Composition, Protein Contents, and Microstructural Characterisation of Grains and Flours of Emmer Wheats (*Triticum turgidum* ssp*. dicoccum*) of the Central Italy Type

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Abstract

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The microstructural characteristics were evaluated of two types of Italian Farro (*Triticum turgidum* ssp. *dicoccum*) with spring and autumn growth habits, the former with a vitreous tendency and the latter with a floury tendency. Common wheat flours and grains (*Triticum aestivum*) were used as controls. Protein fractions such as glutenin and gliadin were extracted from *Triticum turgidum* ssp. *dicoccum* flours and studied by SDS-PAGE in order to make a comparison between the electrophoretic analyses and microstructural studies which were conducted on the same samples using Scanning Electron Microscopy (SEM and Cryo-SEM). The results obtained by SDS-PAGE showed that the gliadin patterns of both emmer samples were similar, while the common wheat gliadins showed a band at 90 kDa that was not present in the gliadin fraction of emmer. When the glutenin patterns were analysed, the autumn emmer did not show the low molecular weight protein bands (16–23 kDa) whilst spring emmer wheat appeared more similar to common wheat. Regarding the microstructural characteristics of the kernels, spring (vitreous tendency) emmer showed starch granules covered by protein to a higher extent than autumn emmer. These differences were also observed in flours. The gluten of spring emmer wheat was observed as a homogeneous structure showing similarities with common wheat gluten, while autumnal emmer gluten appeared more heterogeneous and lacking in structure.

Keywords: Cryo-SEM; gluten; microstructure; SDS-PAGE; SEM

In Italy, the word farro is generally used to define hulled wheats of the *Triticum* species: einkorn (*Triticum monococcum* L. ssp. *monococcum*,), emmer (*T. turgidum* L. ssp. *diccoccum*, Schrank ex Schübler), and spelt (*T. aestivum* L. ssp. *spelta* (L.) Thell.) (Szabó & Hammer 1996). The sole term farro indicates emmer wheat, one of the oldest cultivated grains, which originated in the Middle East and is widely spread in the Mediterranean basin. Emmer is a tetraploid wheat derived from the intersection of the wild species of *Triticum dicoccoides* and *Triticum durum* (Pagnotta *et al.* 2005). Emmer wheat is a rustic cereal which adapts itself to soils which are poor in nutritive elements and is resistant to extreme weather conditions (cold and hot climates), aridity and humidity, thus it is largely grown in the hills and low mountain areas.

Emmer is actually cultivated in Armenia, Turkey, Albania, the Carpathian mountains on the border of the Czech and Slovak Republics, the south-western regions of Germany, Switzerland, Italy, Spain (Asturias), and Morocco (Perrino *et al.* 1996). In Italy, emmer has been cultivated for centuries in the central-southern regions of Tuscany, Umbria, Abruzzo, Molise, Marche, and Puglia (Porfiri *et al.* 1998).

Emmer is generally sown in autumns in spite of having basically spring habits (Perrino *et al.* 1996).

In Italy, spring sowing is generally performed following the failure of the autumnal or winter ones (Bonciarelli & Bonciarelli 1998) even if some landraces are still sown in spring and reported as spring types (Barcaccia *et al.* 2002).

Based on morpho-phenological data and geographic area of cultivation, the Italian emmer materials were classified into three group types: Garfagnana (winter types, large kernel with floury fracture); Central Italy (spring types, small spike medium-size kernel usually with vitreous fracture); Southern Italy (winter types, large spike with long awns, large kernel with both floury and vitreous fractures) (D'Antuono & Pavoni 1993; Porfiri *et al.* 1998).

Because of the social, cultural or economic reasons, the cultivation of emmer wheat, which was historically abandoned due to the introduction of high yield crops, is back again. Today, in fact, the increasing popularity of organic agriculture and health food products has led to a renewed interest for this crop (D'Antuono & Bravi 1996; De Vita *et al.* 2006).

