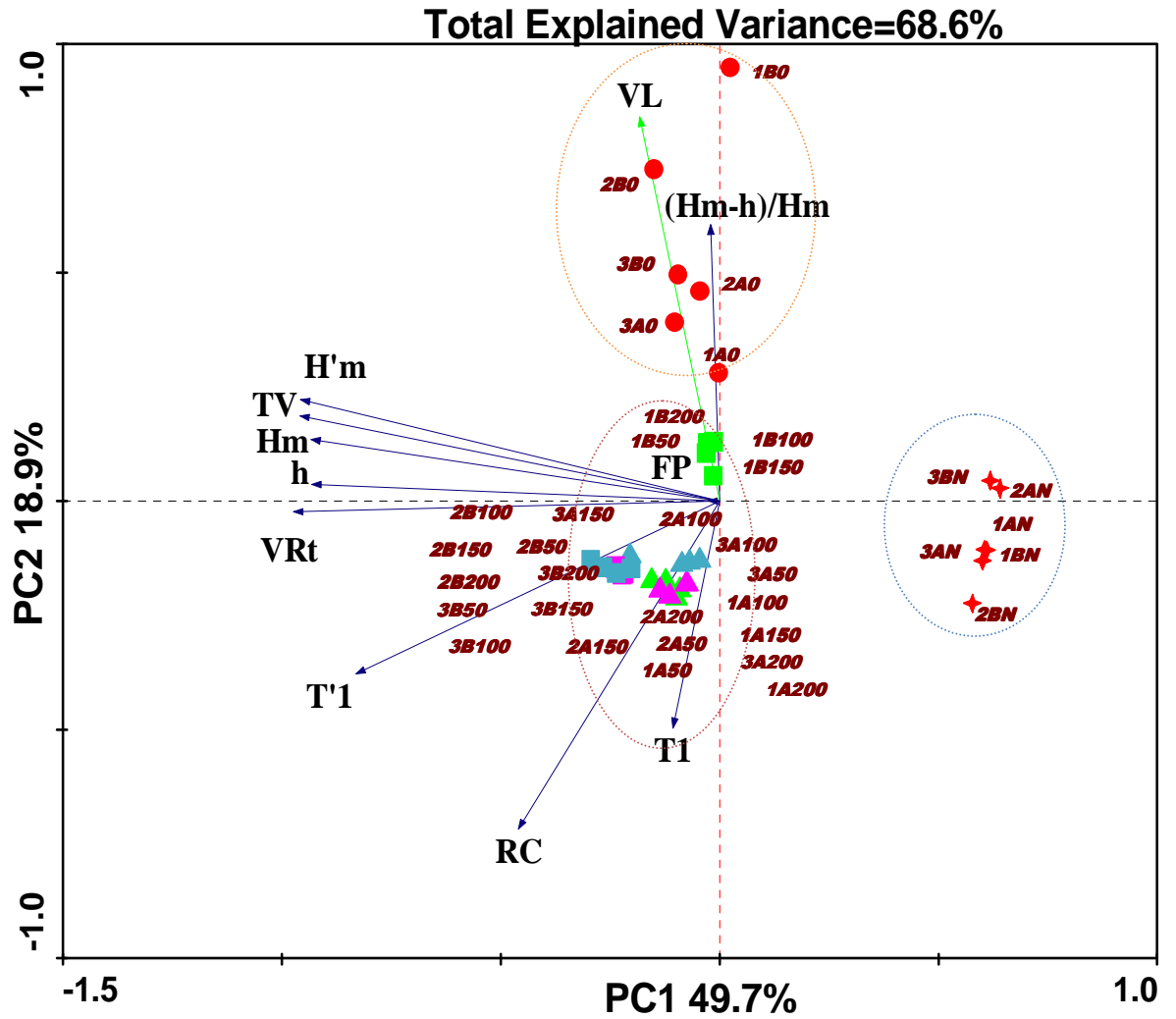


Figure 8. Loading plot of first two principal components based on fermentation properties with flour protein of six commercial wheat flours added with five levels of ascorbic acid. Symbols and definitions: ● -Control samples, ★- Negative controls, ▲- Low protein A flours, ▲-Medium protein A flours, ▲- High protein A flours, ■ - Low protein B flours, ■Medium protein B flours, ■- High protein B flours.

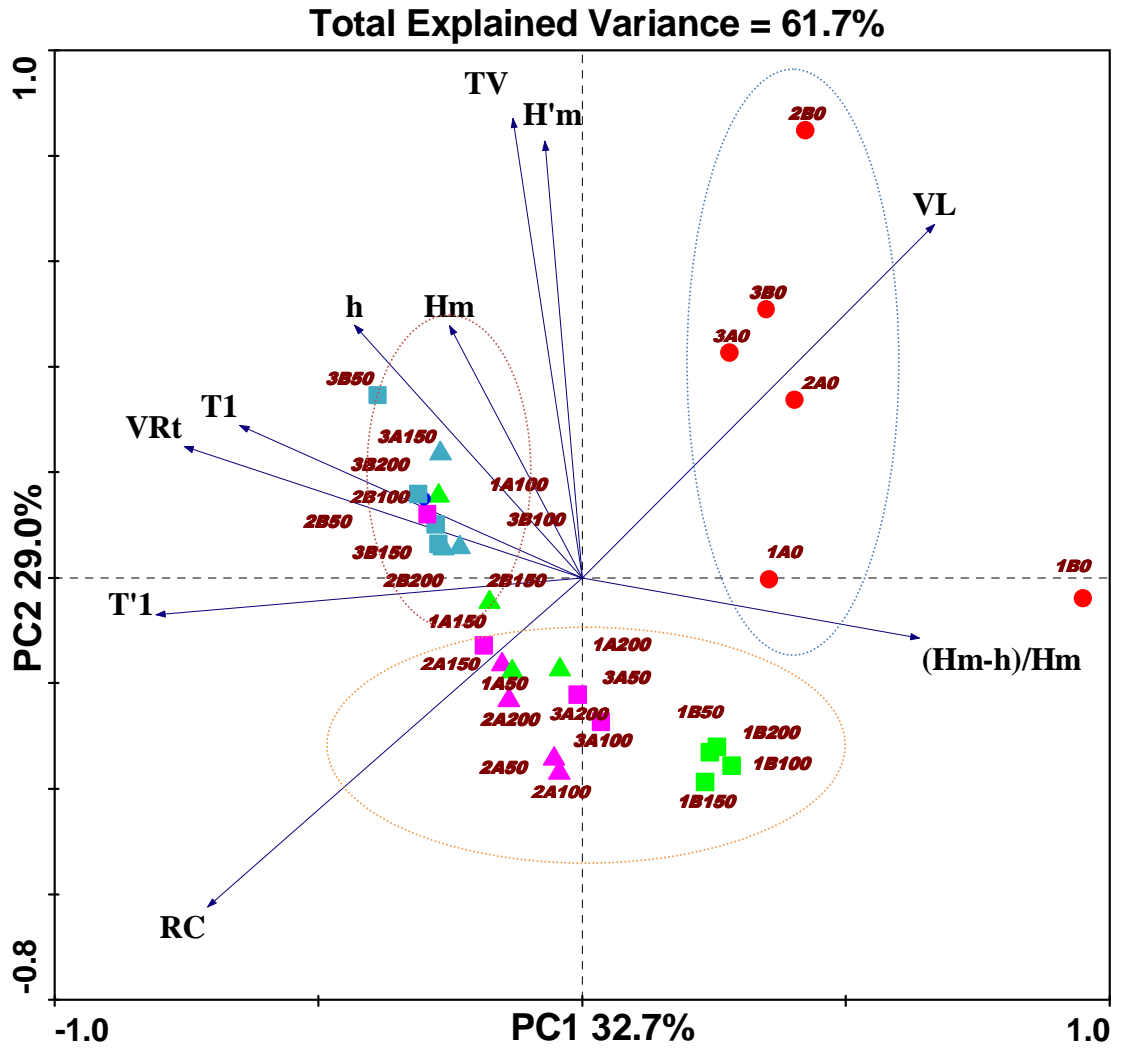


Figure 9. Loading plot of first two principal components based on fermentation properties without negative control of six commercial wheat flours added with five levels of ascorbic acid. Symbols and definitions: ● - Control samples, ▲ - Low protein A flours, ▲ - Medium protein A flours, ▲ - High protein A flours. ■ - Low protein B flours ■ - Medium protein B flours, ■ - High protein B flours.

