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Thermal properties of gluten proteins of two soft wheat varieties

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Abstract

The thermal properties of gluten proteins from two soft wheat varieties showing different rheological properties, ''Amazonas'' and ''Sorraia'', were studied by differential scanning calorimetry. Three endothermic peaks were found in all gluten proteins fractions, exception to ''Amazonas'' gliadins. In this case, the third endotherm is absent. Transition temperature, transition enthalpy and activation energy of the transition reaction were determined. Since gluten development is caused by the breakage and reformulation of sulphur bridges, the higher the energy needed to perform denaturation the more difficult will be the interaction between gliadins and glutenins. ''Amazonas'' wheat needs more energy to onset and to develop the transition. So, it is to be expected that the interactions among the protein fractions will become more difficult and consequently the gluten will appear as a weaker one. Multivariate analysis of the results indicates that the gliadin fraction seems to be the most responsible for the lower bread baking ability shown by ''Amazonas'' wheat.

Keywords: DSC; Gluten protein; Gliadins; Glutenins; Soft wheats

1. Introduction

The knowledge of the conformational changes that occurred in cereal macromolecules, namely proteins, is important for understanding their functionality. Proteins are known to form three dimensional structures mainly stabilized by non-covalent interactions. Gluten proteins (glutenins and gliadins) are the major storage wheat proteins. The unique functional properties of wheat among cereals are due to its ability to form gluten, a protein network showing viscoelastic properties and leading to a desirable texture after baking. These properties are mainly related to insoluble storage proteins. In particular, the glutenin polymer appears to determine dough strength, by forming an elastic network, which interacts with the gliadins by non-covalent forces, mainly hydrogen bonds (Lamacchia et al., 2000). Several research studies showed that the impact of glutenins on bread making quality is more important than the one of gliadins (Odintsova et al., 2000).

Gliadins are present as monomers and are responsible for gluten extensibility and cohesiveness, while the glutenins form high molecular weight polymers and contribute to the elasticity of gluten.

Both the gliadins and glutenins are mixtures of proteins that can be divided into groups. One of such groups of glutenin proteins, called the high molecular weight subunits of glutenin, appears to be particularly important in determining the viscoelastic properties of gluten and differences in this property among cultivars of good and poor bread making performance (Payne, 1987).

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The transitions occurring in protein affect the physical state and textural characteristics of various foods. The thermal behaviour of dough is very important to the quality of the final bakery product which results from two thermally induced phenomena: starch galatinization and protein denaturation. Denaturation is the most important transition occurring in proteins during the baking process, and contributes significantly to the characteristics of the baked products. Protein denaturation is defined as a process or a sequence of processes in which spatial arrangement of the polypeptide chain within the protein molecule is changed from the typical form of the native protein to a more disordered arrangement.

Most proteins denature at temperatures from 50 to 80 °C, being range of protein transition temperature specific for each protein (Slade & Levine, 1995). The denaturation of proteins has been extensively studied by differential scanning calorimetry (DSC), where denaturation is observed as an endothermic peak (Privalov & Khechinashvili, 1974).

The protein fraction of food materials is often composed of several proteins. Therefore, thermograms of a particular food material may exhibit several denaturation endotherms (Donovan, Mapes, Davis, & Garibaldi, 1975).

Wheat protein transition is of major importance in establishing bread structure. Another functional feature of wheat protein is its hydration during dough formation and the transfer of water from gluten to the starch component during baking, to support the swelling of the starch granules. The transition of gluten is accompanied by decreased solubility and proceeds to a point where the gas vesicle walls are fixed and expansion ends. The glass transition temperature (T_g) has been the main studied parameter to understand the mechanical properties of gluten proteins (Kalichevsky & Blanshard, 1992). The glass transition of glutenin and its depression due to water plasticization were observed from studies using thermal and mechanical behaviours of the glutenin fraction by DSC and mechanical spectroscopy (Cocero & Kokini, 1991). Other authors Kalichevsky, Jaroszkiewicz, and Blanshard (1993) confirmed this statement.

