

Protein profile evaluation for some wheat species, food adulteration identification possibilities

Diana Nicoleta Raba^{*a}, V. Tabără^b, Ghe. Mencinicopschi^c, I. Jianu^a

^aFaculty of Food Processing Technology, Banat's University of Agricultural Sciences and Veterinary Medicine
Calea Aradului no.119, RO-300645, Timisoara, Romania

^b Faculty of Agricultural, Banat's University of Agricultural Sciences and Veterinary Medicine
Calea Aradului no.119, RO-300645, Timisoara, Romania

^cFoods Institut Research Bucarest Romania

Abstract

This paper present a potential method of wheat adulteration determination, which has applicability for the identification of *Triticum durum* and *Triticum spelta* wheat species adulterated with *Triticum aestivum*. For this reason, were subjected to SDS PAGE electrophoresis 6 samples of those three wheat species, and their corresponding flours. The applied methodology is: a comparative study sharp bands of glutenin of those wheat species: *T. aestivum*, *T. durum*, and *T. spelta*, and their flours. From point of view of glutenin subunits distributions of studied species was concluded that *T. aestivum* and *T. durum* varieties are quite similar, and some differences were observed to *T. spelta*.

Keywords: adulteration, flour, wheat, proteins, glutenin, SDS PAGE electrophoresis

1. Introduction

At the very beginning of this millennium almost 1 billion of people on the Earth do not have available the necessary food; this reality premerged the appearance of uncertain, low quality, and adulterated food on the market, which have a lower price comparison with the safe, good quality, and authentic food. This category of food can be very easily bought by the citizens with low possibilities; for the elimination of this problem, the authenticity, security, and safety of food are facing challenging problems worldwide since 1992. Identification and elimination of food adulterations is becoming a difficult problem, because the food matrix is very complex, which require a vast range of analytical techniques, other methods, and statistical programs in order to be deeply analyzed with high precision. One of the most frequent problems are related with the wheat authenticity, this problem being associated with the species where are coming from. The differences between normal wheat and hard wheat

Triticum durum is that the last one is significant in semoline and pasta processing. From this issue, the quality concept of pasta, from consumers point of view, was associated with the limited utilisation of regular wheat grain (*T. aestivum*) and the exclusively usage of hard wheat varieties (*T. durum*) in pasta processing. *Spelta* wheat, with its latin denomination *Triticum spelta* is the oldest cereal and the ancestor of common wheat. In contrast with common wheat, spelta wheat has a particular taste, similar with walnuts one; there are some differences between common wheat and spelta one in terms of proteins complexes, protein content-which can achieve 19% for *spelta* comparison with 13% for *aestivum*.

More than that, wheat grains composition is affecting significantly processing properties of different types of wheat species. Some wheat varieties are suitable for bakery, and some others are suitable for pastries and confectionary.

* Corresponding author: e-mail address: dianaraba@yahoo.com

Glutenin proteins represent approximately 85% of total wheat proteins content, and are arranged proteins of endosperm. Those are composed by prolamins (gliadine), and glutenins (glutenine) [1,2,3].

Wheat gluten is a very complex mix of proteins with at least 50 individual components which have been separated by bidimensional isoelectric focusing of SDS-PAGE from total reduced fractions;

Wheat subunits with high molecular mass (HMW subunits) are responsible for the determination of viscous-elastic properties of the dough, and are similar with allelic subunits pairs, being associated with high or low dough resistance; even though many researchers have been started to determine the adjusted structure of high molecular weight subunits by X-ray crystallography of those whole subunits or of the repetitive peptides, but the proposed crystals did not showed any clear diffraction matrices. Similar to that, the synthetic peptides analysis based on repetitive sequences did not proved to be a way of three-dimensional structure determination [4,5].

The current methods for wheat authenticity determination are: visual identification of the grains, chemical analysis common to all grains, specific qualitative characteristic of all grains, proteic gluten particularities characterization, and some other potential methods for wheat authenticity determination like: ELISA and nucleotides methods [6,7,8,9].

The present paper emphasizes wheat species deceleration by mixes with glutenin fractions separation methods by polyacrylamide gel electrophoresis.

2. Materials and Method

Glutenins isolation. The vegetal material consisting of three wheat species: *Triticum aestivum*, *durum* and *spelta* which were harvested from the research place of Banat's University of Agricultural Sciences and Veterinary Medicine were subjected to SDS-PAGE electrophoretic investigations;

the results have been compared with the flour resulted from those wheat species.