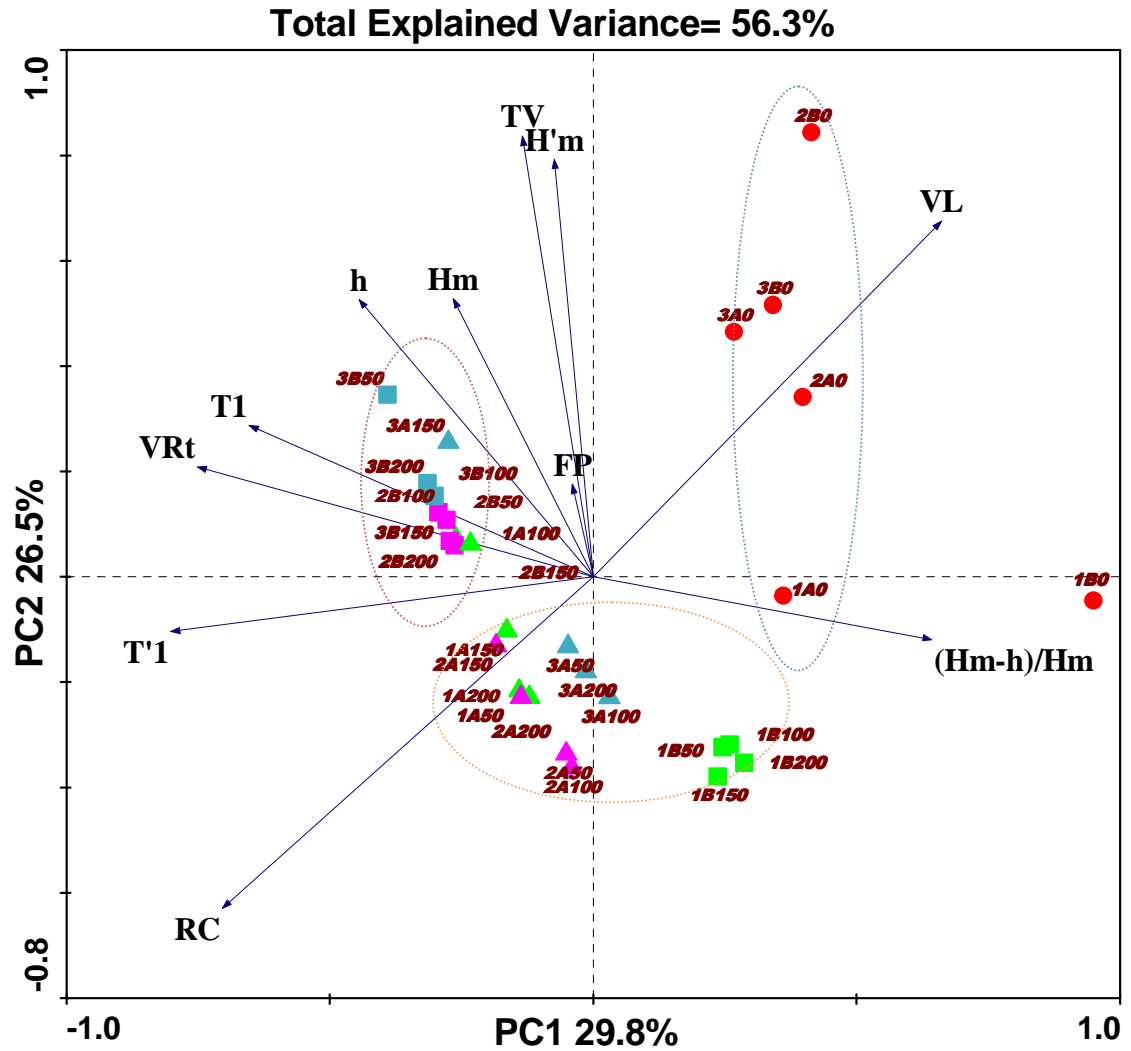


Figure 10. Loading plot of first two principal components based on fermentation properties with flour protein of six commercial wheat flours containing five levels of ascorbic acid. Negative control samples were removed. Symbols and definitions: ● - Control samples, ▲ - Low protein A flours, ▲ - Medium protein A flours, ▲ - High protein A flours. ■ - Low protein B flours, ■ - Medium protein B flours, ■ - High protein B flours.

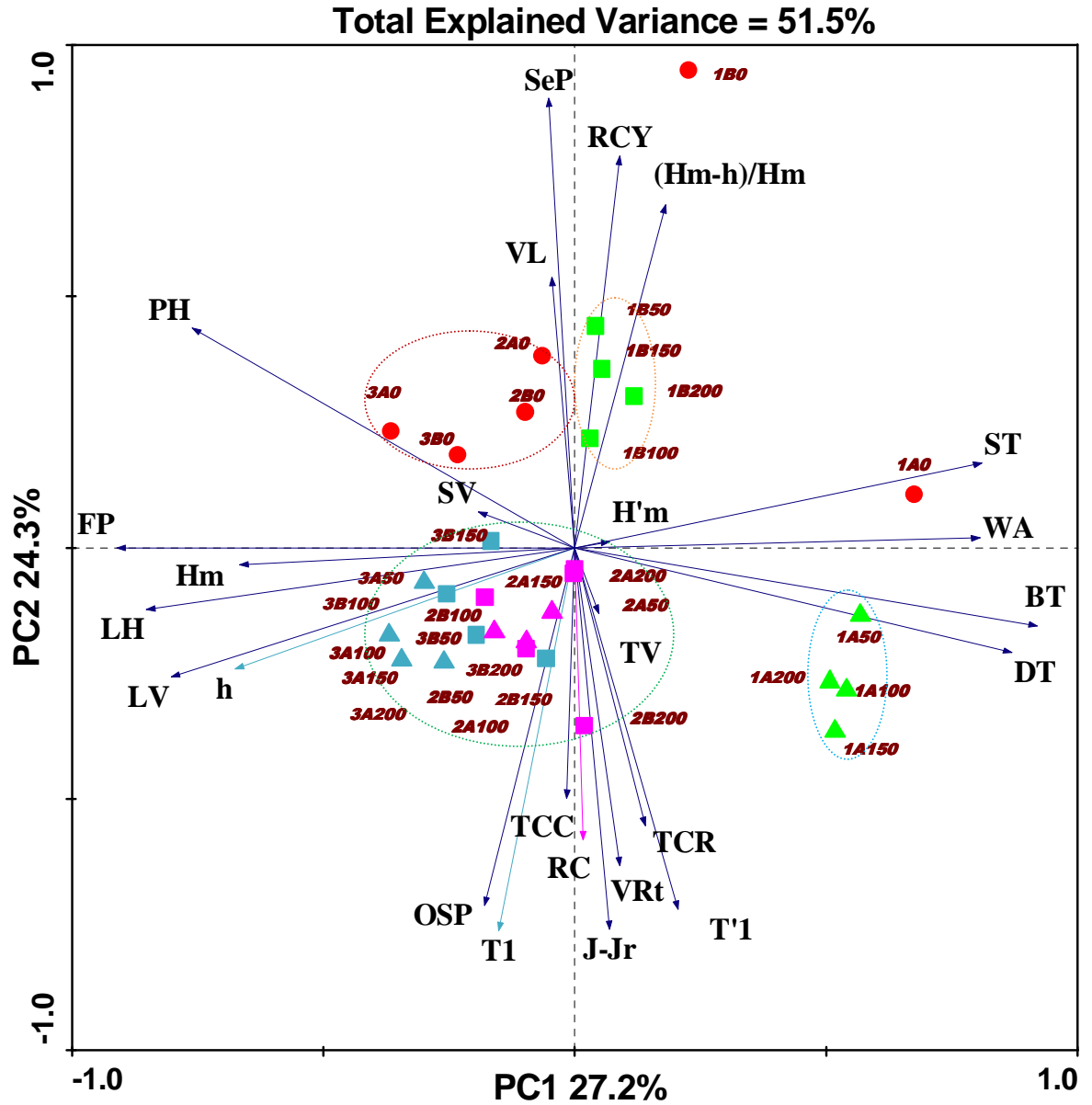


Figure 11. Loading plot of first two principal components based on fermentation, baking, visco-elastic and dough properties of six commercial wheat flours added with five levels of ascorbic acid. Symbols and definitions: ● -Control samples, ▲ - Low protein A flours, ▲ -Medium protein A flours, ▲ - High protein A flours, ■ - Low protein B flours, ■ - Medium protein B flours, ■ - High protein B flours.

