

**Title in English**                    **Analysis of bread making quality parameters of Tajik wheat**

**Title in Swedish**                    **Analys av parametrar för bakkingskvalitet i vete från Tajikistan**



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## 1. ABSTRACT

Wheat is a major crop for human consumption in a number of countries around the world. Because of the large consumption, wheat has a crucial role into the daily diet of human. The seed storage components are important while consumed and also for baking bread or production of other types of food from the grain flour.

This work has focused on the evaluation of wheat quality of Tajik wheat varieties and lines using a numbers of quality parameters. The protein composition and content, thousand kernel weight, wet gluten, gluten deformation index, glassiness, bread volume, total and dry gluten, falling number, water absorption and dough strength was analysed. A high variation in protein composition was found among the investigated wheat varieties and lines. The most widely present HMW-GS were 2\*, 7+9 or 7+8 and 5+10 encoded on *Glu-A1*, *Glu-B1*, *Glu-D1*. The investigated wheat varieties and lines were largely found to be inhomogeneous for the protein composition i.e. several types of HMW-GS were found in the same wheat varieties and lines. Seventeen varieties/lines out of 22 were found to carry glutenin subunits 5+10 encoded on *Glu-D1* which is positively correlated to gluten strength and bread making quality. The lines Vorona and Tnmu/munta showed high protein concentration and the lines Cmn82a, Yn3np, Tam200 and Skauz showed higher gluten strength as compared to the other cultivars and lines evaluated. The lines Yusufi and Sarvar showed the highest bread volume among the investigated varieties/lines and also compared to the standard variety. Also two mentioned lines showed higher value of dough strength as compared to the other lines.

Significantly positive and negative correlations were found between different protein fractions and quality parameters. Significantly positive correlation was found between extractable large monomeric proteins (eLMP), unextractable small monomeric proteins (uSMP) and gluten index. The total extractable proteins (TOTE) also correlated significantly and positively with wet gluten.

**Key words:** wheat, bread making quality, protein, HMW (high molecular weight), gluten, allele.

## 2. ABSTRACT IN TAJIK

Дар бисёр кишварҳои дунё гандум зироати асосии ғизоӣ ба ҳисоб меравад. Аз сабаби истеъмоли зиёди маҳсулоти гандумӣ, ин зироат барои ғизои рӯзмарраи инсоният аҳамияти муҳим дорад. Моддаҳои захиравии таркиби дони гандум барои пухтани нон ва ё истеҳсоли дигар намуди хӯрокаи аз орди гандум муҳим мебошанд.

Кори илмӣ мазкур асосан ба омӯзиши сифати дони навъҳо ва линияҳои гандуми Тоҷикистон бо истифода аз якчанд нишондиҳандаҳои сифати нонӣ бахшида шудааст. Таркиб ва миқдори сафеда, вазни ҳазор дона, миқдори глютен (ширешаи) дон, ёзиши глютен дон, шаффофии дон, ҳаҷми нон, миқдори умумӣ ва миқдори хушки глютен, рақами афтиш, ҷаббиши об ва қувваи хамир ташхис карда шуданд. Дар таркиби сафедаи навъҳо ва линияҳои омӯхташуда гуногунии зиёд муайян карда шуд. Сафедаҳои вазни молекулавиашон зиёд ба мисли, 2\*, 7+9, 7+8 ва 5+10, ки дар шакли *Glu-A1*, *Glu-B1*, *Glu-D1* хеле зиёд мушоҳида карда шуд. Навъ ва линияҳои ташхискардашуда аз ҷиҳати таркиби сафеда яқсонии генетикӣ надошта, дар як навъ ва линия якчанд намуди сафедаи вазни молекулавиаш зиёд дарёфт шуд. Аз 22 навъ ва линияҳои ташхискардашуда дар 17-тои онҳо сафедаи вазни молекулавиаш зиёди 5+10, ки дар *Glu-D1* ҷойгир шудааст, мушоҳида карда шуд, ки бо қувваи глютен ва сифати нонии гандум иртиботи мусбӣ дорад. Миқдори зиёди сафеда дар линияҳои Vorona ва Tnmu/munta дарёфт шуд ва дар таркиби дони линияҳои Cmn82a.1294/2\*kauz//, Yn3nrm/vos83, Tam200/kauz ва Skauz bv92 миқдори зиёди глютен ва қувваи глютен дон ошкор гардид. Дар байни навъ ва линияҳои омӯхташуда танҳо навъҳои Юсуфӣ ва Сарвар калонтарин ҳаҷми нонро нишон доданд. Ҳамзамон нишондиҳандаи зиёди қувваи хамир дар навъҳои дар боло зикргардида мушоҳида карда шуд.

Иртиботи назарраси мусбӣ ва манфӣ байни таркибҳои гуногуни сафеда ва нишондиҳандаҳои сифати нонӣ дарёфт карда шуд. Иртиботи мусбии назаррас дар байни сафедаҳои вазни молекулавиашон калони ҷудокардашаванда, сафедаҳои вазни молекулавиашон хурди ҷудокарданашаванда бо нишондиҳандаи сифати ёзиши глютен дарёфт карда шуд. Ҳамзамон иртиботи назарраси мусбӣ байни миқдори умумии сафедаи ҷудокардашаванда ва миқдори умумии глютен мушоҳида карда шуд.

**Калимаҳои калидӣ:** гандум, сифати нонии дон, сафеда, сафедаи вазни молекулавиаш зиёд, глютен, аллел.

### 3. INTRODUCTION

Since the start of the domestication and cultivation of wheat and until present time, wheat has developed into one of the largest crops both in terms of yield produced and food consumed (Dubcovsky & Dvorak, 2007). The earliest type of consumption of wheat was to harvest and eat the kernels directly from the wild population present in the meadows. When the human society was transferred from hunting and gathering towards agrarian ones, domestication of wheat started in the area of western Asia. Archaeologists have reported this region as being the area of einkorn wheat domestication (Harlan & Zohary, 1966; Zohary & Hopf, 1988). The first domestication of einkorn started in the region of West Diyarbakir in south eastern Turkey (Blumler *et al.*, 1991; Tanno & Willcox, 2006). Wheat has then been used for food mainly through boiling of wheat grain in water or by making flour (Heun *et al.*, 1997). Wheat is considered as being one of the first main sources of protein and energy for human already soon after domestication, and has prevailed to be so also in present time (Anjum *et al.*, 2007). Beside proteins and energy, wheat in the human diet contributes with B vitamins, magnesium, iron, and other compounds like phenols. Several of these compounds, are important for human health (Liangli, 2007). Wheat is a main cereal crop and 620 million tons are produced annually around the world (Dubcovsky & Dvorak, 2007). The wheat quality and adaptability are as crucial as is the yield potential (Slafer *et al.*, 1999). Drought and a hot climate are influencing the wheat production in many countries in the world, but wheat has the ability to withstand many of these climate conditions. Wheat is a widely adaptable cereal crop, at present adapted to different climatic growing conditions and this ability is used in drought tolerance breeding programs (Worland & Snape, 2001). Drought climate conditions can affect the wheat growing process, especially during the grain filling time and thus, the yield is decreased, but under normal growing condition wheat store large amounts of nutritious compounds and have a good yield potential (Turner, 1979; Passioura, 2002; Lin *et al.*, 2007). Wheat quality is influenced by the genetic background of the used wheat cultivars, but also growing conditions and environmental factors are important (Johansson & Svensson, 1999a; Perretant *et al.*, 2000). Specific requirement of wheat quality depends on the specific end-use of the wheat products (Morris *et al.*, 2004).

#### **3.1.WHEAT AND BREAD MAKING QUALITY**

##### **3.1.1. Wheat**

The cereal grass crops were successfully domesticated 10 thousand years ago (Dubcovsky & Dvorak, 2007) and have thereafter been used for food production. Wheat

(*Triticum aestivum*) is a cereal crop that belongs to the tribe of *Triticeae*, which is one of the largest and most important tribes in the grass (*Poaceae*) family. The wild species of wheat are diploids ( $2n = 2x = 14$  chromosomes), with the genome definition AA (*Triticum monococum*), DD (*Triticum tauschii*) and SS (*Triticum speltooides*), or tetraploids ( $2n = 4x = 28$  chromosomes), with the genomes definition AABB (*Triticum turgidum*) or AAGG (*Triticum timopheevii*). *Triticum durum*, durum wheat ( $2n = 4x = 28$  AABB), is a polyploid species that appeared after interspecific hybridisation of two diploid grass species. The A genome originates from *Triticum uratru* ( $2n = 4x = 14$  AA) while the B genome is related to *Aegilops speltooides* ( $2n = 4x = 14$  SS) (Kihara, 1944; McFadden & Sears, 1946; Dubcovsky & Dvorak, 2007). The hexaploid, *Triticum aestivum* - AABBDD ( $2n = 6x = 42$  chromosomes), is the most important wheat species in the world and is also called common wheat (Zohary & Hopf, 1988). Almost all currently grown wheat are either common (*Triticum aestivum*) or durum wheat (*Triticum durum*). One additional species is *Triticum compactum*, which is grown in some few countries. *Triticum compactum* has small, slender grains with low thousand kernel weight and a compact spike (Swaminathan & Rao, 1961; Tomar *et al.*, 2007). Different types of wheat are used for different end-use purposes depending on the variation in quality among them (Pomeranz, 1988). Differences among the various species of wheat leads to variation in a range of characters such as spike morphology, seed shape and quality parameters. According to the botanic and biological features, wheat is divided into different types based on colour and glassiness (*Wheat standard*).