Reagents: TRIS Hydroxymethylamine or TRIS Hydrochloride, sodium dodecyl sulphate (SDS), pyronin Y/G, glycerol, 2-mercaptoetanol, acrilamide, bisacrilamide, TRIS Hydroxy-methylamină sau TRIS Hydrochloride, sodium dodecyl sulphate (SDS), amonium persulphate (APS), NNN'N' - tetramethylethylenediamine (TEMED), tricloracetic acid (TCA), clorhidric acid or sodium hidroxid, acetic acid glacial, glicine, n-buthanol, ethanol, Coomassie Brilliant Blue (CBB) R-250/G-250. The reagents are analyticaly pure, purgheased from Merk.

Instruments: Polyacrylamide gel electrophoresis installation Protean II XI CELL from Bio – Rad, having as main characteristics: central cooling device and adapter tube 26x19x30, buffer tub, air tightening system, platinum electrodes, two glass plates, voltage: 120V – 240V – 100V.

Method. Necessary vegetable material is 50mg. For the wheat case, is necessary a preliminar grinding. Raw material is mixed with 0,75 ml solution of extraction (2,504ml, pH=6,8, 1M TRIS HCl buffer, 8,229 ml mineral free water, 0,2ml SDS 10%, 10mg pyronin Y/G, 10ml glycerol).

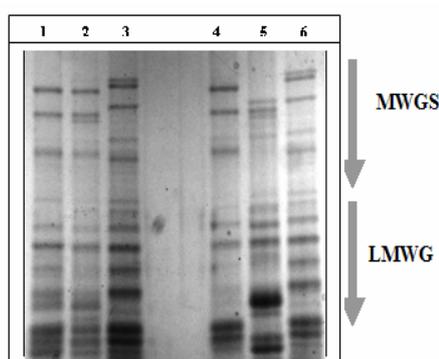
Electrophoresis gel is mantained at 100V for 10-30 seconds, after that the samples is placed in godeaus (around 7,0µl supernatant sample/godeau). Electrophoresis process is taking place to normal temperature, at 100-200 V as long as the migration front goes out of the gel. [10,11].

3. Results and Discussion

The electrophoresis profiles of the high molecular weight gluten subunits (HMWGS) and low molecular weight gluten subunits (LMWGS) correspond to the wheat species studied in the present paper and their corresponding flours, compared to the ones of gliadin and gluten are presented in figure 1.

The gluten profile of different wheat species can be observed in the electrophoresis bands with high molecular weight HMW described in table 1 respecting the genotype.

The electrophoresis bands belonging to the gluten subunits with high molecular weight HMW identified after separation of proteins by SDS-PAGE technique and the binary matrix that certify their frequency in the investigated wheat and flour samples are revealed in table 2.



Legend

- 1= *Triticum aestivum* wheat
- 2= *Triticum durum* wheat
- 3= *Triticum spelta* wheat
- 4= *Triticum aestivum* flour
- 5= *Triticum durum* flour
- 6= *Triticum spelta* flour

Figure 1 The electrophoresis profiles of the high molecular weight gluten subunits (HMWGS) and low molecular weight gluten subunits (LMWGS) corresponding to the three wheat and flour varieties compared to their ones of gliadin and gluten profiles.

According to the informations presented by figures 1 and 2, and 5.3.1. table data respectively, can be concluded that glutenic profile of wheat and flour proteins from *T. aestivum*, *T. durum* and *T. spelta* species presents bands with high molecular weight (HMW):

- *Triticum aestivum* variety is fingerprinted by 5+1 and 7+9 bands, for wheat, and for flour to those bands is added band 8. A comparison between those data's and literature one show that the flour sample has as raw material another type of *T. aestivum* genotype except the studied ones;
- *Triticum durum* species presents 5+10, 7+9 and 20 fingerprints for the analyzed wheat sample, and *durum* flour belongs to some different genotypes of the same species; in this case the comparison with literature data certify the *Triticum durum* samples authenticity;
- Electrophoresis bands with high molecular weight (HMW) of *Triticum spelta* wheat and flour are 2+2*, 6+8 and 12, this being a prove that they are corresponding to the same genotype; by comparison with literature data is noticed their similarity, and the authenticity of investigated samples is confirmed [12,13].

The appearance frequency of the electrophoretic bands corresponding to the high molecular weight gluten subunits (HMWGS) in the studied wheat and flour samples is revealed by figure 2.