In spite of the current knowledge about glass transition of wheat proteins, little is known about other thermal properties of those proteins. However, one can assume that dough constituents react on heating to give rise to intermolecular and intramolecular cleavages which produce highly crosslinked macromolecular structure. These phenomena modify the reological properties of dough and are responsible for the solid-like properties of baked products (Schiraldi, Piazza, Fessas, & Riva, 1999, chap. 16). The lack of knowledge of those thermal properties has been explained by the small or no calorimetric response of wheat proteins (gliadins and glutenins) (Ma, 1990). Arntfield and Murray (1981) did not found a characteristic endotherm of denaturation for gluten proteins. Meanwhile, other authors reported the presence of two or four small peaks on isolated globulins from wheat (Eliasson & Hegg, 1980). The peaks at 88 and 101 °C were attributed to gluten protein transitions but the apparent enthalpies of the protein transition were very small. There is no consistent explanation for the absence of gluten denaturation endotherms. Eliasson and Larsson (1993) proposed different hypotheses: (i) no ordered structure exits in the gluten proteins; (ii) they have an unusual thermostability; (iii) or the DSC technique requires considerable cooperativity to produce a detectable heat flow, which may not be possible with gluten proteins. Nevertheless recently Léon, Rosell, and Barber (2003) concluded that DSC conditions used in previous works for the detection of the thermal properties of wheat proteins were not the most suitable. The same authors also concluded that when thermal properties of wheat proteins are assessed in samples with low water content, peak endotherms are found at temperatures ranging from 50 to 85 °C. The albumins and globulins are the most heat sensitive, followed by the gliadins and finally the glutenins. Based on the encountered high enthalpy values, this effect seems to be related to the most ordered structure of those proteins.

The present study intends to contribute to explain the differences between technological ability of two portuguese soft wheat varieties presenting similar protein content, on the basis of the thermal properties of the gliadins and glutenins fractions (soluble and insoluble in an acid solution).

2. Materials and methods

2.1. Materials

Two portuguese soft wheat varieties – "Amazonas" and "Sorraia" – were supplied by a national germplasm bank (Estação Nacional de Melhoramento de Plantas – Portugal). Those were previously characterized for protein content and rheological properties (Brites & Bagulho, 1999).

Flours were fractionated into three main fractions according to a previously reported method (Czuchajowska & Pomeranz, 1993). The obtained gluten fractions were further fractionated and purified according to Fig. 1.

2.2. Methods

2.2.1. DSC analysis

DSC analysis was performed in a Shimadzu DSC 50 equipped with a TA 50 SI thermal analyser. Helium (99.95% purity) was the purge gas and flowed at approximately 20 mL min^{-1}. The calorimeter was calibrated according to a standard procedure established in the manufacturer user manual. The DSC instrument was