When wheat was domesticated a saddle quern stone was used to produce flour from the wheat grain. An amount of seed was placed on the surface of the saddle stone and pounded with a small stone by hand. Each of the saddle stones were normally used only for one family of approximately six persons. As time went, the saddle querns improved and the size of the stones became bigger. Later on, operation of the stone changed from using human towards using animal power. The ancient Romans used water power in the twelfth century, Europeans started to use wind power for turning the stone. Thereby, turning the stone became faster and to operate the stone became more productive and thus, the saddle querns were used for many families. Nowadays, milling of wheat is carried out by milling companies with modern equipments, although in some countries, still saddle querns are used driven by water or wind power (Reynolds, 1995).

Bread is today commonly made using flour from wheat, although also flour from seed of other plants like barley, buckwheat, corn, oats, beans, peanuts, rice, rye and soybeans.



During processing of the wheat grains, especially to white flour and further to bread, some vitamins and minerals are lost. Thus, the baking industries are often adding vitamins and minerals to the flour to replace the losses during the milling process (*The story of wheat*).

Nowadays, more than 90% of the wheat grown worldwide is common wheat due to its suitability for bread and cookie production (Shewry, 2009). Durum wheat production is 8% of the worldwide wheat production with growing areas of around 20-30 million hectares. Durum wheat is mainly used for preparing pasta and semolina products. Most of the durum wheat is grown in the Mediterranean areas (Bozzini *et al.*, 1988; Liu *et al.*, 1996) Common wheat is adapted to spring and winter growing conditions through the need versus not need of a cold period after sowing in order for spike formation. Wheat kernel colour can be either white or red (Pomeranz, 1988).

### **3.1.2. Wheat for Tajikistan**

Tajikistan was during a numbers of years a part of the former Soviet Union. In 1991, Tajikistan became an independent country in the southeast of Central Asia. The territory of the country is 143.1 thousand km<sup>2</sup>. Tajikistan is a mountainous country and thus 93% of the total area is occupied by mountains, while only 7% are plain lands (Albrecht *et al.*, 2010). Cereals are the main crops in Tajikistan (Mirzoev *et al.*, 2007). The total agricultural arable land is close to 900.000 ha and the land under the cereals and legumes is 459.000 ha. The rest of the agricultural land is used for cultivation of different crops including industrial crops e.g. cotton, potatoes, vegetables, melons and gourds, and forage crops. Wheat is considered as one of the main food crops in Tajikistan (Morgunov *et al.*, 2007; *Statistical Agency under the President of the Republic of Tajikistan.*, 2011).The demand of the Tajikistan population for cereal crops is 2 million tons while the local production is around 800 to 900 thousand tons and thus the production is by far less than the demand. The deficit amount of wheat is mainly imported from Russia and Kazakhstan. Wheat self-supply has become one of the current tasks for food security and thus there is a need for wheat breeding in the country (Gladstone, 2001; Van Anrooy *et al.*, 2008).

Tajikistan is one of the centres of origins for many of the cereals. The area seems to be one of the places where the domestication of wild crop including cereals was started. After independence of the country, the production of cereals has increased. The amount and variety of cereal products have also increased, although the economic situation together with some problems within the agrarian sector has lead to a still low yield of wheat (around 1.5-1.6 t/ha). The breeders and research institutes are working together on increasing the yield potential and

improving quality parameters of new wheat varieties. Wheat breeding in Tajikistan started in 1926 and since then wheat varieties with improved quality, resistant to different diseases and pests and with increased yield has been created and released. Most varieties that have been released have become widely grown in the country (Andreas, 2010; Doukas *et al.*, 2012). Since the independence of the country, new collaborations with International Centres such as CIMMYT (International Maize and Wheat Improvement Centre), ICARDA (International Centre Agricultural Research on Dry Areas) and other research programs have been developed. New wheat lines from these programs have annually been received and tested under Tajik condition. The material has been tested as related to quality, disease and pest resistance and yield. From these lines some varieties like Norman, Alex, Ziroat 70. Somoni, Tsicar, Ormon, Sadokat, Iqbol, Oriyon, Sarvar, Ysufi, Vahdat and Isfara have been selected and submitted for official testing and some of them have already been released (Morgunov *et al.*, 2007).

### **3.1.3. Bread making quality**

Wheat is considered as one of the major agricultural and dietary component crops in the world. The seed endosperm of wheat contains carbohydrates, proteins, starch, fibre and other components. The most common use of wheat as a food is to make flour and bake bread from the wheat grain (Zhou *et al.*, 2004). When human started to use wheat as a food, they mixed flour with water to make dough which they thereafter used to produce special dishes on the fire. To produce bread, different kind of baking techniques have been adopted over time. One of the most common and widely used ovens was the domed clay ovens, still in use in Central Asian countries. These ovens are made by hand and for production of the clay, soil is used. In some places hair of goat or sheep is mixed with the soil to make the oven stronger. When the oven or tandur is ready, it is placed in a special place and heated by fire and the bread is baked quickly (Reynolds, 1995).

Flour is commonly the bases for a variety of wheat products used. Requirement of the wheat flour quality depends on several aspects like the production process and kind of bread products designed. Several quality parameters need to be evaluated in order to meet the requirements of the bread making industry. Different methods are used in various countries to evaluate quality parameters and examples of such methods are farinograph, extensograph, alveograph, and baking tests (Miralbés, 2004; Dogan *et al.*, 2010). Improvement of the bread-making quality has been a main purpose for breeders and researchers working with bread wheat (*Triticum aestivum*). In some cases, the researchers have been able to improve the

quality parameters by exploiting the genetic variation as to the potential of the gluten, the proteins and other flour components concentrations and structures (Carrillo *et al.*, 1990). The high molecular weight glutenin subunits (HMW-GS) are one type of grain proteins which are produced during the grain filling time (Mifflin *et al.*, 1980; Payne & Lawrence, 1983) and these glutenin subunits are highly influencing the bread making quality (Payne *et al.*, 1987a). Dough properties and bread making quality are highly dependent on these components (MacRitchie, 1992; Johansson & Svensson, 1999a; Johansson & Svensson, 1999b). The storage protein is divided mainly into gliadins, HMW-GS and low molecular weight glutenin subunits (LMW-GS). Wheat grain protein composition is one of the most important genetic factors influencing the wheat quality (Williams, 1979). The responsible genes for synthesis and controlling of the HMW glutenin subunit are located on the long arm of the 1A, 1B, and 1D chromosomes (Payne *et al.*, 1984). Improving wheat with quality suitable for bread can be carried out by using the specific alleles for HMW-GS (Shewry *et al.*, 1995; Anjum *et al.*, 2007).

#### **3.1.4. *Wheat seed storage proteins***

The grain filling process is the final stage of the growing cycle of a plant and the seeds are normally formed at the end of the plant growing stages of the plant (Barlow *et al.*, 1980). The seed is a small embryonic plant covered with a seed coat. The seed is formed after the development of flowers that are pollinated to fertilize and create an embryo. The embryo develops from the zygote while the seed coat develops from the integuments of the ovule. The embryo is like an immature plant and from the embryo a new plant starts to develop. The endosperm of the wheat seed is a result of a double fertilization and serves as a reservoir of oil, starch, proteins and other components for the developing wheat plant (*Seed Structure and Anatomy*, 2000). The proteins are mainly accumulated in the seed during the grain filling process. The seed is used by human in three aspects: for food consumption, for feeding animals and as raw materials for different purposes (Matz, 1991).

The total protein has a great impact on the end use of wheat grain and the total protein of cereals is about 10-15% of the grain dry weight (Shewry & Halford, 2002). Studies of the seed storage protein components started some 250 years ago and until now many studies have been carried out around the world. Protein components will remain in focus also in the future as they are useful in breeding for improved seed protein composition and concentration which is related to bread making quality. The gluten allocation was described already by Beccari in 1745 (Benvenuti *et al.*, 1754; Shewry & Halford, 2002). Gluten is the part of the protein

fraction in wheat that is not soluble in water. Several factors such as the genetic background and environmental growing conditions are influencing the quality and quantity of gluten (Pomeranz, 1988).

Protein content of the grain is one of the most important components for the grain quality. Bread making quality is also influenced by the physical and chemical properties of the storage proteins. The wheat gluten protein is subdivided into two main groups' polymeric (glutenin) and monomeric (gliadin) protein, both having a crucial role for dough properties and bread-making quality (Figure 1) (Osborne, 1909). The gliadins of protein have a molecular weight of up to 100000 Da (daltons) and the molecular weight of the glutenins levels up to ten millions of daltons. The HMW-GS have received more attention than the LMW-GS because of their better separation on SDS gel electrophoresis. HMW-GS also have higher impact during the dough process than has LMW-GS. LMW-GS influence dough strength, but their great importance relates to their relevance for the extensibility of dough (Lawrence & Shepherd, 1981; Pomeranz, 1988; Cornish *et al.*, 2001; Carver, 2009).