Table 1. The electrophoretic bands corresponding to the gluten subunits with high molecular weight (HMW) for the *Triticum aestivum*, *durum* and *spelta* species

Wheat species	Band										
<i>T.aestivum</i>	1	2*	5	7	8	9	10	12	17	18	
<i>T.durum</i>	1	2*	6	7	8	13	16	18	19	20	22
<i>T.spelta</i>	1	2*	2	5	6	7	8	10	12	13	16

Table 2. The binary matrix of the large molecular weight gluten subunits corresponding to the wheat and flour samples investigated by SDS PAGE

Sample origin	Band	2	2*	5	6	7	8	9	10	12	20
	Sample										
<i>Triticum aestivum wheat</i>	1	0	0	1	0	1	0	1	1	0	0
<i>Triticum durum wheat</i>	2	0	0	1	0	1	0	1	1	0	1
<i>Triticum spelta wheat</i>	3	1	1	0	1	0	1	0	0	1	0
<i>Triticum aestivum flour</i>	4	0	0	1	0	1	1	0	1	0	0
<i>Triticum durum flour</i>	5	0	0	0	1	1	1	0	0	0	1
<i>Triticum spelta flour</i>	6	1	1	0	1	0	1	0	0	1	0

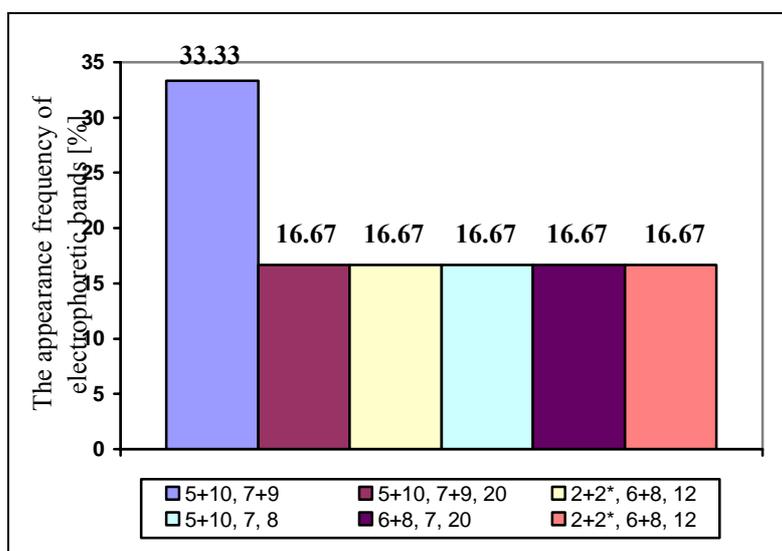


Figure 2. The appearance frequency of the electrophoretic bands in the wheat and flour samples