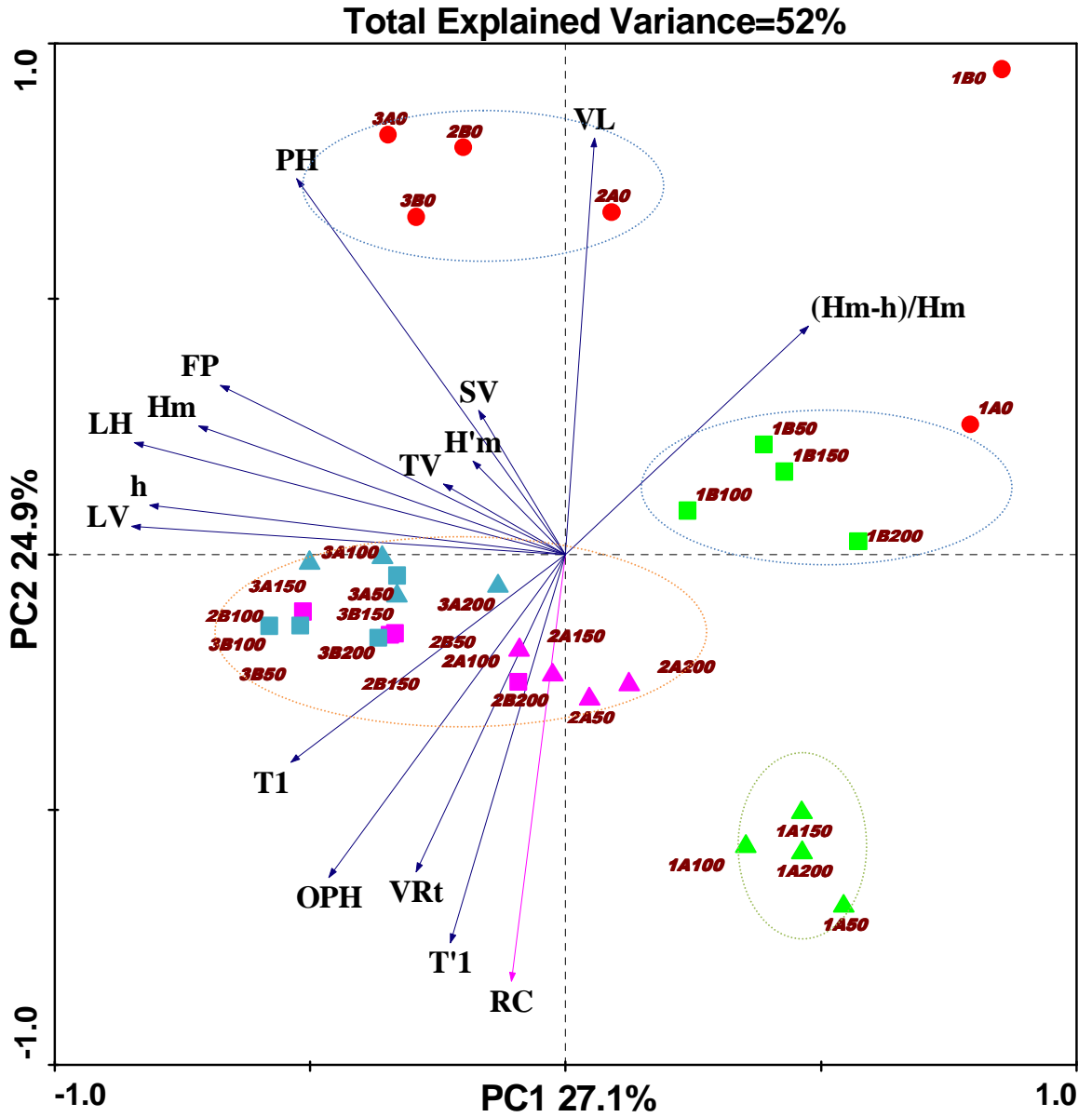


Figure 12. Loading plot of first two principal components based on fermentation and baking properties of six commercial wheat flours added with five levels of ascorbic acid. Symbols and definitions: ● -Control samples, ▲ - Low protein A flours, ▲ - Medium protein A flours, ▲ - High protein A flours. ■ - Low protein B flours, ■ - Medium protein B flours, ■ - High protein B flours.

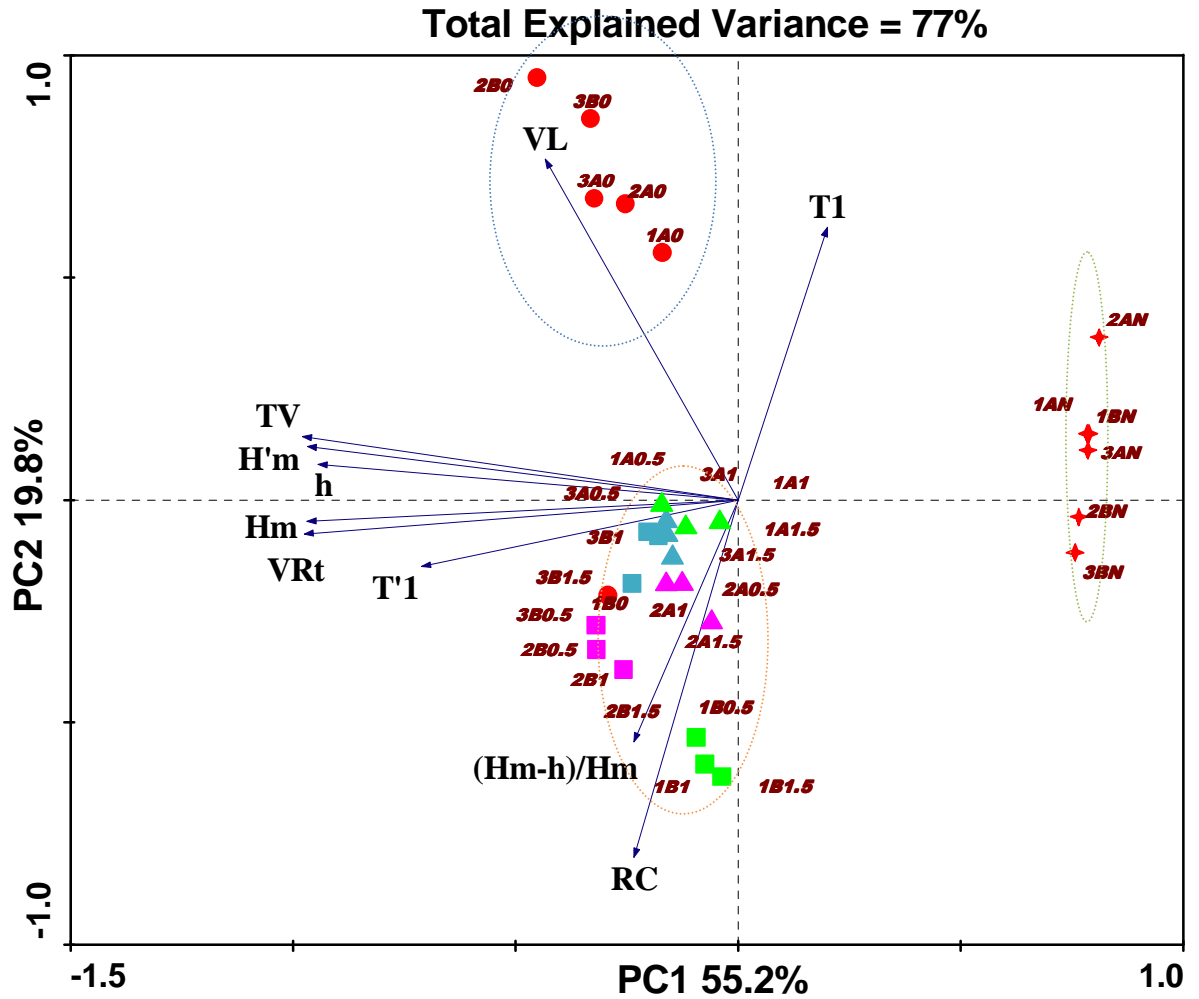


Figure.13. Loading plot of first two principal components based on fermentation properties with negative control of six commercial wheat flours, added with four levels of urea. Definitions of fermentation, visco-elastic, mixing and baking variables explained in Table 2 and 3. Flour protein content (%), 1A = 7.95, 2A = 11.19, 3A = 13.68, 1B = 10.4, 2B = 10.59 and 3B = 11.38, respectively. Symbols and definitions: ● -Control samples, ★ - Negative controls, ▲ - Low protein A flours, ▲ - Medium protein A flours, ▲ - High protein A flours. ■ - Low protein B flours, ■ - Medium protein B flours, ■ - High protein B flours.