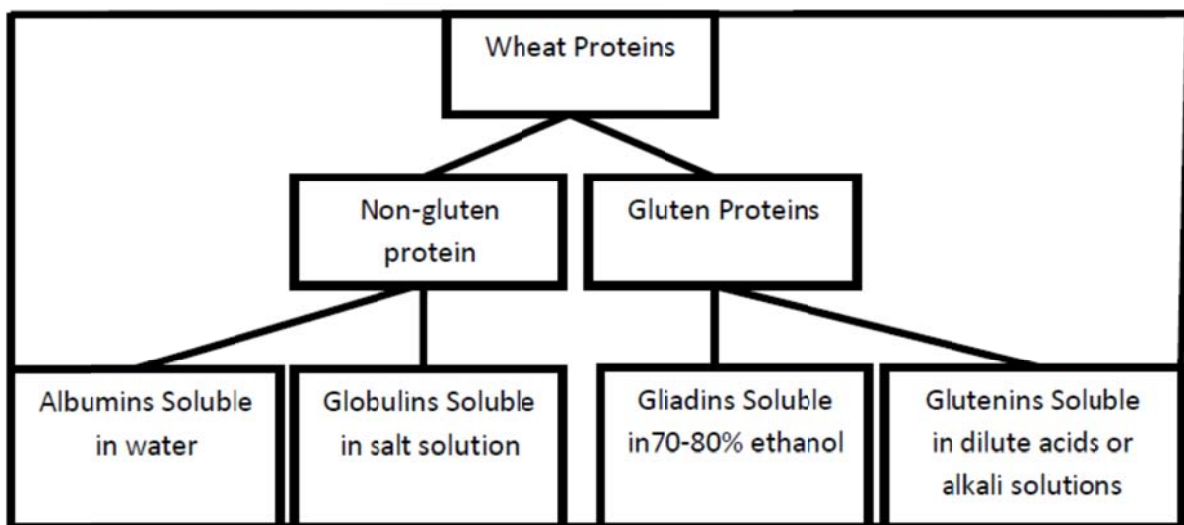


Figure 1. Wheat proteins classification according to their solubility, as described by (Osborne, 1909).

### 3.1.5. Aim of the study

In the first part of this study, 23 wheat varieties were used, while in the second part nine varieties were used. All wheat varieties investigated, originated from the national wheat breeding program of Tajikistan. The research work aimed to investigate bread-making and protein quality of Tajik wheat varieties/lines. The goal was to identify differences among wheat genotypes related to variation in bread making quality parameters and to give advises

as to which of the investigated wheat genotypes that were suitable for further breeding through improved bread-making quality.

### **3.1.6. Hypothesis**

Through the analyzes of baking quality parameters, protein composition and protein content it will be possible to identify wheat varieties suitable to be used in breeding programs, and being aimed towards improved bread-making quality.

## **3.2. MATERIALS AND METHODS**

### **3.2.1. Plant materials**

Twenty three different varieties and lines from the national wheat breeding program were selected for this study in 2009 (Table 1). The wheat samples were analysed for thousand kernel weight, wet gluten content, gluten deformation index, glassiness, protein composition and total protein. The samples were collected from two different geographical locations of Tajikistan where these varieties were included in multi-location yield trials in the Tajik national wheat breeding program. One of the locations is situated in the central-western part of the country in the Hisor valley at 850 m above sea level (38°31'16N; 68°34'21E, 788 masl). The second location is situated in the north-eastern part of the country in the Isfara district and is also 850 m above sea level (40°09'N; 70°43'E, 822 masl).

In addition to the 23 tested varieties, the local variety Navruz released by the Farming Institute in Tajikistan, was included as a reference for laboratory analyses in Tajikistan and the Swedish variety Dragon with known HMW-GS composition (*Glu-A1-2\**, *Glu-B1-7+9* and *Glu-D1-2+12*) was included as a reference for SDS-PAGE electrophoresis in Sweden. During the period of 1975-1996 Dragon was one of the most widely grown varieties in Sweden (Johansson *et al.*, 1999).

Table 1. Twenty three varieties and lines used for analyses of bread making quality parameters.

№	Variety/Line
1	Navruz
2	Alex
3	Jagger
4	Tnmu/munta
5	Prinia/star
6	Shark/f4105w2.1
7	Vorona/kauz//1d13.1/mlt
8	Tam200/kauz
9	1d13.1/mlt//tui
10	Arilw pronghorn
11	Eskina-8
12	Yn/3npm/vos83
13	Pastor/3/vorona/cn079
14	Skauz bv 92
15	Vorona sn079
16	Soroca
17	Otus toba 97
18	Kauz2/chew//bcn/3milan
19	Chen/aegilops
20	Cbrd/kauz
21	Huavun inia
22	Cmn82a.1294/2*kauz//
23	Starshina

For further analyses of bread-making quality, 9 different varieties and lines including a reference variety were selected from the national wheat breeding program in 2009 (Table 2). This material was analysed for bread volume, protein content, falling number, water absorption and dough strength. For these analyses the released variety Alex was used as a reference. The samples were analysed using the above mentioned parameters in the laboratory for variety testing at the state commission of Kyrgyzstan in Bishkek. The lines evaluated were selected for the mentioned analyses based on their good performance during field trials, including high yield potential, good quality parameters and resistance to diseases.

Table 2. Nine wheat varieties and lines used for further analyses of bread-making quality.

№	Cultivars lines	Origin nursery	Cross	Released year
1	Alex	1WWERYT	PYN/BAU	2007
2	Vahdat	25ESWYT	VORONA SN079	
3	Ziroat	Special nursery		2009
4	Isfara	25ESWYT	SW89.5181/KAUZ	
5	Norman	5FAWWON	OR F1.158/FDL//BLO/3/SHI4414/CROW	2007
6	Ormon	8FAWWON	NWT/3/TAST/SPRW//TAW12399.75	2008
7	Sarvar	25ESWYT	CHEN\AEGILOPS SQUARROSA (TAUS0//BCN/3/BAV92	
8	Somoni	Special nursery		2008
9	Yusufi	25ESWYT	SOROCA	

### 3.3. PARAMETERS MEASURED AND METHODS USED

#### 3.3.1. Thousand Kernel Weight

Thousand kernel weight (TKW) of the wheat samples was estimated by the traditionally used method of kernel counting and weighting with an ordinary balance. For this evaluation two replicates of 500 seed were counted manually from each sample. All dirt and broken kernels were removed. Afterwards, the two portions of 500 seeds were weighted and the results noted. Differences between the three portions of 500 kernels should not be more than 0.5 g. For some samples the differences between portions were more than the 0.5 g and these samples were repeated from the beginning of counting the seed. In such cases the mean value from the three measurements was noted as the final result.

#### 3.3.2. Glassiness

The glassiness is connected with presence of certain components of the wheat grain such as proteins and starch (*Library of cereals*). Glassiness was identified by using a Diafanaskop ДС3-2. Hundred seeds were counted and placed in a glass net with separate places for each seed. The percentage of glassiness was estimated for hundred seeds based on transferring light from the lamp under the glass through each seed (*Grain elevators*; Luzev & Sorokin, 2010).

#### 3.3.3. Gluten quantity and quality

The percentage of total wheat gluten was analyzed by washing dough under running water using a MOK-1M-gluten washing machine. Three separate dough samples of 25 g flour portions were used for the analyses. Three replication of 25 g flour was mixed separately with water in a laboratory dough mixing equipment (Y1-ETK), to obtain the dough. The dough

was kept in water at room temperature for 15 to 20 minutes. Thereafter the dough was placed inside the gluten washing machine and then the dough was washed under running water. The washing process was accomplished five times, changing the pressure of the water and the washing duration (Table 3; Machexina *et al.*, 2010).

Table 3. Wheat gluten washing process through MOK-1M-gluten washing machine.

№	Washing process	Units
1	Gap (mm)	7
	Time (min)	3
	Position of pouring valve	1
	Water demand (dm <sup>3</sup> )	0.35
2	Gap (mm)	7
	Time (min)	2
	Position of pouring valve	1
	Water demand (dm <sup>3</sup> )	0.5
3	Gap (mm)	2
	Time (min)	4
	Position of pouring valve	1
	Water demand (dm <sup>3</sup> )	0.35
4	Gap (mm)	2
	Time (min)	2
	Position of pouring valve	2
	Water demand (dm <sup>3</sup> )	0.35
5	Gap (mm)	7
	Time (min)	2
	Position of pouring valve	1
	Water demand (dm <sup>3</sup> )	0.35

The gluten washing process is shown in Figure 2. The gluten is not dissolved in water but most other components are and thus rather pure gluten remains in the gluten washing machine after the washing. The gluten obtained from the washing machine was weighted and thus the percentage of the total gluten of wheat was estimated. The percentage of gluten was calculated according to the commonly used formulae:

$$\% = \frac{Ax100}{B}$$

A – Total weight of gluten after washing (g), B – Total weight of dough (g)



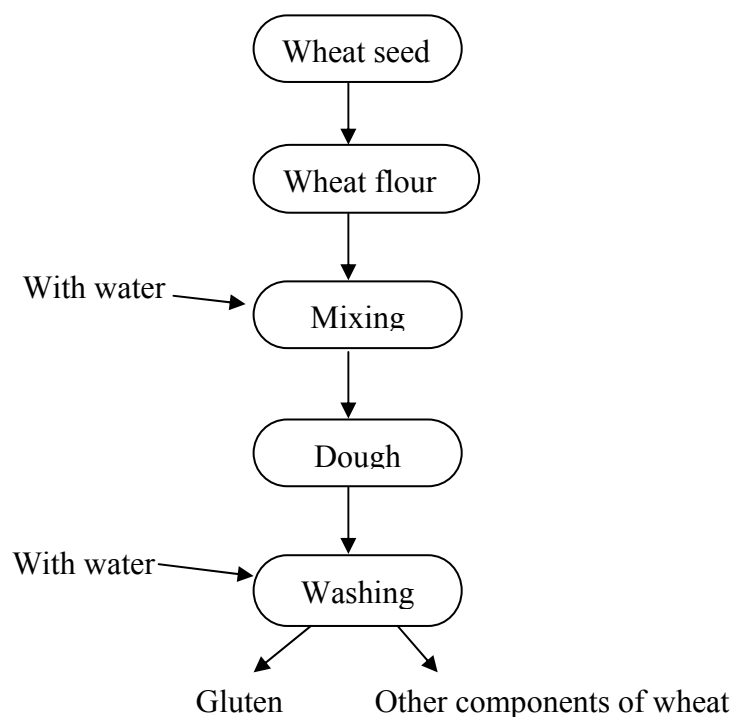


Figure 2. Gluten washing process.