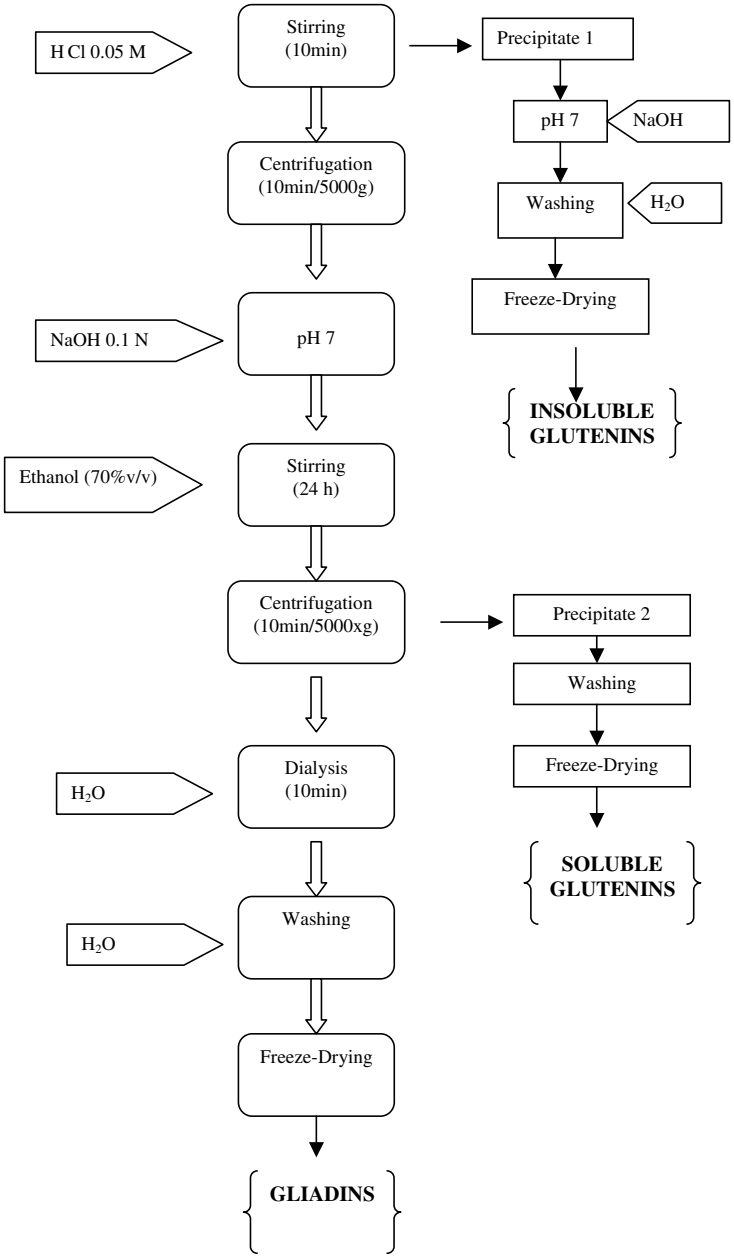

Fig. 1. Fractionation and purification diagram to produce gluten proteins fractions.

calibrated using indium (m.p.: 156.6 °C, $\Delta H_f = 28.45$ J g^{-1}) and deionised water (m.p.: 0 °C, ΔH_m: 333 J g^{-1}). The calibration procedure was completed with the scanning rate to be used in the trials.

Samples of 7.0 ± 0.1 mg were weighed into aluminium pans (inner volume: ca.30 mL), and covers were hermetically sealed into place. An empty, hermetically sealed aluminium pan was used as reference. Prior to the analysis of samples, the baseline was defined with an empty, hermetically sealed aluminium pan.

In a previous trial samples were equilibrated at different water activity values (0.05, 0.3, 0.79 and 0.9) and samples scanned in the calorimeter. On the bases of these results $a_w = 0.79$ (about 50% moisture) was selected as the most suitable. Gliadins and glutenins were subjected to the following temperature programme: heating from 20 to 80 °C at a scanning rate of 2 °C min^{-1}.

Thermal transition was defined in terms of peak or transition temperature (T_n). Heat of transition or enthalpy ΔH (J g^{-1}) was evaluated from peak areas and results expressed per weight (g) of protein. The manufacturer's software programme was used to analyse and plot the thermal data (Anon, 1995).

The transition kinetics were studied by DSC, using the heat evolution method of Borchardt and Daniels as reported by Danielenko et al. (1985). The method assumes that the reaction obeys the relationship

$$d\alpha/dt = k(1-\alpha)^n,$$

where $d\alpha/dt$ is the reaction rate; k is the rate constant (s^{-1}); n is reaction order.

The reaction rate $d\alpha/dt$ is obtained by dividing the peak height at a temperature T by the total area, and the fraction unreacted $(1-\alpha)$ obtained by measuring the ratio of the partial area at temperature T to the total peak area, and then subtracting this value from unity.

The method also assumes that the dependence of the reaction rate follows the Arrhenius expression:

$$v = v_0 e^{E_a/RT},$$

where v is $d\alpha/dt$; v_0 is the constant rate; E_A is the activation energy (J mol^{-1}); R is the gas constant; and T is the absolute temperature.

All the results are the average of at least four runs.