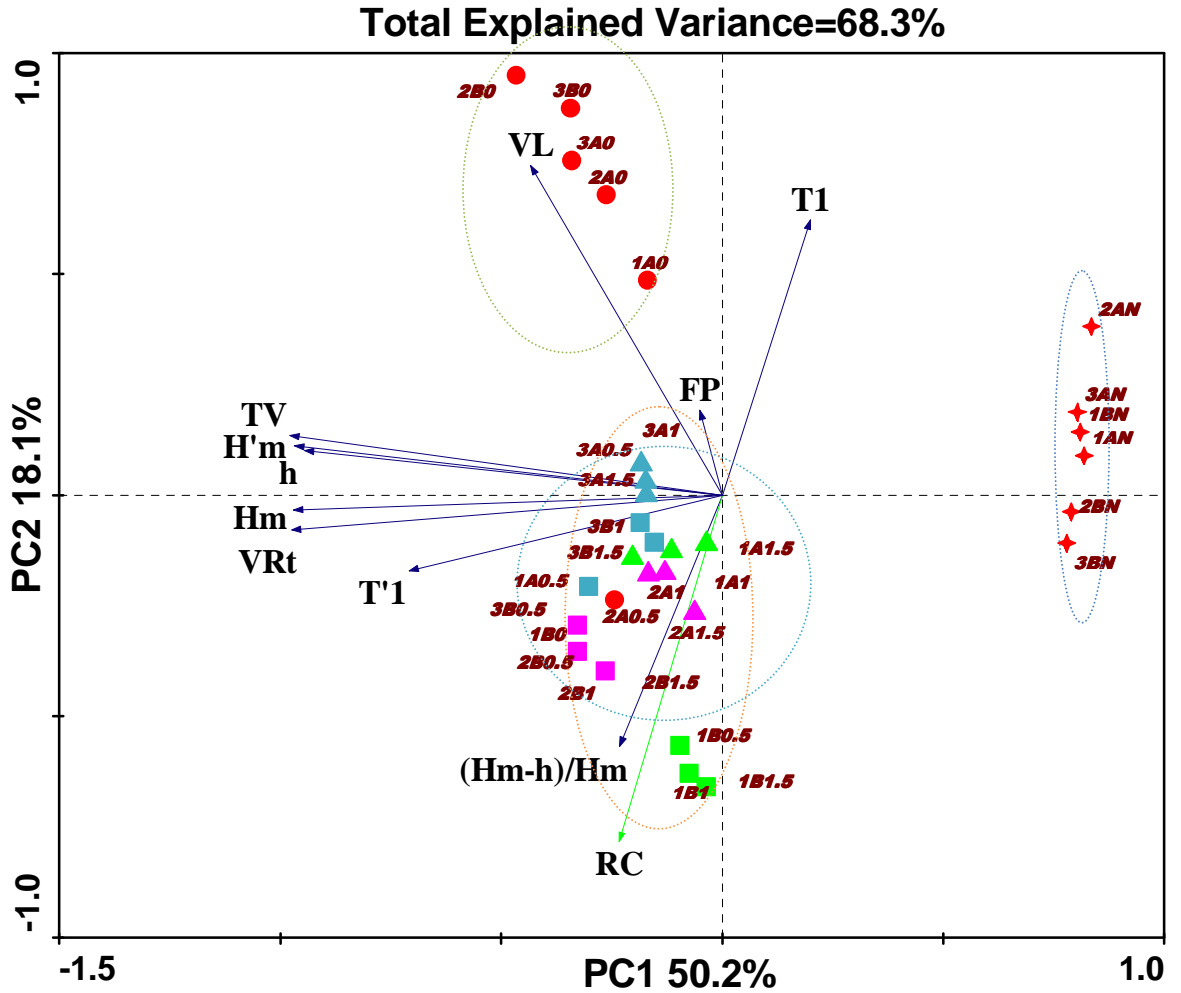


Figure.14. Loading plot of first two principal components based on fermentation properties with flour protein of six commercial wheat flours added with four levels of urea. Symbols and definitions: ● -Control samples, ★ - Negative controls, ▲ – Low protein A flours, ▲ -Medium protein A flours, ▲ - High protein A flours. ■ – Low protein B flours, ■ - Medium protein B flours, ■ - High protein B flours.

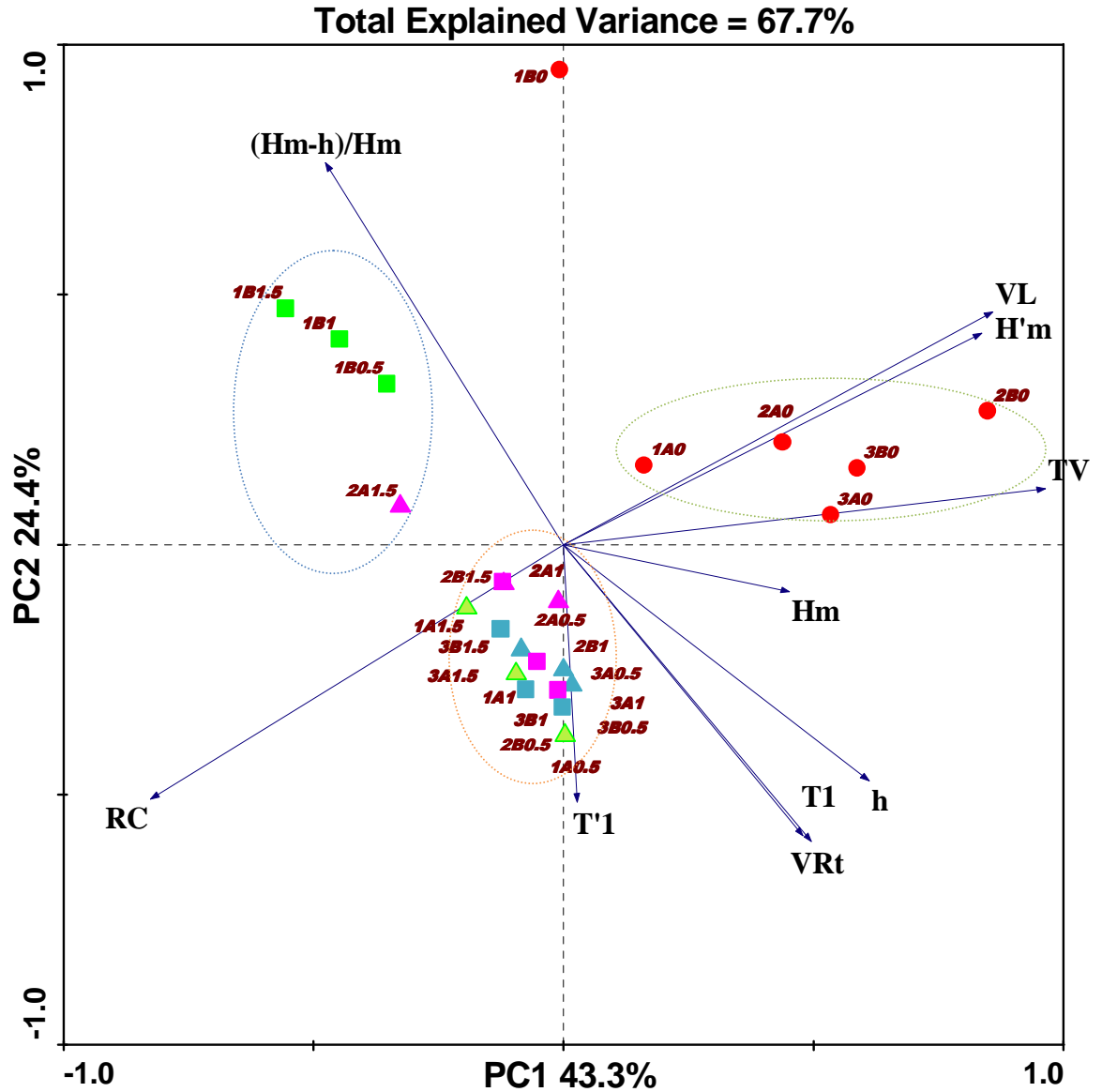


Figure. 15. Loading plot of first two principal components based on fermentation properties without negative control of six commercial wheat flours added with four levels of urea. Symbols and definitions: ● -Control samples, ▲ - Low protein A flours, ▲ - Medium protein A flours, ▲ - High protein A flours. ■ - Low protein B flours, ■ - Medium protein B flours, ■ - High protein B flours.