The same gluten was used for identification of gluten elasticity using the equipment ИДК-3М-measurement of gluten deformation (Osborne, 1909). After obtaining the pure gluten, the gluten was placed under pressing part of the equipment ИДК-3М for measurements of the gluten deformation. The equipment is pressing the gluten and measures the deformation index (Bespalova *et al.*, 2006).

#### 3.3.4. SDS-PAGE

The protein composition of the HMW-GS from the wheat grain samples was identified by sodium dodecyl sulphate polyacrylamide gel electrophoresis (SDS-PAGE) the technique for separating proteins based on their molecular weight (Payne *et al.*, 1984). Total proteins of wheat were extracted from each kernel and thereafter the proteins were separated on 10% SDS-PAGE (Payne *et al.*, 1980). Eleven seeds of each wheat samples were analysed and therefore these eleven seeds were crashed and 0.5 ml extraction buffer (0.06M Tris, 2% SDS and 2% DTT with small amount of pyronin) was added to each of the sample. The mixture was vortexed four times in 1h. After preparation of the gel 20 µl from each sample was loaded on to the gel. The extracted samples were kept in the refrigerator before loading, and just previous to loading the samples were incubated 5 min in boiling water (90-95°C). Two-dimensional 12.5 cm high, 14 cm wide and 1 mm thick gels were prepared. The gels were run vertically 16 h at 15mA. After the run, the gels were kept in a staining solution with

Coomassie brilliant blue R250, for 24 h and destained with distilled water and trichloroacetic acid (TCA) according to (Johansson *et al.*, 1993). After the destaining, the gels were scanned (by Epson Perfection V200 Photo). The presence of protein subunits was evaluated according to the descriptions of Payne & Lawrence (1983).

### **3.3.5. Measuring moisture content of samples for SE-HPLC**

Flour of the 22 different varieties and lines with three replications were weighed lyophilized and weighed again to calculate the moisture content. The same sample was thereafter used for SE-HPLC analyses. Wheat grain moisture content is expressed as a percentage of moisture of dry matter. The grain moisture is depending from high relative humidity and the humidity in storage conditions (Le Patourel, 1986; Fields & Korunic, 2000).

### **3.3.6. SE-HPLC**

For evaluation of amount and size distribution of proteins, the wheat samples were analysed by high-performance liquid chromatography (SE-HPLC) (Gupta *et al.*, 1993; Kuktaite *et al.*, 2004). Three replications of  $16.5 \pm 0.05$  mg lyophilized flour from each sample were used for the analyses. To each sample 1.4 ml of extraction SDS phosphate buffer (0.5% SDS, 0.05M  $\text{NaH}_2\text{PO}_4$ , pH 6.9) was added. The samples were vortexed for 10 s and centrifuged for 30 min at 10000 rpm and the supernatant was transferred to a vial for further HPLC analyses. A sequential extraction was made by adding 1.4 ml of the same extraction buffer to the pellet. The samples were thereafter sonicated for 45 s at amplitude 5 with Soniprep 150, centrifuged for 30 min at 10000 rpm and the supernatant of this extraction was transferred to a vial for further SE-HPLC analyses. The separation by HPLC analyses was accomplished in 30 min for each sample and 20  $\mu\text{L}$  of sample was injected into the SE-HPLC. The SE-HPLC analyzes were carried out using a Biosep-SEC-S4000 Peak Phenomenex Column with isocratic flow of 0.2 ml/min (50% Acetonitrile, 0.1% TFA, 50%  $\text{H}_2\text{O}$ -millipore water). Depending on molecular size of the protein the SE-HPLC chromatograms were divided into four sections. The four sections were large and small polymeric proteins, (LPP), (SPP), and also large and small monomeric proteins (LMP) and (SMP) respectively. For evaluation of the protein parameters, SAS statistical program was used. The protein composition and molecular weight of the protein separated by SE-HPLC are described by Larroque *et al.* (1996).

### 3.3.7. *Evaluation of bread wheat varieties for quality parameters in Bishkek*

From the national wheat breeding program of Tajikistan, one released variety Alex, and eight lines: Vahdad, Ziroat, Isfara, Norman, Ormon, Sarvar, Somoni and Yusufi, were selected and sent to Bishkek for further evaluation of baking quality parameters of these samples. The analyses were performed at the variety testing state commission laboratory of Kyrgyzstan, Bishkek, and included analyses of bread volume, gluten index, total gluten, dry gluten, falling number, water absorption, and dough strength. The mentioned analyses are further described below.

### 3.3.8. *Bread volume*

To evaluate the bread volume, the method described by Finney (1984) was adopted. According to this method, three replication of wheat flour was produced from each sample using a laboratory Cyclone Sample Mill (M 20 IKA). Thereafter, from each replication of wheat flour sample, preparation of dough was made using 100 g of flour, 5 g sugar, 1 g salt, 3 g yeast and 50 ml of water. The dough was made with a laboratory dough mixer (U1-ETK) by mixing the dough for 7 to 10 minutes. The dough was kept on 40°C inside of a bake for 15 minutes before being baked. After 15 minutes the bubbles of the dough were removed and the dough was placed in a bake for baking according to Finney (1984). Inside of the bake, the temperature was 130°C and after 25 minutes the bread was ready. The volume of the bread was measured with a volumeter according to Shogren & Finney (1984).

### 3.3.9. *Gluten quality (gluten index, total gluten, dry gluten)*

The gluten quality was identified through measuring the gluten index (%) the total gluten (%) and total dry gluten (%) by the use of a Perten instrument DA 7200, by the method of manual gluten washing according to the (*AACCI Method 38-12.02 Wheat Gluten, Dry Gluten, Water-Binding Capacity, and gluten index 1969*).

### 3.3.10. *Falling number*

Falling number was measured on the samples using the (*Wheat and Flour Testing Methods*). According to this method three replications of 7 g flour for each sample was mixed with 25 ml distilled water in a glass falling number tube using a termomixer. The glass falling number tube was placed inside of the falling number measuring instrument (falling number 1800) in a boiling water bath (100°C) and after 1 minute was taken out. The time of an iron stirrer to drop down through the sample was measured and recorded as the falling number value. The falling number normally amount to in between 55 and 600 s.

### 3.3.11. *Water absorption*

Water absorption is the water amount which is needed for flour in order to be obtained the end product of wheat flour. Water absorption was analysed by the use of a farinograph following the method of Wheat and Flour Testing Methods (*Wheat and Flour Testing Methods: A Guide to Understanding Wheat and Flour Quality*). According to this method three replicates of 50 g flour was placed in a farinograph mixing bowl. Water amount which is added to the flour during the mixing can change the position of the curve on the graph. The less amount of water results increase of dough consistency and moves curve upper. During the dough mixing, the farinograph recorded a curve on graph paper. By adding the optimal amount of water the curve is centred on the 500-Brabender units (BU) line  $\pm 20$  BU.

### 3.3.12. *Dough strength*

Dough strength was analysed by an alveograph according to the method of Wheat and Flour Testing Methods: A Guide to Understanding Wheat and Flour Quality: Version 2 p 49-50 (*Wheat and Flour Testing Methods: A Guide to Understanding Wheat and Flour Quality*). The alveograph method is built on that air is blown into dough and then the dough expands into a bubble that is finally broken. The air pressure needed to blow the dough creates a curve on a graph paper. A bigger bubble from the dough indicates higher extensibility. According to the method triplicates of each sample was produced weighting 60 g for each replicate of flour and then adding about 34 ml of salt solution depending on the moisture content of the flour. The flour was mixed with the salt solution to form dough. Before analyses, the dough was rested inside the bake in 25°C for 30 minutes. After the resting each dough party was tested by alveograph separately.

## 4. RESULTS

### **4.1. EVALUATION OF BREAD MAKING QUALITY PARAMETERS OF WHEAT VARIETIES/LINES FROM NATIONAL WHEAT BREEDING PROGRAM OF TAJIKISTAN**

Thousand kernel weight (TKW) of the standard variety Navruz was 24.8 g. All the rest of the varieties/lines showed higher TKW as related to the standard variety. The TKW of 42.0 g was shown by the line Chen/aegilops, and this TKW is the 17.2 g higher than that of the standard variety.

The glassiness varied among the tested varieties and the standard variety Navruz had a value of 45%. The variety Starshina had 87% glassiness and it is the highest value among all the samples.

The wheat gluten content of the standard variety Navruz was 8.5%, while the gluten deformation index of the same variety was 65 (Table 4). Fifteen out of 21 wheat varieties/lines in the present study showed a higher level of gluten content than the standard variety, while four varieties/lines showed a lower wet gluten content (Table 3). The variety Starshina has a high wet gluten content of 17.6%, which is a doubling of the content as related to the standard variety. The gluten deformation index varied between 27 and 85 in the varieties/lines evaluated. The line Cbard/kauz showed the highest gluten deformation index of 85 while the line Tnmu/munta showed the lowest gluten deformation index of 27 (Table 4).

#### **4.2. SPECIFIC PROTEIN COMPOSITION**

A high variation in protein composition was found among the analysed varieties/lines. Only one investigated variety was found homogeneous for composition of HMW-GS (Table 5). The most commonly found HMW-GS encoded on *Glu-A1* was 2\*, which was present in all varieties. Six varieties out of all investigated varieties showed only 2\* encoded on *Glu-A1*. Also HMW-GS 1 was common in the varieties/lines and fifteen of the varieties/lines consisted of grains with a mix of protein compositions of 1 and/or 2\* encoded on *Glu-A1*. Gluten subunit “null” was found only in one sample of a heterogeneous line in the present study and this variety showed three alternative protein compositions e.g. 0/1/2\* encoded on *Glu-A1*.