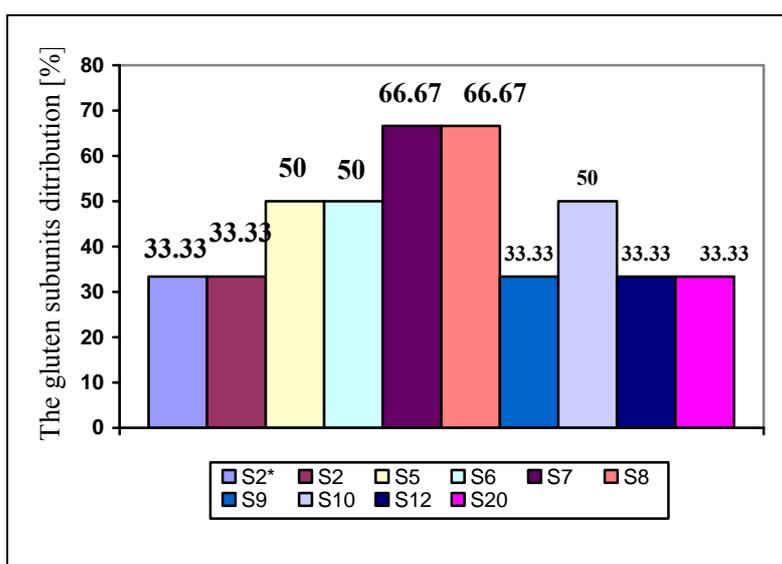


Figure 3. The gluten subunits distribution for the wheat and flour samples

The previous data and a comparison between literature and electrophoresis profile of studied samples show that the glutenic profile of different types of wheat species is certified by the presence of personalized bands; in the present case the presence of band 20 testify the *Triticum durum* authenticity, and 2+2* and 12 bands certify *Triticum spelta*. The affirmation is available in both identifications: for wheat varieties or their flours. Moreover, depending of genotype, the same wheat variety can present different electrophoresis sharp bands, with possible differences between 10 and 11, most of them being common with the three studied species. According to this, the wheat species and flour samples from *T. aestivum* and *T. durum* present common bands 5+10, 7+9, and differences on band 20p-specific to *T. durum*. Another similarity is represented by the presence of 7, 6+8 and 8 bands to all the three species. Unique bands like 20, 12 and 2+2* are present wheat species and flours from *T. durum* and *T. spelta*. Detailed analysis of electrophoresis gels analysis testify the presence of 10 glutenic subunits with high molecular weight, and 7 of them with higher molecular weight placed on superior part of the gel, and the other 3 with lower molecular weight placed on the bottom of polyacrylamide gel. The band frequency graph of those wheat and flour species show that while subunits pairs 5+10, 7+9 are present at 33% of the cases, all the rest of the pairs are individual bands present in equal percent at two of studied samples.

The chart representation of the gluten subunits distribution for the wheat and flour samples belonging to *T. aestivum*, *T. durum* and *T. spelta* is presented in figure 3.

From graphical distribution of glutenin distribution of wheat species *T.aestivum*, *T.durum*, and *T.spelta* and their flours can be concluded that bands 7 and 8 have migrated in most of the cases (66,67%), subunits 5, 6, 10 were identified in 50% of the samples, and sequences 2, 2*, 9, 12 and 20 at just 33,33% of them. Also, glutenin subunits distribution analysis from species

point of view, was evidenced that the highest similitudiny is between *T. aestivum* and *T. durum*, and the only difference found was the presence of 6 and 20 bands at the last one, the rest of glutenin subunits (5,7,9,10) beeing present to both of them. A different glutenic profile was present for the wheat anf flour of *T. spelta* species, fingerprinted by two species.

The dendrogram obtained by the simple link method, based on the 'euclidian distances' is presented in figure 4.

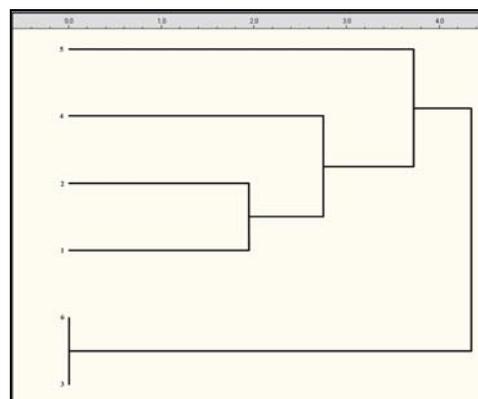


Figure 4. The dendrogram of the *Triticum aestivum*, *durum* and *spelta* wheat samples and of the correspondent flours studied using gluten stamps.

The dendrogram presented in figure 4 certify that the wheat and flour of *T. aestivum* și *T. durum* species presents an obvious similitude in case of glutenic fingerprintings. In this way, is observed that the flours group (5,4) and the wheats group (1,2) are forming a cluster based on the minimum distance between them. At evident distance was placed the cluster formed by the wheat and flour of *T. spelta* species.

4. Conclusions

After the SDS PAGE analysis of glutenic extracts of 9 glutenin sequences with different high molecular weight, some of them beeing specific to particular wheat species, those representing a prove to their authenticity.

Were identified the next bands: 2*, 5, 6, 7, 8, 9, 10, 12 and 20, glutenic profile of

different wheat species is personalized by the presence of a specific band.

As a conclusion, band 9 was identified only in the case of *Triticum aestivum*, band 12 is specific to *Triticum spelta*, and band 20 for *Triticum durum*. Bands: 2*, 5, 6, 7, 8, and 10 were identified distributively in the matrix of all 6 analyzed samples, but each variety has also its specific band present. According to these results, it can be clearly concluded that the substitution of one of them with another one can be identified based on this data.

Due to the fact that the specific bands for one genus is very limited, for the precise adulteration identification of those varieties for purpose or involuntary, it is necessary for the identification to be done by mixing SDS PAGE electrophoresis with another quantitative method, more sensitive, for example High performance liquid chromatography HPLC or Infrared spectroscopy NIR.

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