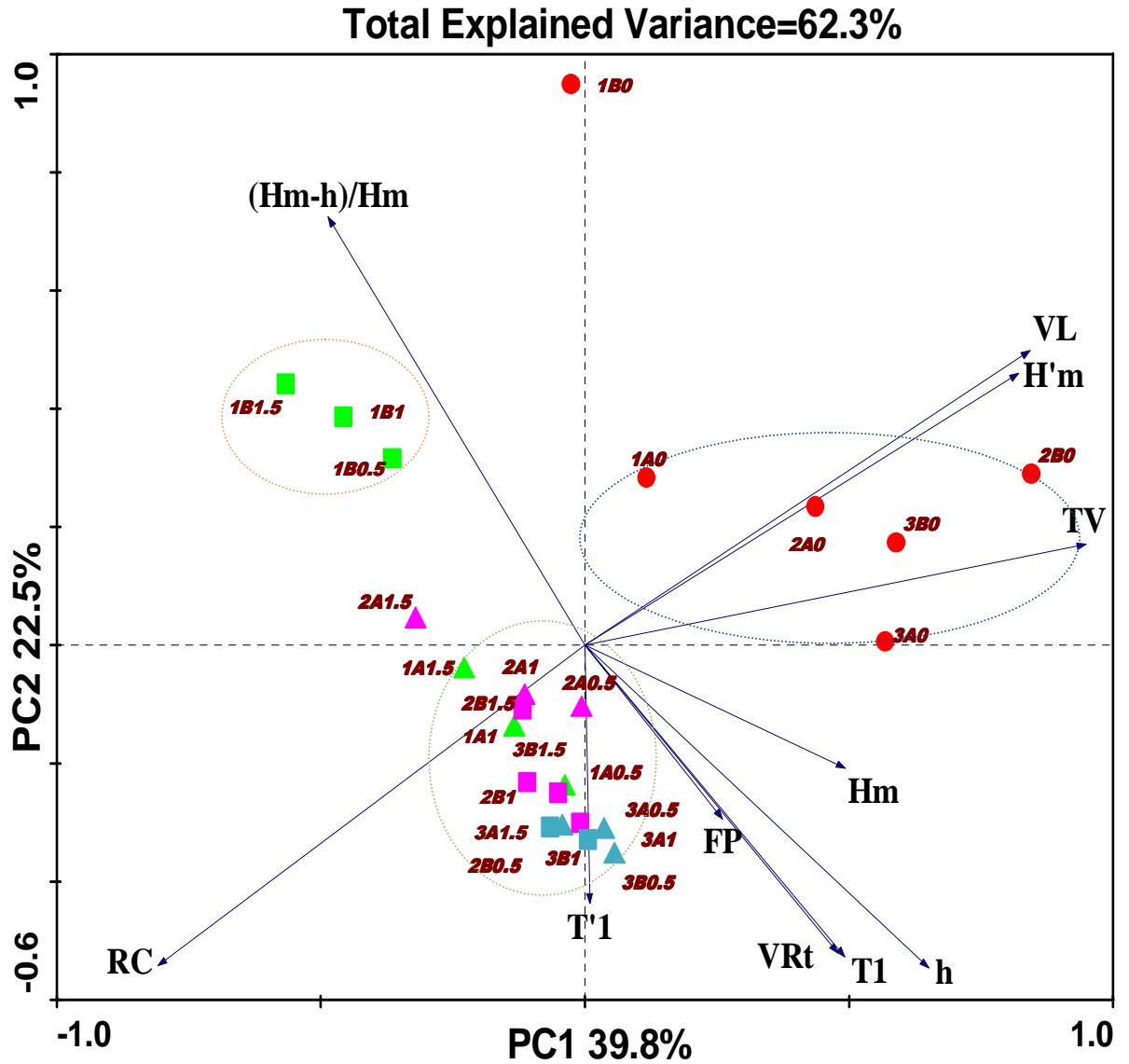


Figure. 16. Loading plot of first two principal components based on fermentation properties with flour protein of six commercial wheat flours containing four levels of urea. Negative control samples were removed. Symbols and definitions: ● -Control samples, ▲ - Low protein A flours, ▲ - Medium protein A flours, ▲ - High protein A flours. ■ Low protein B flours, ■ - Medium protein B flours, ■ - High protein B flours.

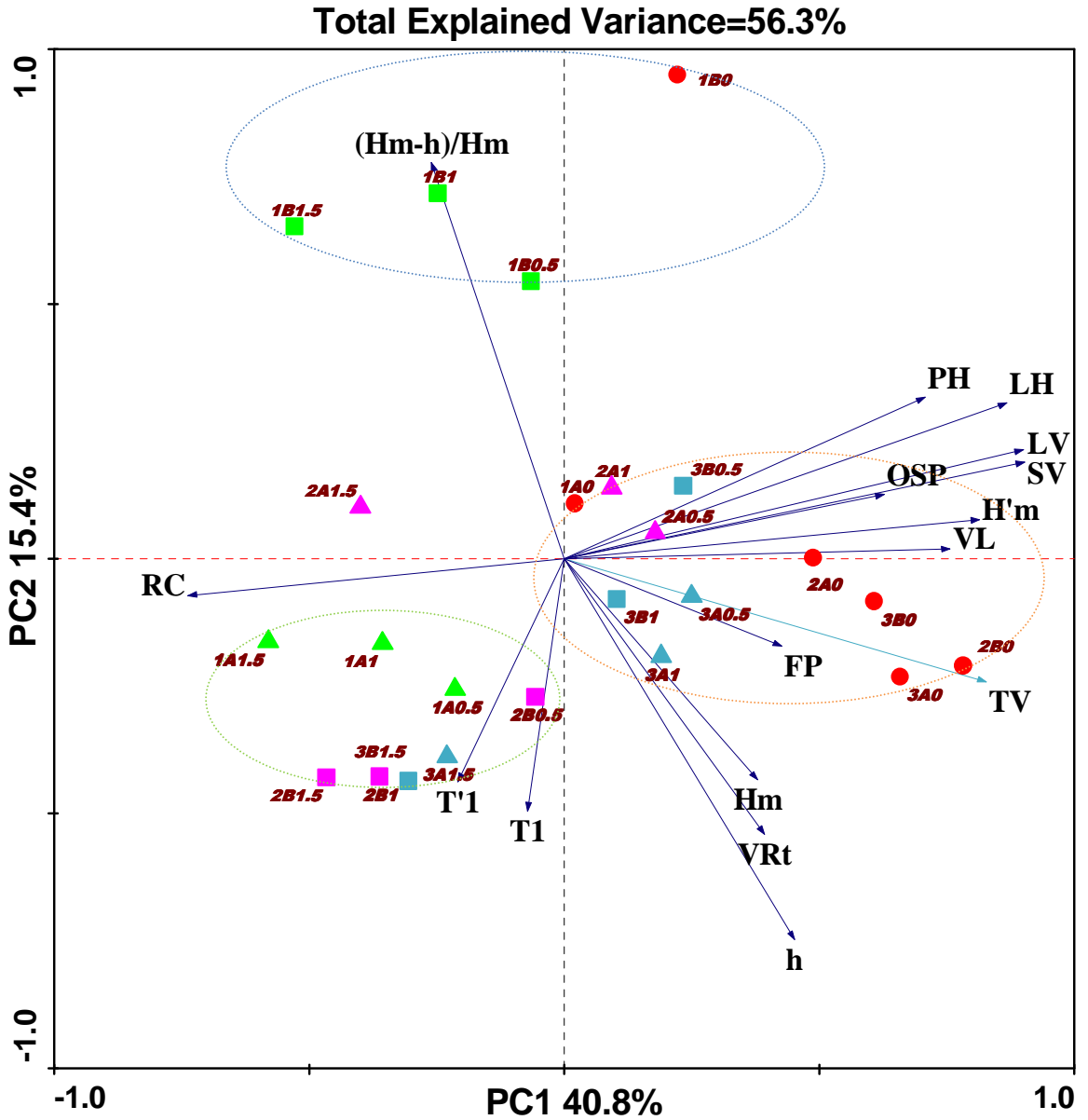


Figure.18. Loading plot of first two principal components based on fermentation and baking properties of six commercial wheat flours added with four levels of urea. Symbols and definitions: ●-Control samples, ▲- Low protein A flours, ▲-Medium protein A flours, ▲- High protein A flours. ■- Low protein B flours, ■ - Medium protein B flours, ■ High protein B flours.