Only two varieties out of all investigated varieties were homogenous for the protein composition 7+9, encoded on *Glu-B1*. The rest of the investigated varieties showed two, three or four alternative protein compositions encoded from *Glu-B1*. Thus, a number of alternative protein compositions were found within the same sample in most of investigated materials, e.g. 7+8, 7+9, 13+16, and 17+18, encoded on *Glu-B1*. The most commonly detected HMW-GS encoded on *Glu-B1* were 7+9, although also 7+8 were common. HMW-GS such as 13+16 and 17+18 encoded on *Glu-B1*, were found to be relatively common in the Tajik wheat material (Table 5).

The HMW-GS 5+10 were the most commonly found subunit encoded by *Glu-D1* and 5+10 was found in seventeen varieties out of all tested materials of Tajik wheat breeding program. Five varieties out of all investigated varieties were homogenous for a certain protein composition encoded on *Glu-D1*, e.g. protein composition 5+10 or 4 +12. The rest of the

investigated varieties/lines showed heterogeneous protein composition encoded on *Glu-D1*, e.g. combinations of 5+10, 2+12 and 4+12 or 2+10, 3+10, 5+10, 2+12, respectively. The variety Eskina-8 was the only variety with having homogeneous protein composition HMW-GS 4+12 encoded on *Glu-D1*, while three additional varieties showed the same protein composition from *Glu-D1* but in combination with other protein compositions. The only variety found to be totally homogenous for HMW-GS composition was the variety Eskina with 2\* encoded on *Glu-A1*, 7+9 encoded on *Glu-B1*, and 4 +12 encoded on *Glu-D1* (Table 5).

Table 4. Thousand kernel weight (TKW), wet gluten content, deformation of gluten and glassiness of one standard variety, Navruz, and 21 variety/lines from the national wheat breeding program.

# Variety/line	Thousand kernel weight (g) (Mean ± StDev)	Wet gluten (%) (Mean ± StDev)	Gluten deformation index (Mean ± StDev)	Glassiness (%) (Mean ± StDev)
1 Navruz	24,7 ± 1,2	8,5 ± 0,5	65,3 ± 2,5	45,0 ± 4,0
2 Alex	34,9 ± 3,4	11,3 ± 1,0	64,0 ± 3,0	65,0 ± 3,0
3 Starshina	32,4 ± 2,2	17,4 ± 0,7	44,0 ± 4,0	87,0 ± 2,0
4 Tnmu/munta	34,6 ± 3,0	17,5 ± 1,2	27,0 ± 1,0	80,3 ± 2,5
5 Prinia/star	32,2 ± 2,2	15,4 ± 0,4	50,6 ± 4,0	68,0 ± 3,0
6 Shark/f4105w2.1	35,7 ± 2,6	7,7 ± 0,5	69,0 ± 1,0	35,0 ± 4,0
7 Vorona/kauz//1d13.1/mlt	30,4 ± 3,0	11,4 ± 1,0	52,3 ± 6,0	47,0 ± 6,0
8 Tam200/kauz	33,7 ± 3,2	9,3 ± 0,3	77,0 ± 6,0	35,0 ± 1,0
9 1d13.1/mlt//tui	43,5 ± 6,6	10,1 ± 0,9	51,6 ± 1,5	68,3 ± 0,5
10 Arilw pronghorn	28,2 ± 2,1	11,4 ± 0,4	62,0 ± 3,0	74,3 ± 1,5
11 Eskina-8	30,2 ± 0,3	13,4 ± 2,9	48,0 ± 3,0	78,3 ± 0,5
12 Yn/3npm/vos83	37,5 ± 0,4	9,0 ± 1,0	68,0 ± 2,0	65,0 ± 4,0
13 Pastor/3/vorona/cn079	40,3 ± 0,5	7,0 ± 0,1	79,0 ± 4,0	41,0 ± 1,0
14 Skauz bv 92	32,6 ± 0,5	10,2 ± 0,2	58,0 ± 7,0	54,0 ± 1,0
15 Vorona sn079	34,5 ± 0,4	8,6 ± 0,3	77,6 ± 2,5	42,0 ± 2,0
16 Soroca	36,1 ± 0,2	10,0 ± 0,1	54,3 ± 4,5	41,0 ± 1,0
17 Otus toba 97	35,8 ± 0,2	7,4 ± 0,4	80,0 ± 1,0	46,0 ± 1,0
18 Kauz2/chew//bcn/3milan	39,4 ± 1,2	14,2 ± 0,3	62,0 ± 7,0	72,0 ± 2,0
19 Chen/aegilops	42,0 ± 1,3	12,2 ± 0,2	71,0 ± 7,0	62,6 ± 1,5
20 Cbrd/kauz	39,3 ± 4,2	7,5 ± 0,5	85,0 ± 1,0	47,0 ± 1,0
21 Huavun inia	32,1 ± 1,8	9,5 ± 0,5	70,0 ± 2,0	49,0 ± 2,0
22 Cmn82a.1294/2*kauz//	33,6 ± 0,5	10,4 ± 0,4	62,0 ± 2,0	64,0 ± 2,0

Table 5. Protein composition of Tajik wheat varieties/lines from both locations.

#	Variety/Line	Location 1			Location 2		
		<i>Glu-A1</i>	<i>Glu-B1</i>	<i>Glu-D1</i>	<i>Glu-A2</i>	<i>Glu-B2</i>	<i>Glu-D2</i>
1	Navruz	2*	7+9	5+10	2*	7+9/20	5+10/2+12
2	Alex	2*	7+9	5+10/2+12	1	17+18/13+16	5+10
3	Jagger	1	7+9/13+16	5+10/2+12	1/2*	7+9	5+10
4	Tnmu/munta	1/2*	17+18/7+8	5+10/2+12	2*	17+18/7+9/13+16	5+10/4+12
5	Prina/star	1/2*	7+9	5+10/2+12	2*	7+9/7+8	5+10
6	Shark/f4105w2.1	2*	7+9	5+10	2*	7+8	5+10/2+12
7	Vorona/kauz//1d13.1/mlt	2*	7+8	5+10/2+12	1/2*	7+8/7+9	5+10/4+12
8	Tam200/kauz	2*	7+8/7+9	2+12	2*	7+8/17+18	2+12/3+12
9	1d13.1/mlt//tui	1	7+9	5+10/2+12	1/2*	7+9/7+8	5+10
10	Arilw pronghor	1/2*	7+9	5+10	1	7+9	5+10
11	Eskina-8	2*	7+9	4+12	2*	7+9	4+12
12	Yn/3npm/vos83	2*	7+8/7+9	5+10	2*	7+8	5+10
13	Pastor/3/vorona/cn079	1/2*	7+9	5+10/4+12	1	7+9/7+8	5+10/2+12
14	Skauz bv 92	1/2*	7+9/3+16	5+10	2*/0	7+9	5+10/2+12
15	Vorona sn079	2*	7+8/7+9	5+10/2+12	1/2*	7+8/13+16/17+18	5+10
16	Soroca	2*	13+16/17+18	2+12/5+10	2*	13+16/7+8	2+12
17	Otus toba 97	1/2*	7+9	2+12	2*	7+9/7+8	5+10/2+12
18	Kauz2/chew//bcn3milan	1	7+9/7+8	2+12/5+10	1/2*	7+9/13+16	5+10
19	Chen/aegilops	1/2*	7+9/13+16	2+10/3+10/5+10	1	7+9	2+10/2+12
20	Cbrd/kauz	1	17+18	5+10	2*	7+8/7+9	5+10
21	Huavun inia	2*	7+8/13+16	5+10/2+12	1/2*	7+9/17+18	5+10
22	Cmn82a.1294/2*kauz//	2*	7+8/7+9	5+10	1	17+18	5+10

### 4.3. WHEAT GRAIN MOISTURE CONTENT

In this study the grain moisture content of the samples varied from 8,3 to 9,3% in analysed samples although no significant difference were present among most of the samples (Table 6).

Table 6. Moisture content of wheat variety/line.

#	Variable Variety/Line	Moisture content (mean± StDev)
1	Navruz	8,5 ± 0,4
2	Alex	8,8 ± 0,3
3	Jagger	8,3 ± 0,5
4	Tnmu/munta	8,6 ± 0,2
5	Prinia/star	9,5 ± 0,5
6	Shark/f4105w2.1	9,4 ± 0,3
7	Vorona/kauz//1d13.1/mlt	8,5 ± 0,4
8	Tam200/kauz	9,3 ± 0,4
9	1d13.1/mlt//tui	9,0 ± 0,7
10	Arilw pronghorn	8,8 ± 0,3
11	Eskina-8	9,7 ± 0,2
12	Yn/3npm/vos83	8,5 ± 0,4
13	Pastor/3/vorona/cn079	8,5 ± 0,3
14	Skauz bv 92	8,6 ± 0,4
15	Vorona sn079	9,0 ± 0,3
16	Sorooca	8,8 ± 0,7
17	Otus toba 97	9,0 ± 0,4
18	Kauz2/chew//bcn/3milan	9,1 ± 0,2
19	Chen/aegilops	8,7 ± 0,1
20	Cbrd/kauz	9,1 ± 0,3
21	Huavun inia	8,4 ± 0,4
22	Cmn82a.1294/2*kauz//	8,8 ± 0,3
23	Starshina	8,6 ± 0,5



#### ***4.4. AMOUNT AND SIZE DISTRIBUTION OF POLYMERIC AND MONOMERIC PROTEINS OF THE WHEAT VARIETIES/LINES***

As to amount and size distribution of proteins, all measured protein parameters were found to vary significantly as related to cultivation location and investigated variety (Table 7). The small difference was found in protein parameters between two locations and location one shows little bit higher results compare to second location (Table 8). Only for uLPP, a non-significant difference was found for varieties (Table 7). For all protein parameters except for eSMP, uSMP, TUPP and LUPP, the amounts were higher in the first location (Hisor valley) as compared to the second location (Isfara district). Large variation was found for all protein parameters according to the investigated cultivars and lines. Highest amount of TOTE was found for Jagger 1.44, Tnmu/munta 1.43 and Vorona sn079 1.44 and the lowest amount of TOTE was found for Yn3npm/vos83 1.15. The highest values of TOTU and %TUPP were found for Cmn82a.1294/2\*kauz// 7.15 and 0.55. In both locations, the protein content varied between all investigated varieties and lines.