3. Statistical analysis

Data were statistically analysed by Principal Component Analysis (PCA) and Clusters Analysis using the software "Statistica™", version 5 (from Statsoft, USA).

4. Results and discussion

Previous studies (Table 1) on gluten from both wheat varieties show high alveographic strength ($W > 200 \times 10^{-4}$ J) but different viscoelastic properties. The water absorption ability of both flours (to attain the same consistency), is 53.5% and 58.2% for "Amazonas" and "Sorraia", respectively. Protein content is quite similar in both varieties. This fact can be explained by the different composition in both low and high molecular weight glutenins, as verified by separation by electrophoresis in SDS polyacrilamide gel (SDS–PAGE) (Brites & Bagulho, 1999). The works published in this domain (Payne, Nightingale, Krattiger, & Holt, 1987) suggested that "Amazonas" variety possesses glutenins HMW subunits (5 + 10) that provide larger force to the gluten than those presented in the "Sorraia" variety (2* + 12). However, the obtained results do not explain the rheological behaviour of the two varieties based on the HMW glutenins. According to the Alveographic results "Sorraia" gluten shows a higher tenacity and a lower extensibility than "Amazonas" gluten, and so a P/L higher than one. On the other hand "Sorraia" W value is higher than that of the "Amazonas".

Table 1
Physical-chemical properties of the two tested soft wheat varieties

	Protein (%)	W (J 10^{-4})	P (mm H_2O)	L (m 10^{-3})	P/L
"Sorraia"	12.0 ± 0.1	398	156	80	1.99
"Amazonas"	12.9 ± 0.1	318	70	125	0.57

W, strength; P, tenacity; L, extensibility.

4.1. DSC analysis of gluten fractions

The main scope of this research was the assessment by DSC analysis of the thermal behaviour of two soft

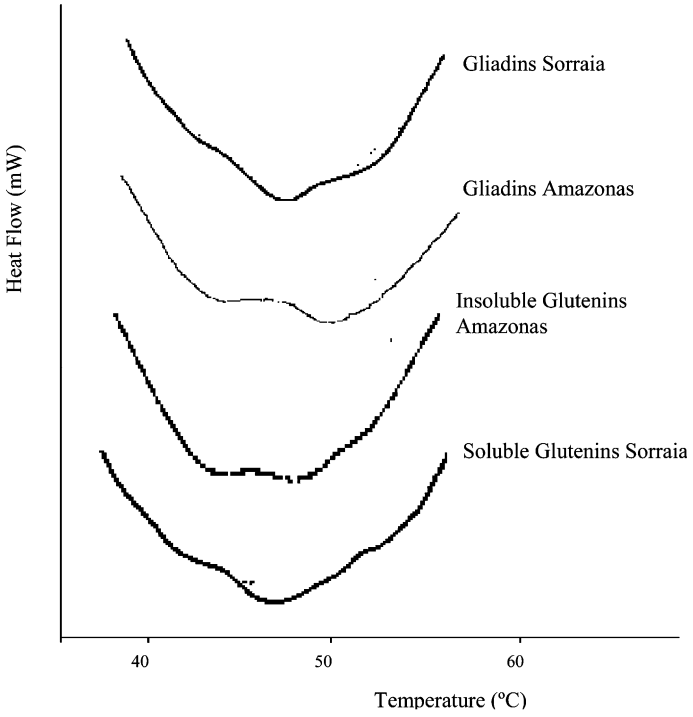

Fig. 2. Examples of Amazonas and Soraia wheat gluten proteins DSC thermograms.

wheat varieties showing different bread making abilities as determined. Fig. 2 presents examples of thermograms for wheats gliadins and glutenins.

As already found by Léon et al. (2003) all the fractions presented endothermic transitions and that water content may have played a role in these transitions. In order to determine whether this factor was responsible for the absence of protein endotherms in previous reports the thermal properties of gluten protein fractions were assessed in the presence of different moisture contents. Only at a relative humidity around 80% (about 50% moisture) it was possible to detect transition phenomena. Those results are also in agreement with other authors who found that water contents around 50% were the most suitable conditions. When increasing or decreasing water content, no endotherms could be registered. Therefore, from our results and Léon et al. (2003) it seems that the absence of wheat protein endotherm was not real and could be due to unsuitable DSC conditions. DSC data of "Amazonas" and "Sorraia" wheat gliadins are summarized in Table 2.