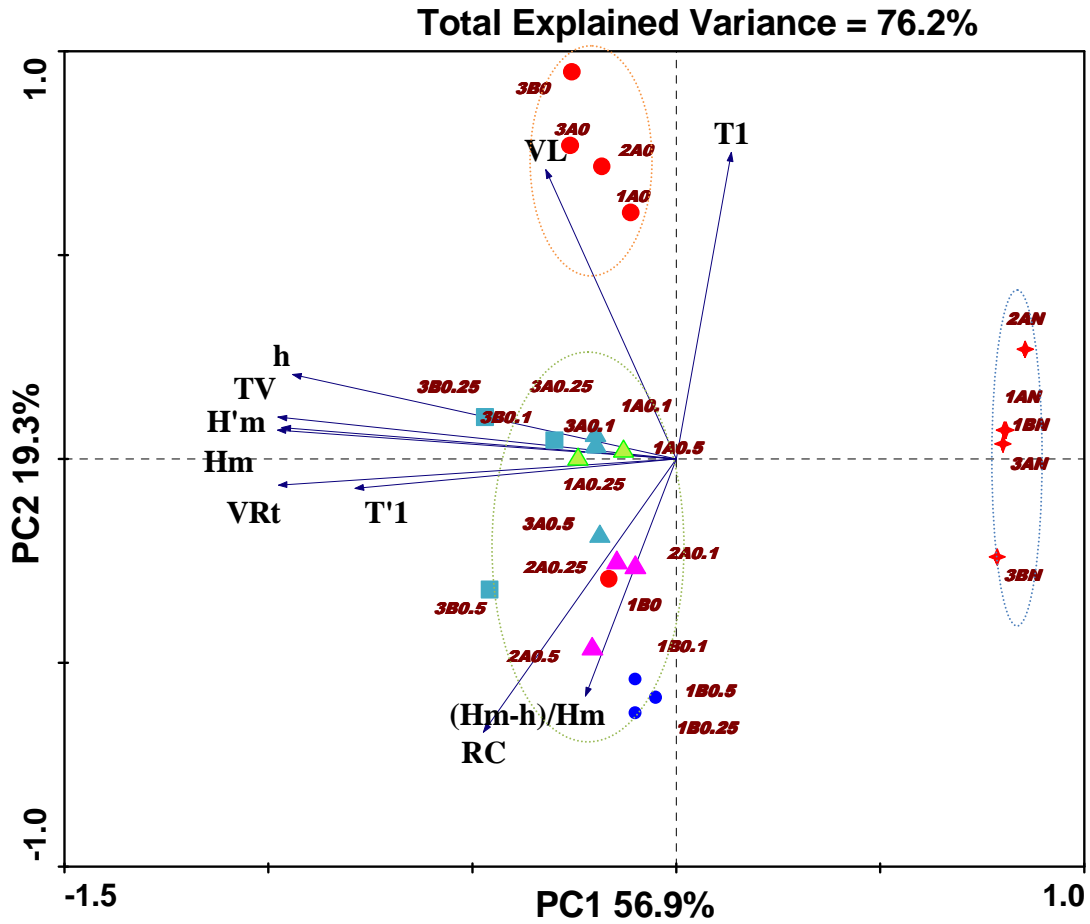


Figure 19. Loading plot of first two principal components based on fermentation properties with negative control of five commercial wheat flours, added with four levels of DTT. Definitions of fermentation, visco-elastic, mixing and baking variables explained in Table 2 and 3 (Chapter III). Flour protein content (%), 1A = 7.95, 2A = 11.19, 3A = 13.68, 1B = 10.4 and 3B = 11.38, respectively. Symbols and definitions: ● -Control samples, ★ - Negative controls, ▲ - Low protein A flours, ▲ - Medium protein A flours, ▲ - High protein A flours. ■ - Low protein B flours, ■ - Medium protein B flours, ■ - High protein B flours.

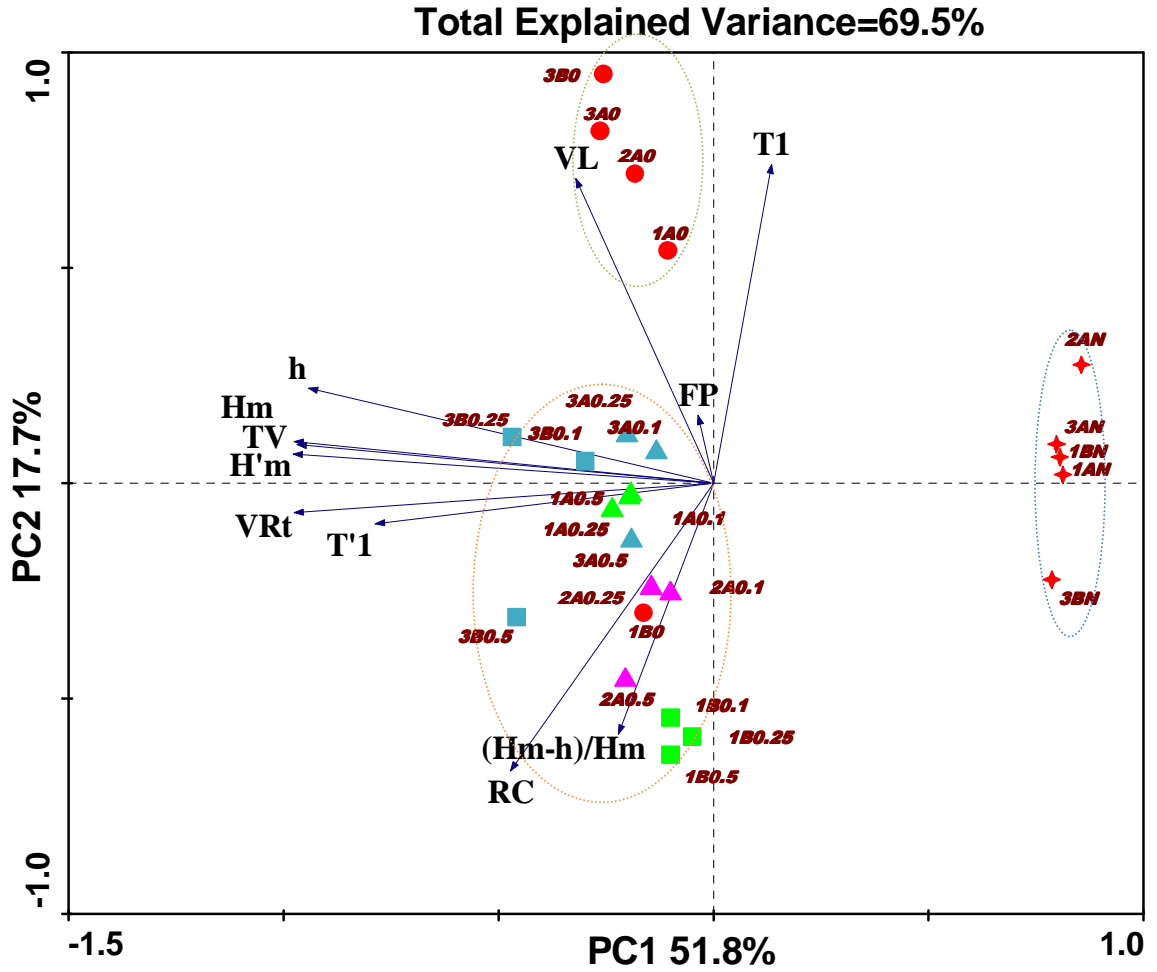


Figure 20. Loading plot of first two principal components based on fermentation properties with flour protein of five commercial wheat flours added with four levels of DTT. Symbols and definitions: ● -Control samples, ★ - Negative controls, ▲ - Low protein A flours, ▲ -Medium protein A flours, ▲ - High protein A flours. ■ - Low protein B flours, ■ - Medium protein B flours, ■ - High protein B flours.