Table 7. Mean square values from ANNOVA of relative amounts of protein fractions of twenty three wheat lines in two different locations.

Source	Df	eLPP	eSPP	eLMP	eSMP	uLPP	uSPP	uLMP	uSMP	TOTE	TOTU	TUPP	LUPP	LUMP	Monopol
Location (L)	1	0.35***	15.4***	32.6***	0.54***	0.45***	0.63***	7.23***	2.25***	90.2***	16.9***	0.04***	0.03***	0.007***	170.7***
Cultivar (C)	21	0.97***	0.36***	2.42***	0.07***	0.09ns	0.70***	0.33***	2.44***	3.35***	1.63***	0.005***	0.01***	0.002***	4.92***
LxC	21	0.70***	0.34***	1.37***	0.04***	0.06ns	0.17***	0.28***	0.08***	2.91***	0.84***	0.002***	0.004***	0.001***	4.25***
Error	90	1.76	0.01	0.11	0.02	0.06	0.02	0.05	0.02	0.23	0.07	0.0002	0.001	0.0004	0.16
Total	134														

\*, \*\*, \*\*\*=Significant at P<0.05, 0.01, 0.001. DF= degree of freedom ns=Non significant

Table 8. Mean values of relative amounts of different protein fractions of twenty two wheat lines/varieties in two different locations.

Source	eLPP	eSPP	eLMP	eSMP	uLPP	uSPP	uLMP	uSMP	TOTE	TOTU	TUPP	LUPP	LUMP	Monopol
	(10 <sup>6</sup> )	(10 <sup>7</sup> )	(10 <sup>7</sup> )	(10 <sup>6</sup> )	(10 <sup>6</sup> )	(10 <sup>7</sup> )	(10 <sup>7</sup> )	(10 <sup>6</sup> )	(10 <sup>8</sup> )	(10 <sup>7</sup> )	(%)	(%)	(%)	(10 <sup>8</sup> )
Location 1 (Hisor)	3.46a	3.47a	9.44a	8.38b	5.05a	2.75a	2.42a	4.97b	1.41a	6.17a	0.49b	0.57b	0.20a	1.94a
Location 2 (Isfara)	2.44b	2.79b	8.45b	9.66a	3.88b	2.61b	1.95b	5.06a	1.24b	5.46b	0.45a	0.61a	0.18b	1.71b

Numbers followed by the same letter within a column and for specific protein parameters do not differ significantly (LSD method < 0.05)

Table 9. Mean values of twenty three different wheat lines from both locations, at maturity.

No	Variety/lines	eLPP	eSPP	eLMP	eSMP	uLPP	uSPP	uLMP	uSMP	TOTE	TOTU	TUPP	LUPP	LUMP	Monopol
1	Navruz	3.38b	3.08fghi	7.71m	8.35g	3.69bc	2.51ji	2.30bcde	5.06bcd	1.19jk	5.69fghi	0.45fg	0.51ji	0.23a	1.80jki
2	Alex	4.00a	3.58a	9.25cd	8.45g	4.54bc	2.75fgh	2.19cdefg	4.70gh	1.40ab	5.87efgh	0.45gh	0.54hji	0.19efgh	1.79jkil
3	Jagger	3.42b	2.96jkl	1.02a	8.36g	4.14bc	2.21kl	1.93ghji	4.58h	1.44a	5.02kl	0.44gh	0.55hi	0.16jk	2.27a
4	Tnmu/munta	3.04c	2.96hijkl	1.02a	8.56fg	4.83bc	2.66hi	2.40abc	4.81fg	1.43a	6.02cdef	0.49c	0.61cdef	0.18fghi	2.17c
5	Prina/star	3.30b	3.29cde	8.78ghijkl	8.76efg	4.86bc	2.87cdef	2.08defghi	5.05bcd	1.32cdefg	5.95defg	0.48cd	0.60defg	0.19efghi	1.77kl
6	Shark/f4105w2.1	2.84ed	3.28cde	9.16cdef	8.37g	5.07bc	2.70fghi	1.69k	4.70gh	1.35bcde	5.37ji	0.47cdef	0.64bc	0.15k	1.80jki
7	Vorona/kauz//1d13.1/mlt	2.79ef	3.00hji	9.48bc	9.82c	4.41bc	2.36jk	2.13defgh	4.68gh	1.37bc	5.41ji	0.46defg	0.61cdef	0.18ghji	2.18bc
8	Tam200/kauz	3.06c	3.23def	8.40kl	8.78efg	9.34a	2.82efgh	1.83jki	5.13abcd	1.28ghi	6.11cde	0.51b	0.66ab	0.17hjki	1.62m
9	1D13.1/mlt//tui	2.67fg	3.05hji	8.52jkli	7.63h	4.16bc	2.67ghi	1.90hjki	4.97def	1.26hi	5.49i	0.48cd	0.60cdefg	0.18ghji	1.83ghji
10	Airilw pronhorn	3.04c	3.20efg	8.35l	8.71efg	4.09bc	2.73fgh	2.04efghji	4.98cdef	1.27ghi	5.68ghi	0.47cde	0.57fgh	0.19defgh	1.77kl
11	Eskina-8	2.76ef	2.97hjki	8.94defgh	1.31a	2.74c	2.17l	1.80jk	5.17abc	1.35cdef	4.77l	0.43hi	0.50j	0.16jki	2.24ab
12	Yn/3npm/vos83	2.18i	2.65m	7.76m	9.08def	4.01bc	2.78fgh	2.24bcdef	5.30a	1.15k	5.96defg	0.52b	0.64bc	0.22abc	1.88fgh
13	Pastor/3/vorona/cn097	2.47h	2.71lm	8.62hjki	9.44cd	3.31bc	2.10l	2.23bcdef	4.99cde	1.25ji	5.16jk	0.45gh	0.57gh	0.20bcdefg	2.27a
14	Skauz bv 92	2.53gh	3.01hji	9.11cdef	8.77efg	4.45bc	2.98cde	2.34abcd	5.05bcd	1.32cdefg	6.27bcd	0.51b	0.63bcd	0.20bcdefg	1.91f
15	Vorona sn079	3.39b	3.53ab	9.74b	8.66efg	4.16bc	2.85defg	2.34abcd	5.19ab	1.44a	6.13bcde	0.45efg	0.55hi	0.19efgh	1.88fg
16	Soroca	3.08c	3.41bc	9.08defg	9.11de	4.19bc	3.05bc	2.30bcde	5.13abcd	1.37bcd	6.29bc	0.48c	0.57fgh	0.20bcdefg	1.78jkl
17	Otus toba 97	2.98cd	2.91jk	8.86efghi	8.99def	3.79bc	2.39jk	2.33bcd	5.02bcde	1.29fghi	5.60hi	0.46efg	0.55h	0.20bcdefg	2.12d
18	Kauz2/chew//bcn/3milan	3.08c	3.37cd	9.19cde	8.98def	4.59bc	3.02bcd	2.44abc	5.12abcd	1.37bc	6.44b	0.48c	0.60defg	0.20abcdef	1.82hijk
19	Chen/aegilops	3.08c	3.42bc	8.84efghji	1.06b	3.28bc	2.36jk	2.41abc	5.26a	1.36bcd	5.63ghi	0.42i	0.51ji	0.21abcde	2.00e
20	Cbrd/kauz	2.77ef	3.22ef	8.47jkl	8.62efg	4.06bc	2.53ji	2.09defghi	5.16abc	1.28ghi	5.55hi	0.45efg	0.59efg	0.19cdefgh	1.85ghi
21	Huavun inia	2.52gh	2.85kl	9.15cdef	8.99def	4.26bc	2.53ji	2.59a	5.15abcd	1.31defgh	6.07cde	0.48c	0.62bcde	0.21abcd	2.17cd
22	Cmn82a. 1294/2*kauz//	2.44h	3.11fgh	8.70ghjki	8.79efg	4.66b	3.57a	2.48ab	5.25a	1.29ghi	7.15a	0.55a	0.69a	0.22ab	1.67m
23	Starshina	2.56fg	3.06ghi	8.89defghi	8.32g	5.13bc	3.17b	2.00fghji	4.58h	1.30efghi	6.18bcde	0.52b	0.65b	0.18ghji	1.73l

Numbers followed by the same letter within a column and for specific protein parameters do not differ significantly (LSD method < 0.05)

Significantly positive correlation was found between eLMP as well as uSMP and gluten index. TOTE correlated significantly negatively with gluten index while significantly positively with wet gluten. For the rest of the evaluated protein fractions, no significant correlation was found with the quality parameters (Table 10).

Table 10. Significant correlation between specific protein fractions and quality parameters of 22 wheat varieties/lines.