"Amazonas" and "Sorraia" gliadins showed different thermal behaviour in terms of the numbers of endotherms, two and three for "Amazonas" and "Sorraia", respectively. Nevertheless, the transition temperatures encountered for the two first endotherms are similar for both wheat varieties. In "Amazonas" wheat the enthalpy involved in transition is higher than in "Sorraia" presumably due to higher ordered structure in "Amazonas" wheat that may also explain its poorer contribution to the variety's rheological properties. Activation energy is higher in "Sorraia", which means that this wheat needs more energy to onset the transition process, due to its higher heat stability (Eliasson & Hegg, 1980). However, once denaturation started the energy needed to accomplish the process is smaller then in "Amazonas" wheat.

Concerning the soluble glutenins of both varieties (Table 3), the same number of endotherms was observed. Higher transition temperatures were found for "Amazonas" fractions, thus making the transition more difficult. The enthalpy values involved in fractions transitions and activation energies are also higher in "Amazonas" fraction, meaning that this phenomenon is more difficult to achieve in this wheat cultivar.

The values of transition temperature, enthalpy and activation energy of the insoluble glutenins from "Amazonas" and "Sorraia" wheats are shown in Table 4.

The insoluble glutenins showed similar pattern in both wheat cultivars in regard to the number of endotherms and transition temperatures. Again the energy needed to develop the transition is higher in "Amazonas" wheat.

Principal Component Analysis (PCA) and Cluster Analysis (CA) were applied trying to explain the difference in the whet variety's functionality based on transition parameters. PCA reduced space of six dimensions (transition temperatures and enthalpies) to a plane defined by two principal components (Factor 1 and Factor 2) accounting for 78.5 % of the total variance of the original data (Fig. 3). The enthalpies (ΔH_1 and ΔH_2) are negative and significantly related to the first principal component, transition temperatures (T_1 and T_2) are negative and significantly related to the second principal component and ΔH_3 are positively related to the third component. It is apparent that "Sorraia" is mainly defined by the third transitions found in all protein fractions.

Table 2
Transition temperature (T °C), enthalpy (ΔH J g^{-1}) and activation energy (E_A J mol^{-1} °C^{-1}) of the gliadins from "Amazonas" and "Sorraia" wheat

	T_1	T_2	T_3	E_{A1}	E_{A2}	E_{A3}	ΔH_1	ΔH_2	ΔH_3
"Amazonas"	47.7 ± 3.6	49.7 ± 3.9		18674 ± 670	19167 ± 298		21 ± 3.2	12.3 ± 6.7	
"Sorraia"	44.4 ± 0.2	45.6 ± 0.5	46.5 ± 0.8	45165 ± 616	38981 ± 208	42416 ± 136	1.4 ± 0.1	3.7 ± 1.1	3.5 ± 2

Table 3
Transition temperature (T °C), enthalpy (ΔH J g^{-1}) and activation energy (E_A J mol^{-1} °C^{-1}) of the soluble glutenins in an acid solution from "Amazonas" and "Sorraia" wheat

	T_1	T_2	T_3	E_{A1}	E_{A2}	E_{A3}	ΔH_1	ΔH_2	ΔH_3
"Amazonas"	48.0 ± 1.3	49.1 ± 1.04	53.1 ± 1.1	50427 ± 815	55116 ± 919	30157 ± 328	5.6 ± 2.6	7.8 ± 0.9	3.1 ± 0.5
"Sorraia"	36.8 ± 0.8	37.3 ± 0.8	38.8 ± 1.3	30332 ± 879	25305 ± 844	45813 382	4.4 ± 2.4	2.4 ± 0.7	3.2 ± 0.6