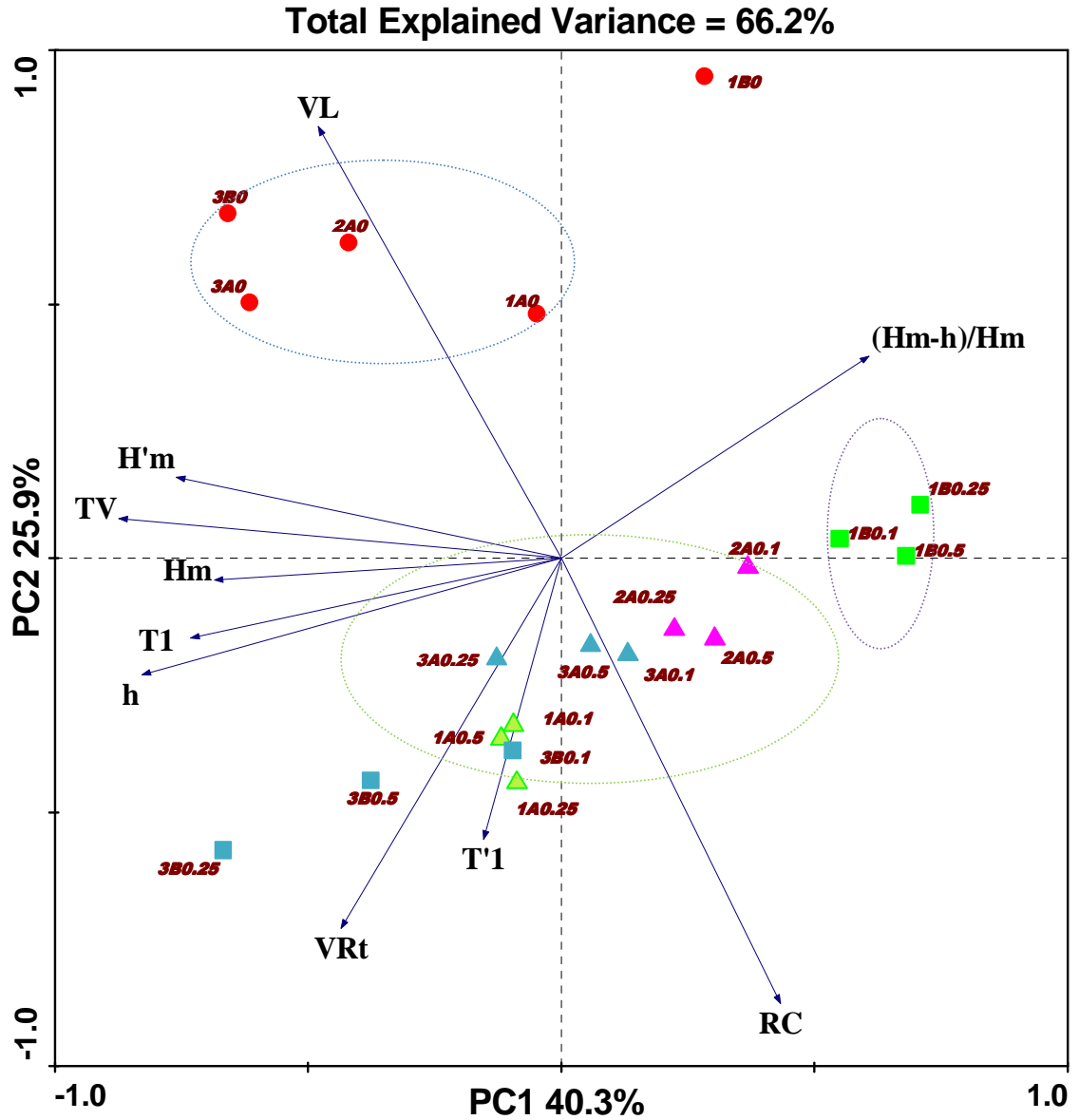


Figure 21. Loading plot of first two principal components based on fermentation properties without negative control of five commercial wheat flours added with four levels of DTT. Symbols and definitions: ● -Control samples, ▲ – Low protein A flours, ▲ -Medium protein A flours, ▲ - High protein A flours. ■ – Low protein B flours, ■ - Medium protein B flours, ■ - High protein B flours.

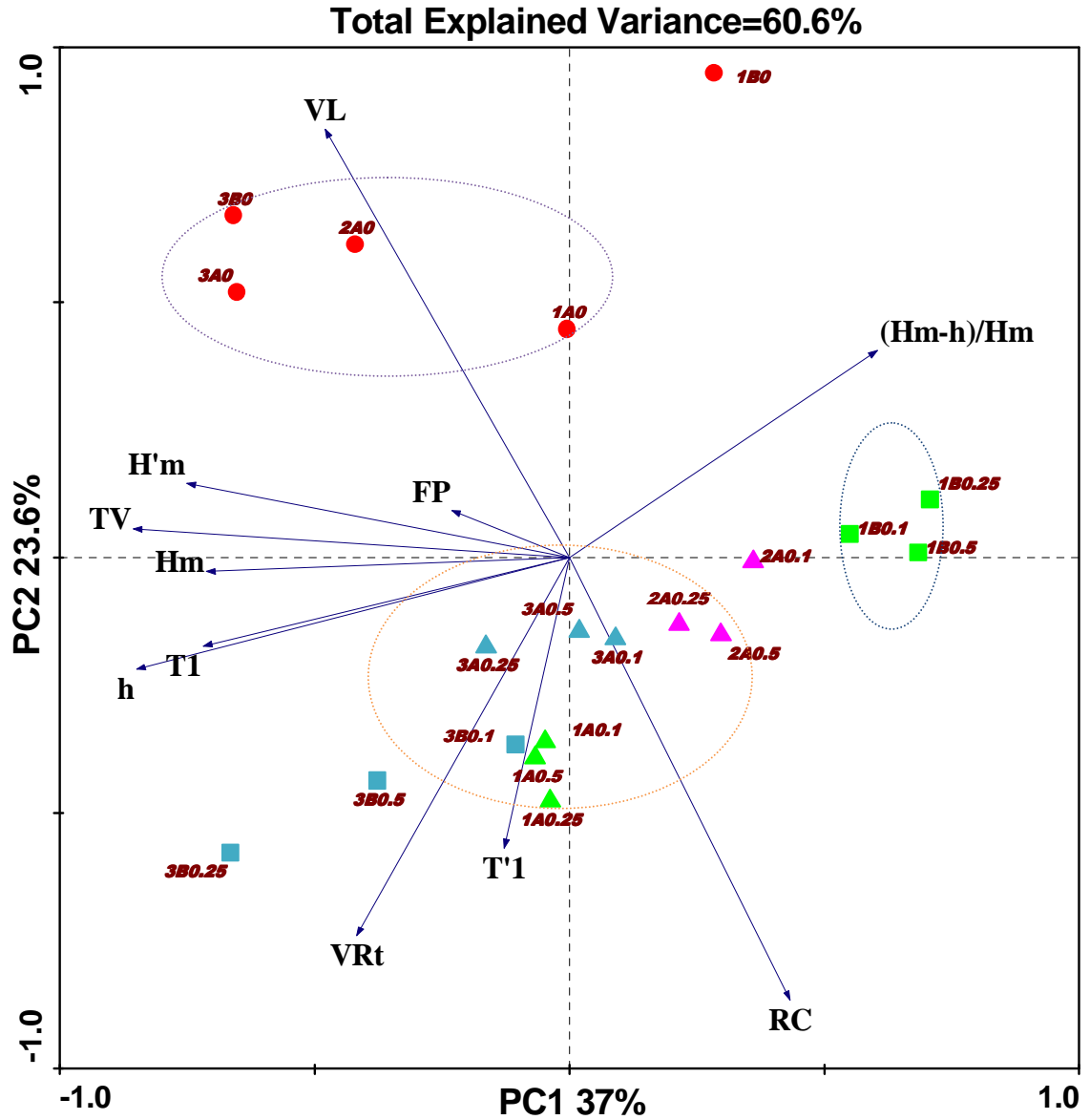


Figure 22. Loading plot of first two principal components based on fermentation properties with flour protein of five commercial wheat flours containing four levels of DTT. Negative control samples were removed. Symbols and definitions: ● -Control samples, ▲ - Low protein A flours, ▲ - Medium protein A flours, ▲ - High protein A flours. ■ - Low protein B flours, ■ - Medium protein B flours, ■ - High protein B flours.