#	Protein fractions	Quality parameters	Correlations	P-Value
1	eLMP	Gluten index	0,601	0,003
2	uSMP	Gluten index	0,414	0,055
3	TOTE	Wet gluten	0,522	0,013
4	TOTE	Gluten index	-0,401	0,064
5	TOTE	TKW	0,056	0,806
6	TOTE	Glassiness	0,241	0,279
7	TOTU	Wet gluten	-0,040	0,858
8	TOTU	Gluten index	0,050	0,826
9	TOTU	TKW	0,066	0,770
10	TOTU	Glassiness	-0,100	0,658
11	LUMP	Wet gluten	-0,281	0,205
12	LUMP	Gluten index	0,296	0,180
13	LUMP	TKW	0,038	0,866
14	LUMP	Glassiness	-0,127	0,573
15	LUPP	Wet gluten	-0,125	0,580
16	LUPP	Gluten index	0,007	0,974
17	LUPP	TKW	0,103	0,649
18	LUPP	Glassiness	-0,179	0,425
19	TUPP	Wet gluten	-0,078	0,731
20	TUPP	Gluten index	-0,095	0,673
21	TUPP	TKW	-0,024	0,917
22	TUPP	Glassiness	-0,068	0,762

eLMP = extractable large monomeric proteins, uSMP = unextractable small monomeric proteins, TOTE = total extractable proteins, TOTU = total unextractable proteins, LUMP = large unextractable monomeric proteins, LUPP = large unextractable polymeric proteins, TUPP = total unextractable polymeric proteins, TKW = thousand kernel weight.

#### **4.5. EVALUATION OF BREAD WHEAT VARIETIES FOR QUALITY PARAMETERS**

The standard variety Alex showed bread volume of 590 ml from 100 g flour. Only one variety, Vahdat, showed a lower bread volume of 560 ml as compared to the standard variety. The other seven tested varieties showed higher bread volumes than the standard variety. The highest bread volume of 650 ml was from the varieties Sarvar and Yusufi (Table 11).

The gluten index of the standard variety Alex was 57% and three of the other varieties, Vahdat, Ziroat and Ormon, showed higher values than the standard variety. The remaining

five varieties showed lower gluten index values than that of the standard variety. The variety Ormon showed the highest gluten index of 96 compared to the standard and other varieties (Table 11). Total gluten and dry gluten was 24.3 and 7.6, in the standard variety Alex. Two of the lines, Ziroat and Ormon showed lower values for total gluten and dry gluten than the standard variety while the remaining varieties had higher values than the standard one. The variety Yusufi showed the highest values, 33.1% of total gluten and 10.7% of dry gluten, of the investigated lines.

Falling number of the standard variety Alex, was 370 s and four lines, Vahdat, Ziroat, Norman and Somoni, had higher falling number than the standard variety whereas the other four lines showed lower falling number values (Table 11).

Water absorption of the standard variety, Alex was 59.5% and two lines Norman and Ormon showed higher water absorption while the remaining lines showed lower water absorption than the standard variety (Table 11).

The standard variety Alex and the line Vahdat showed similar results for dough strength measured by alveograph. All the other lines showed lower dough strength than the standard variety (Table 11).

Table 11. Wheat quality parameters (bread volume, gluten index, total gluten, dry gluten, falling numbers and water absorption and dough development time) of nine wheat varieties from the National breeding program of Tajikistan.

№	Varieties	Bread volume from (100 g flour, ml)	Gluten index	Total gluten (%)	Dry gluten (%)	Falling numbers (seconds)	Water absorption (%)	Dough development time (min)
1	Alex	590	57	24.3	7.6	370	59.5	4.5
2	Vahdat	560	94	25.8	8.8	386	60.5	4.5
3	Ziroat	620	82	23.1	7.3	389	61.2	1.5
4	Isfara	600	17	25.4	8.8	358	61.1	0.5
5	Norman	610	45	25.3	8.0	374	57.7	2.5
6	Ormon	610	96	19.6	6.5	362	57.0	0.5
7	Sarvar	650	19	28.3	9.2	362	63.0	2.5
8	Somoni	600	38	25.4	8.2	415	63.0	1.0
9	Yusufi	650	51	33.1	10.7	358	64.1	3.5

## 5. DISCUSSION

Many breeders have the aim to combine a suitable protein composition in the bread wheat in order to improve the bread making quality with high yield. Therefore selection is carried out for optimal composition of proteins subunits for new varieties aimed for different products (Bushuk, 1998). The desire is a dough that is highly elastic for bread production, but more extensible for cakes and biscuits production (Edwards *et al.*, 2001). The elasticity of the dough is also one of the main bread making quality determining factors (Payne *et al.*, 1987a). Good elasticity results in gas holding capacity of the dough and thereby elasticity of the dough determines the amount of air that can be included and the size of the air bubbles in the dough (Bloksma, 1990; Mani *et al.*, 1992). A high loaf volume is obtained from wheat having desirable quality and by the use of the most suitable combination of yeast, salt and other components (Lai *et al.*, 1989). According to Švec & Hrušková (2009) one of the main baking quality parameters is the specific bread volume and it is affected by protein properties such as content and composition. Deformation measurements of wheat gluten can be used to divide the wheat into three groups: lower than 75, between 75 and 105, and higher than 105. Wheat belonging to the second group, having gluten deformation from 75 to 105 has been found to have more suitable quality for making bread than wheat from the other two groups (Stepicheva, 2007). In this investigation, 5 varieties/lines belonging to the second group, with gluten deformation between 75 and 105 were found.

The thousand kernel weight is dependent of seed size. The thousand kernel weight of wheat is a parameter used by wheat breeders and flour millers to understand the potential of extractable flour of the wheat. The potential of flour extraction is increased in wheat with higher thousand kernel weight (*Wheat four methods*). Thousand kernel weights have also close correlation with the genetically determined yield potential of a variety (Richards, 2000). The thousand kernel weight depends on the duration of grain filling and the photosynthesis process during the grain filling time. The biomass in terms of amount of green leaves during the grain filling time, correlates positively with filling of the grains and the thousand kernel weight (Simpson, 1968; Mohammadi *et al.*, 2009). Thousand kernel weight is also influenced by agro-ecological conditions, agro-technical practices like irrigation, fertility of soil, fertilizers etc. (Protic *et al.*, 2007). Also farmers prefer to grow wheat if they can obtain a full grain with high thousand kernel weight (Sharma & Duveiller, 2003). Wheat with higher thousand kernel weight normally has better milling and baking quality and also improved germination (Campbell *et al.*, 2001). Wheat with high thousand kernel weight has an

increased proportion of endosperm compared to wheat with small kernels (Plaut *et al.*, 2004). Protic *et al.* (2007) reported the variety Pobeda to have the highest thousand kernel weight of 44 g within the Serbian wheat materials investigated in their study. Among the Serbian wheat, the thousand kernel weights varied from 33 to 44 g with an average at 38 g (Protic *et al.*, 2007). The highest value of thousand kernel weight in our investigated samples was 42 g showed by the line Chen/aegilops.

Wheat with higher glassines has better milling properties (Yinian *et al.*, 2008). Among the wheat varieties in the present study several showed higher glassines than the standard variety, showing glassines of 45%. The wheat glassines can also be used as bread making quality parameter. The wheat glassiness also determines which purpose wheat is used for; bread baking require a minimum of 30% glassines while pastry baking require a minimum of 60% glassines (Sperdea, 2008; Sperdea *et al.*, 2010).

Deng *et al.* (2005) reported that wheat lines carrying the HMW-GS 14+15 encoded on *Glu-B1*, and 5+10 encoded on *Glu-D1*, had higher values of flour quality, dough rheological parameters and bread-making quality compared to lines containing other protein compositions. Bradová & Štočková (2010), found that, HMW-GS 7+9 encoded on *Glu-B1*, and 5+10 encoded on *Glu-D1*, were present in varieties known as bread wheat varieties, while HMW-GS 6+8 encoded on *Glu-B1*, and 2+12 encoded on *Glu-D1*, occurred in varieties not suitable for bread-making. Among varieties containing 2+12 and 5+10 encoded on *Glu-D1*, gluten strength was found to be higher in varieties with HMW-GS 5+10, than in those with HMW-GS 2+12 (Johansson *et al.*, 1999). According to Payne *et al.* (1987b) the HMW-GS 5+10 are contributing to better bread making quality than HMW-GS 2+12. In a previous study, HMW-GS 5+10 was the most common subunits encoded on *Glu-D1* followed by HMW-GS 2+12. The HMW-GS 4+12 and 3+12 were relatively common among the investigated wheat in the present study as related to what is present in wheat from many other countries (Johansson *et al.*, 1995). The HMW-GS 13+16, 17+18 and 7+8, encoded on *Glu-B1* were common in the investigated wheat materials in the present study and are all considered to have a positive correlation to bread-making quality (Gianibelli *et al.*, 2001).

Table 12. Quality score assigned to individual or pairs of HMW-GS (Payne *et al.*, 1987b).

Score	Chromosome		
	1A	1B	1D
4	-	-	5+10
3	1	17+18	-
3	2*	7+8	-
2	-	7+9	2+12
2	-	-	3+12
1	null	7	4+12
1	-	6+8	-

Most of the bread wheat varieties in former USSR, were based on the variety Bezostaya 1, having subunits 2\*, 7+9 and 5+10 in its three *Glu-A1*, *Glu-B1*, *Glu-D1* loci. Most of the current Tajik varieties/lines used in breeding program are also based on this variety (Morgunov *et al.*, 1990). The most commonly found HMW-GS in the Tajik wheat samples were 2\*, 7+9 and 5+10, respectively, encoded on the three main loci, *Glu-A1*, *Glu-B1*, *Glu-D1*. In the investigated material 17 varieties/lines out of 22 tested were found to carry 5+10 encoded on *Glu-D1*. Lines and varieties containing subunits 5+10 can be used in breeding for high gluten strength if that are desired. For future good bread making quality, varieties with good quality parameters and good combinations of alleles responsible for quality parameters (Payne & Lawrence, 1983; Payne *et al.*, 1984) can be combined within the breeding. Also in durum wheat HMW-GS 7+8 was found to contribute to high elastic and quality compared with HMW-GS 6+8 or 20 (Pogna *et al.*, 1990; Peña *et al.*, 1994).