Table 4
Transition temperature (T °C), enthalpy (ΔH J g^{-1}) and activation energy (E_A J mol^{-1} °C^{-1}) of the insoluble glutenins in an acid solution from "Amazonas" and "Sorraia" wheat

	T_1	T_2	T_3	E_{A1}	E_{A2}	E_{A3}	ΔH_1	ΔH_2	ΔH_3
"Amazonas"	41.0 ± 1.2	43.5 ± 2.3	44.5 ± 2.4	2734 ± 146	19537 539	74567 ± 358	7.5 ± 1.5	13.7 ± 8.7	0.8 ± 0.3
"Sorraia"	42.0 ± 3.0	43.6 ± 3.4	44.9 ± 4.5	3693 ± 824	12421 ± 246	16691 ± 762	6.6 ± 2.6	6.7 ± 3.3	2.4 ± 1.0

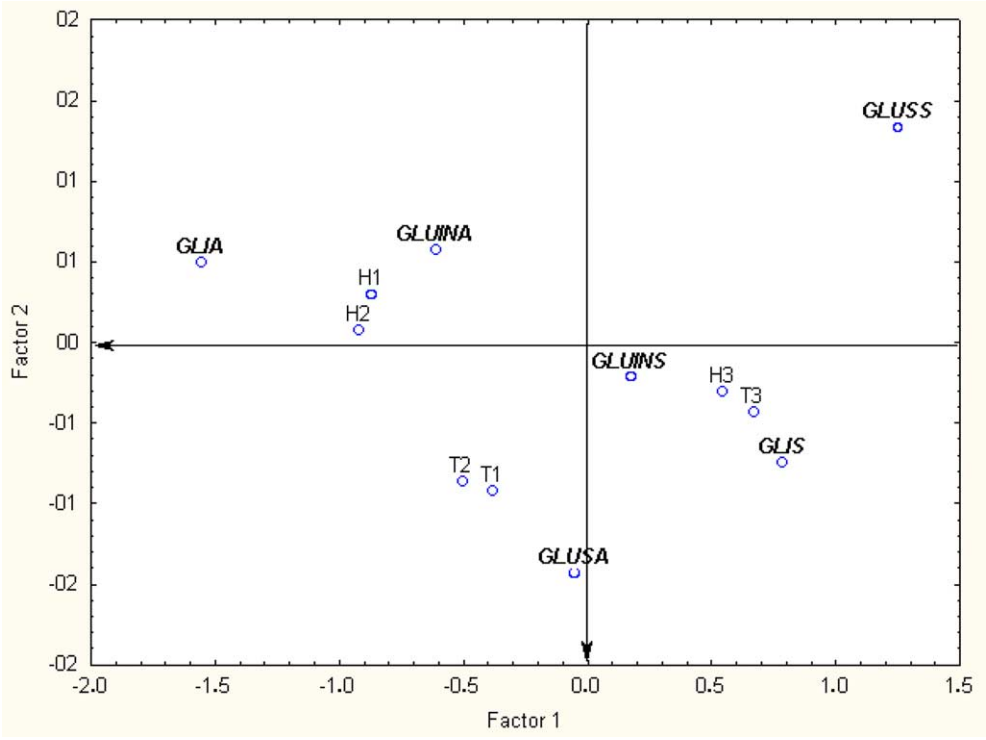

Fig. 3. Projection of thermal attributes and different protein fractions in the plane of the two principal components (Factors 1 and 2). *Legend:* GLIS, Gliadin "Sorraia"; GLIA, Gliadin "Amazonas"; GLUSS, Soluble glutenin "Sorraia"; GLUSA, Soluble glutenin "Amazonas"; GLUINS, Insoluble glutenin "Sorraia"; GLUINA, Insoluble glutenin "Amazonas"; T_n, Transition temperature; H_n, Entalpy; n, number of the endotherm.