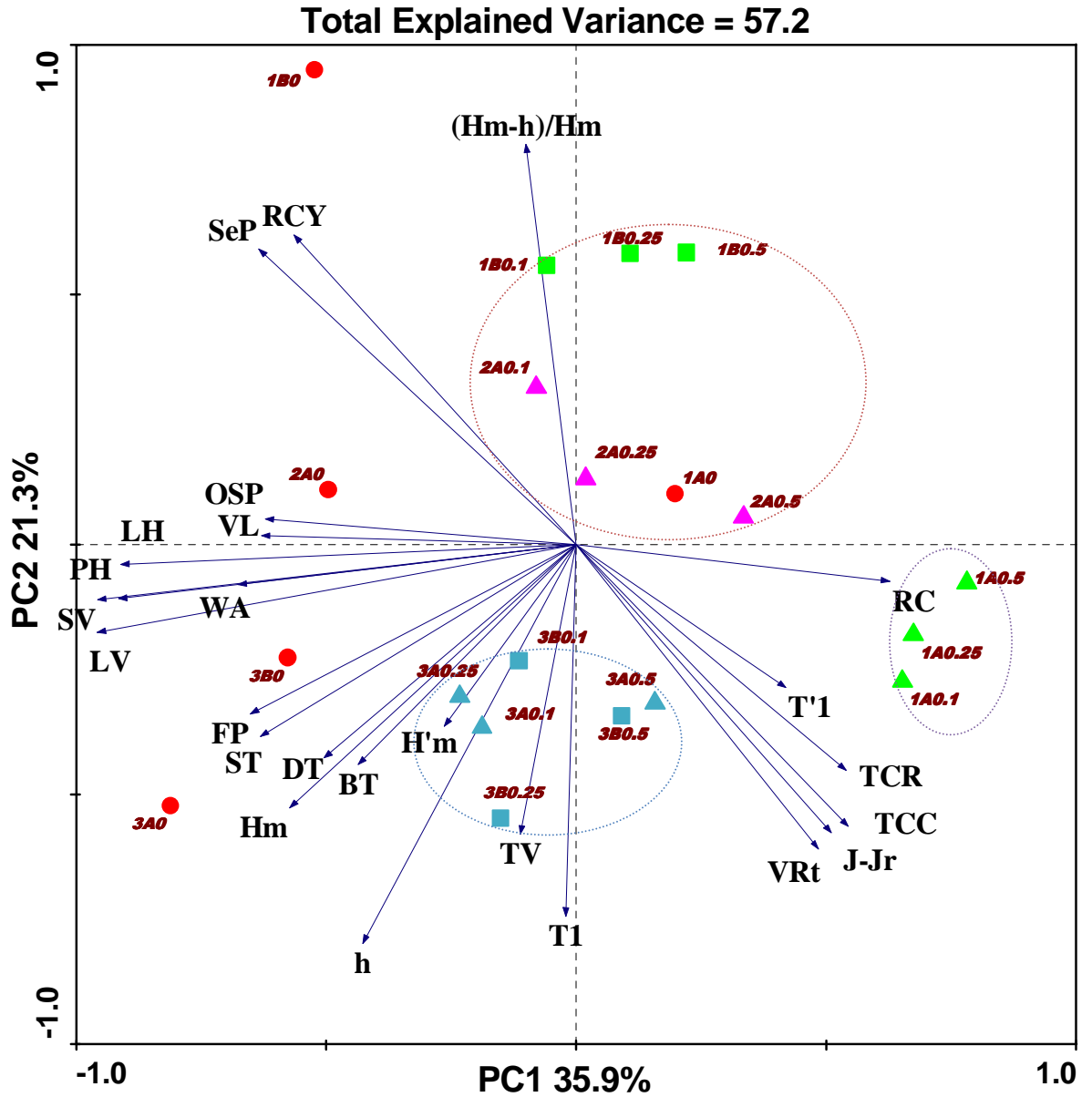


Figure 23. Loading plot of first two principal components based on fermentation, baking, visco-elastic and dough properties of five commercial wheat flours added with four levels of DTT. Symbols and definitions: ● - Control samples, ▲ - Low protein A flours, ▲ - Medium protein A flours, ▲ - High protein A flours. ■ - Low protein B flours, ■ - Medium protein B flours, ■ - High protein B flours.

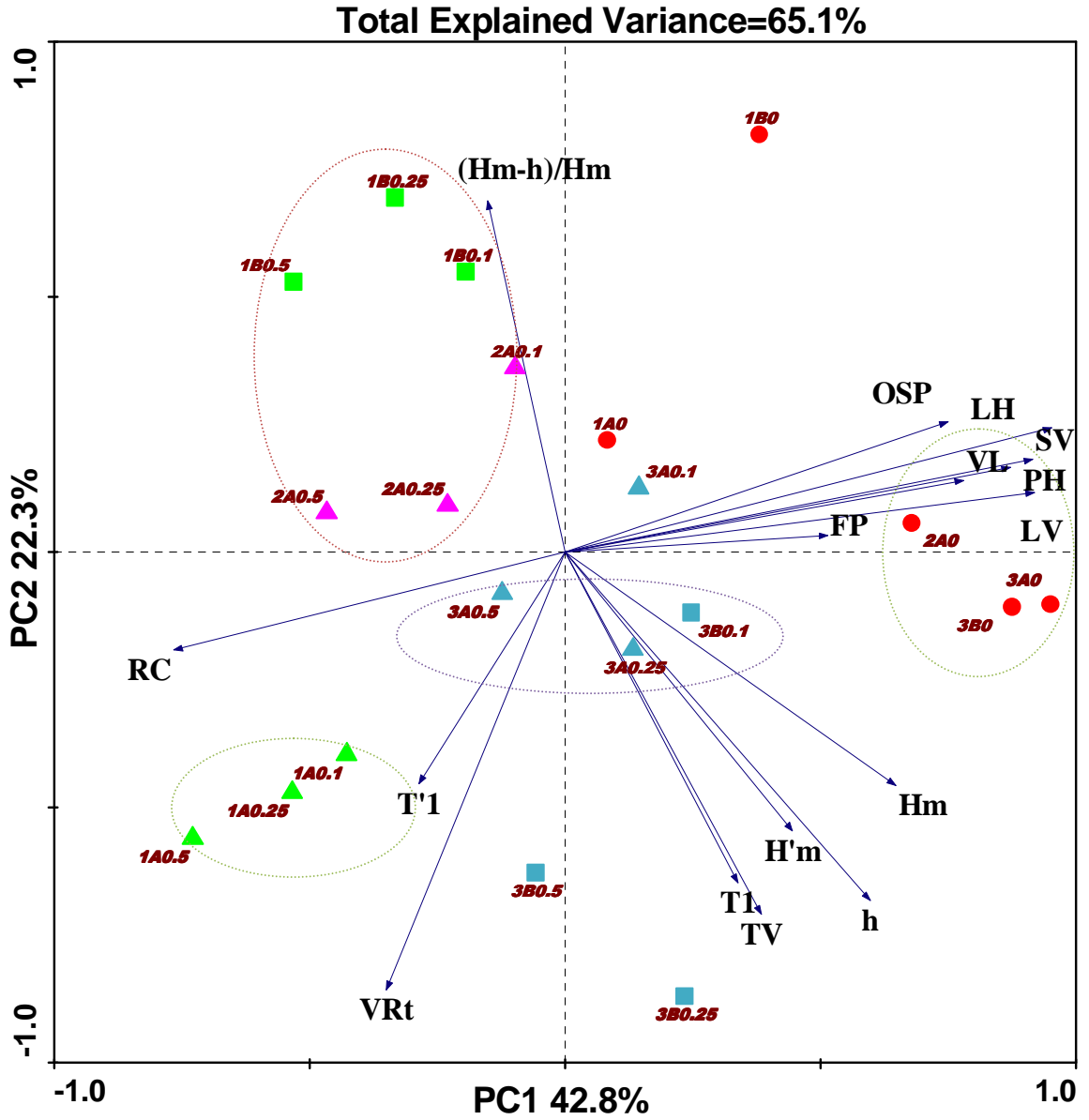


Figure 24. Loading plot of first two principal components based on fermentation and baking properties of five commercial wheat flours added with four levels of DTT. Symbols and definitions: ● -Control samples, ▲ -Low protein A flours, ▲ -Medium protein A flours, ▲ - High protein A flours, ■ - Low protein B flours, ■ - Medium protein B flours, ■ - High protein B flours.

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Scope and Method of Study: Fermentation properties of wheat dough are key factors in the production of yeasted bread products. The purpose of this study was to investigate the effect of physical and chemical changes of dough on fermentation properties and to find possible correlations of fermentation properties with visco-elastic, mixing and baking properties. Six commercial hard red winter wheat flour samples with different protein content and quality were used. The physical and chemical changes were achieved by modifying the surface tension, red-ox state and hydrogen and hydrophobic bonds by the addition of different levels of DATEM, ascorbic acid, dithiothreitol (DTT), and urea. The fermentation properties were measured with a Rheofermentometer.

Findings and Conclusions: By reducing the surface tension of the dough, height of the dough is significantly improved in 1A and 3B flours and retention volume of the gas is increased (11.3-52.1%). Volume of gas lost is reduced (7.3-16.4%) and retention coefficient is increased (21-38%). Overall, gas retention was improved with reducing surface tension obtained with 0.3 and 0.6% DATEM compared to 1% DATEM. By oxidizing the dough, maximum height of the dough development decreases for A flours and increases for B flours. Maximum height of gaseous release shows an increase in low protein A flours. Highest percentage of retention volume of gas was observed in low protein A flour (1A100). Highest percentage of retention coefficient of gas was observed in medium protein flours. The effect of disruption of hydrogen and hydrophobic bonds decreased maximum height of gaseous release, total volume of gas and volume lost. Disruption of disulfide bonds decreases maximum height of the dough and maximum height of the gaseous release for most flours. The ratio of dough heights $[(H_m-h)/H_m]$ is closely related to elastic properties. In both reduction and oxidation states of dough, time taken to reach maximum height of dough development (T1) is closely related to viscous properties and height of the dough development (h) is closely related to flour protein. Retention coefficient (RC) is negatively related to flour protein and baking. Mixing properties are not related to fermentation.

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