The main protein parameters determining bread making quality are protein content, composition of specific protein subunits, and amount and size distribution of polymeric proteins. The protein composition of specific protein subunits and protein content is mainly dependent on the genetic background of the wheat variety (Daniel & Triboi, 2000; Johansson *et al.*, 2001), but protein content and amount and size distribution of polymeric proteins is also influenced by environmental aspects (Finney, 1948; Payne *et al.*, 1984; Branlard & Dardevet, 1985; Johansson *et al.*, 2001; 2002; 2003; 2004). Amount and size distribution of polymeric proteins is normally measured by SE-HPLC (Batey *et al.*, 1991). Several recent investigations have paid increasingly attention on protein polymers in order to understand the principles behind bread making quality (Carceller & Aussenac, 2001; Sivam *et al.*, 2010). Quantity of the total HMW and LMW glutenin subunits has been measured trough RP-HPLC



and was found closely correlated with quality parameters, such as dough strength and extensibility. Increasing the quantity of the protein also leads to greater bread volume (Sutton *et al.*, 1990; Andrews *et al.*, 1994; Johansson *et al.*, 2003). According to previous investigations an increase in protein content can positively influence the bread volume, although gluten strength of dough might be negatively influenced (Finney, 1984; Johansson & Svensson, 1999b). In the present work, the variations in amount and size distribution of proteins were evaluated using SE-HPLC, in wheat varieties and lines from two different climatic growing locations. A number of wheat quality parameters such as protein content, hardness, kernel colour, flour volume and other parameters are known not only to depend on the evaluated cultivars but also on the environment (Pomeranz *et al.*, 1985; Bassett *et al.*, 1989; Peterson *et al.*, 1992; Matus-Cádiz *et al.*, 2003). According to previous investigations, the protein content is influenced to a higher extent by the environment, than by the used wheat varieties (Pomeranz *et al.*, 1985; Zhu & Khan, 2001). In the present investigation, TOTE known to correlate with protein concentration (Johansson, 2002; Johansson *et al.*, 2004) was found higher in the first location, than in the second location. Also, TOTU and %UPP, the later correlated with gluten strength (Marchylo *et al.*, 1989; Johansson, 2002), were found higher in the first location than in the second location. The line Cmn82A.1294/2\*kauz// showed the highest percentage of UPP and TOTU in both locations. This line also carried HMW-GS 5+10 encoded on *Glu-D1* as shown by SDS-PAGE. HMW-GS 5+10 is closely correlated with quality parameters such as gluten strength (Johansson *et al.*, 1999; Deng *et al.*, 2005; Bradová & Štočková, 2010). According to previous investigations, varieties with HMW-GS 5+10 encoded on *Glu-D1* were also found to have higher % UPP (Uhlen, 1990; Gupta & MacRitchie, 1994).

According to Holmes & Hosenev (1987), the bread volume also depends on what ingredients are added to the dough, e.g. salt reduces the yeast activity, but improves the dough strength. Furthermore the wheat loaf volume is dependent not only on protein present in the flour, ingredients and water added, but also from mixing time and intensity (Montgomery & Bettencourt, 1977). Cultivars requiring short mixing time normally have weak gluten and the cultivars with medium or long mixing time requirement, normally have stronger gluten resulting in higher bread volume (Finney *et al.*, 1987). In our investigation, the bread volume was measured based on 100 g flour and the highest value of 560 ml were found in two lines, Sarvar and Yusufi. In Zonius & Quail (1997), investigations on wheat samples were analysed for bread volume by taking 100 g flour and the results varied between 648 and 848 ml.

According to Enrique *et al.*, (2003) wheat with gluten index between 60-90 has an optimal value, resulting in good baking quality. There is no correlation between wet gluten and gluten index (Curic *et al.*, 2001). Our results identified only one line, Ziroat, which had a gluten index of 82, which is within the range for an optimal gluten index. The other investigated lines had gluten index higher than 90 or less than 60. As reported by Curic *et al.*, (2001) in the analyses of seven Croatian wheat samples (Divana, Zitarka, Srpanjka, Sana, Ana, Marija, Patria) with Perten Instruments DA 7200 (AB, Stockholm, Sweden), the gluten index varied between 55.92 and 99.60. Our results showed a variation among the varieties, most likely explained by differences in genetic background of the wheat varieties and lines.

Wheat with high gluten content has good bread making properties and can give high bread volume. To obtain optimal flour for making bread wheat, the flour should have 25-30% of gluten content (Enriquez *et al.*, 2003). In our study the total gluten varied from 19.6% to 33.1%. The standard variety Alex and two lines Ormon and Ziroat, showed the lowest percentage of gluten content while the other lines showed gluten content between 25 and 30%. In the investigated varieties and lines the lowest value for the dry gluten was observed in the line Ormon (6.5%). The highest percentage of dry gluten, 10.7%, was found in line Yusufi. According to Curic *et al.* (2001) the gluten quantity has positive correlation with protein quantity and also with bread volume (Sutton *et al.*, 1990; Andrews *et al.*, 1994; Curic *et al.*, 2001; Wieser & Kieffer, 2001; Johansson *et al.*, 2003).

Wheat with good baking quality normally has a low alpha-amylase activity. Alpha-amylase is first produced in the scutellum and aleurone adjacent to the embryo of the grain and moves into the endosperm during germination (Marchylo *et al.*, 1980). Increased alpha-amylase activity in the wheat grain causes enzymatic starch hydrolysis which may disrupt the quality in relation to processing. Wheat grains express three different alpha-amylases during grain development, named *a-AMY-1*, *a-AMY-2* and *a-AMY-3* (Gale & Ainsworth, 1984). Falling number is measured by the Hagberg falling number (HFN) test. A high alpha-amylase activity causes a low HFN, although the falling number is not a measure of alpha-amylase activity, but of viscosity. By the use of HFN, a plunger is falling slowly if dough is thick and it falls quickly if the starch of dough has been converted into sugar by alpha-amylase. According to Lunn *et al.*, (2001) wet weather condition before harvesting can increase the moisture content of the grain. Under these conditions grain may sprout and thus, alpha-amylase activity will increase. The HFNs required in the UK are above 250 s for bread making, 220-225 s for export or intervention, and 180 s for biscuit making. The HFN values below 250 s are thus not good for bread making (*Keeping Hagberg falling number high*).

Falling numbers in this study varied from 358 s to 415 s. According to wheat and flour testing methods (*Wheat and Flour Testing Methods*, 2007) a high falling number above 300 s indicate less alpha-amylase activity and high quality of wheat. A low falling number below 250 s indicates more alpha-amylase activity and weak quality of wheat. The HFN number above 250, 300 s or even 350 s compared to a high quality of a wheat variety (Mares & Mrva, 2008)

According to Lei *et al.*, (1989) water absorption has a positive correlation with dough volume. In our results, the water absorption of the investigated varieties and lines varied from 57.0% to 64.1%. Greer & Stewart, (1959), reported in their investigated materials that water absorption varied from 47.1 to 58.9 %, and they pointed out that hydrolysis enzymes can reduce the water absorption of wheat flour.

The lines Isfara and Ormon showed the lowest dough strength of 0.5 min. According to previous investigations the gluten strength is influenced by genotype of wheat variety and environment interaction (Johansson & Svensson, 1999a; Perretant *et al.*, 2000). The standard variety Alex and the line Vahdat showed the highest dough development time 4.5 min.

In several previous investigations correlation has been reported among specific gliadins and glutenins as well as with amount and size distribution of polymeric proteins, and bread making quality parameters (Johansson, 1996; Johansson *et al.*, 1993; 2013;).

In the present investigation positive correlation was found between eLMP as well as uSMP and gluten index. Furthermore, TOTE also correlated positively with wet gluten, but negatively with gluten index.

## 6. CONCLUSIONS

Due to varietal difference and environmental influence all the quality parameters including, thousand kernel weight, wet gluten content, gluten deformation index, glassiness and protein parameters, varied among all the investigated varieties/lines.

In this research work, glutenin subunits were found in a number of genotypes/lines that normally influence the bread making quality positively due to their correlation to high gluten strength and high protein concentration. In our investigated material 17 varieties/lines out of 22 tested were found to carry glutenin subunits 5+10 encoded on *Glu-D1*, which has been positively correlated to bread making quality parameters and gluten strength. The investigated wheat varieties/lines with protein compositions relevant for baking quality can be

used to improve baking quality in new genotypes through breeding. Suitable protein combination can be selected depending on types of bread made from the flour, to obtain superior combinations in one genotype. For example, varieties/lines which are carrying 5+10 encoded on *Glu-D1*, can be used for wheat breeding programs in order to develop new varieties with increased gluten strength.

The lines Vorona sn079 and Tnmu/munta, were the most interesting varieties/lines as related to their results from SE-HPLC, indicating their relevance for further wheat breeding programs to increase grain protein concentration. Similarly, the lines Cmn82a.1294/2\*kauz//, Yn/3npm/vos83, Tam200/kauz and Skauz bv 92, were of highest relevance in breeding for increased gluten strength based on their results from SE-HPLC. The lines Yusufi and Sarvar, were of relevance based on their results from quality evaluations and can be used in breeding programs in order to develop new varieties with higher bread volume and stronger dough.

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