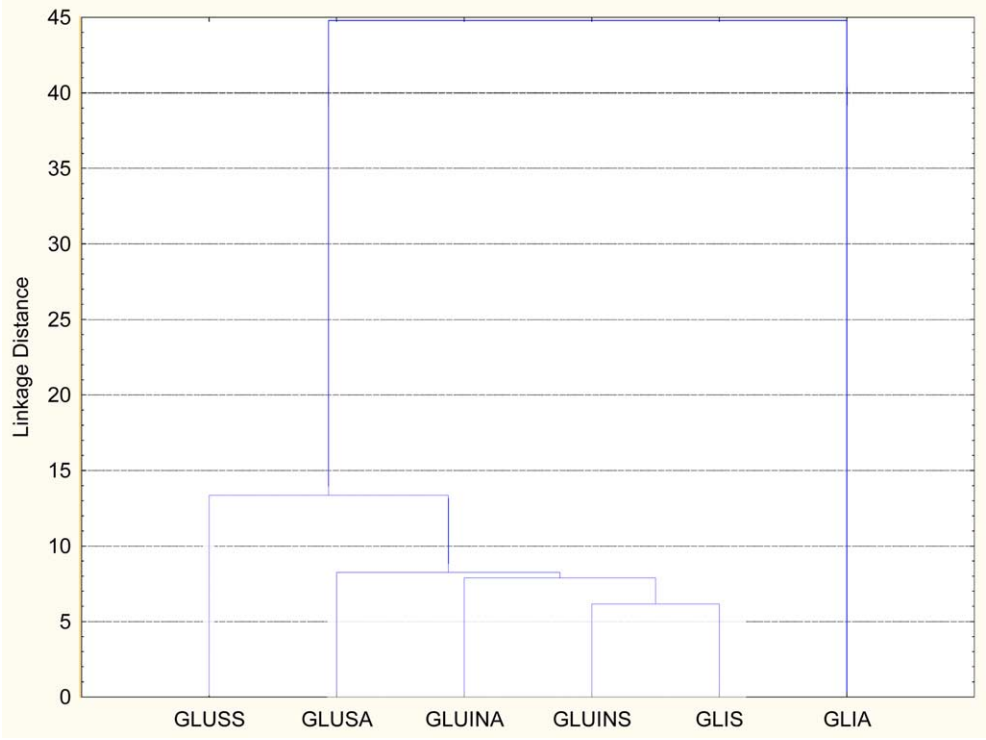

Fig. 4. Dendrogram from a clusters analysis of different protein fractions. *Legend:* GLIS, Gliadin "Sorraia"; GLIA, Gliadin "Amazonas"; GLUSS, Soluble glutenin "Sorraia"; GLUSA, Soluble glutenin "Amazonas"; GLUINS, Insoluble glutenin "Sorraia"; GLUINA, Insoluble glutenin "Amazonas".

The Cluster Analysis (Fig. 4) showed that, at the higher linkage distance, gliadins from "Amazonas" wheat (GLIA) form a subset and the other protein fractions form another one. At a low and quite similar linkage distance are found the "Sorraia" gliadins, "Sorraia" insoluble glutenis, "Amazonas" soluble glutenins and "Amazonas" insoluble glutenins fractions allowing concluding that their thermal behaviour is not significantly different. So, it seems possible to infer that "Amazonas" gliadins and "Sorraia" soluble glutenins are the fractions mainly responsible for the different rheological properties. From those, probably the gliadin fraction plays a significant role by imparting a higher viscosity.

Gluten matrix is produced by kneading gluten proteins with water, being the structure produced due to the breakage and reformulation of sulphur bridges (both intermolecular and intramolecular). So, probably, the higher the energy needed for protein fractions denaturation the more difficult will become the interaction between gliadins and glutenins.

As "Amazonas" wheat is the one that needs more energy to onset and develops the transitions, probably due to a more ordered structure, the interactions among the protein fractions become more difficult and consequently the gluten appear as a weaker one. Major difference among "Sorraia" and "Amazonas" varieties are found in thermal properties of gliadins.

On the basis of these results it seems possible to assume that thermal properties of gluten fractions may contribute to the understanding of the rheological properties and technological ability of different soft wheat varieties.

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