The increasing attention to sustainable agriculture and the demand for organic foods have rekindled the interest in emmer wheat (Galterio *et al.* 2003) thanks to the ability of this crop to grow in soils with low fertility using low-input techniques (D'Antuono & Minelli 1998). The renewed interest in the cultivation of emmer wheat is also dictated by its nutritional and functional properties such as its contents of resistant starch, fiber and antioxidant compounds (Strehlow *et al.* 1994).

Even though emmer possesses interesting nutritional characteristics, its generally low gluten content and poor gluten quality limit its technological performances and its eventual use in the food industry even though it is used in the pasta production (Galterio *et al.* 2001).

The aim of this study was to test the technological performance of two landraces of emmer intended for bread making. To this purpose, two emmer wheat landraces (Central Italy types) from Abruzzo with different growth habits (spring and autumn) and showing different kernel fracture (vitreous and non vitreous/floury) were analysed and compared to common wheat (*T. aestivum* L. ssp. *aestivum*) which is used for bread making. Kernel properties (vitreousness, hardness, and microstructure), kernel and flour proximate compositions and gluten content were determined and the gluten fraction was further analysed by SDS-PAGE. Finally, in order to understand the gluten forming characteristics of the different emmer wheats, the microstructures of flours and gluten were investigated.

MATERIAL AND METHODS

Materials. Two different emmer wheat (*Triticum turgidum* ssp. *dicoccum*) landraces of the central Italy group grown in the Gran Sasso e della Laga national park, Abruzzo region, were used: emmer with spring growth habits and tendency to vitreous fracture, and that with autumn growth habits and tendency to non vitreous/floury fracture. A *Triticum aestivum* L. spp. *aestivum* sample provided from Molino Alimonti s.p.a. (Ortona, Italy) was used as a control and will be referred to as 'common wheat' from now on in this paper.

Emmer flours were obtained by milling de-hulled grains which were wetted to 16% fresh weight (FW) moisture content. The milling process was carried out in a Bona (Monza, Italy) laboratory mill with two breaking and a regrinding rolls. Common wheat flour was ground in the Molino Alimonti industrial mill.

Analytical determinations. Vitreousness was evaluated on 50 kernels by means of visual inspection by trained personnel after cutting. Hardness was measured on 50 kernels by a compression test carried out using an Instron (Wycombe, UK) mod. 5542 equipped with a 50 N load cell and set to a 60 mm/min speed. Fracture force (N), corresponding to the force at the major failure event, was considered as a measure of hardness. Grains were characterised for thousand kernel weight and moisture, which were determined according to the official EU methods of analysis (EEC 2000), whilst ash, fat, and protein contents were determined according to the official Italian methods of analysis (Repubblica Italiana 1994). Neutral detergent fiber (NDF) was determined and considered as insoluble fiber (Escarnot *et al.* 2010).

Flours were analysed for moisture, ash, and protein contents (Repubblica Italiana 1994).

Dry gluten content was determined using ICC-standard No. 137/1 method (ICC 1994) and expressed in dry weight. Gluten for microstructural analysis was extracted from the dough using the ICC-Standard No. 106/2 method (ICC 1984). Gliadins and glutenins were separated following Osborne method (Osborne 1907) modified by Bean *et al.* (1998), recovered and quantified or further submitted to electrophoresis.

Sodium dodecyl sulphate polyacrylamide gel electrophoresis (SDS-PAGE). Electrophoresis was performed on a Multiphor II Electrophoresis System (Pharmacia Biotech, Piscataway, USA), using 12.5% polyacrylamide precast gels (ExcelGel SDS Homogeneous; GE Healthcare Bio-Sciences AB, Uppsala, Sweden) at 600 V, 38 mA, 23 W, and 15°C for 1 h 30 minutes. 8 µl of each sample were loaded in each

well in duplicate. The standard was an Amersham low molecular weight calibration kit (GE Healthcare, Buckinghamshire, UK) consisting of: phosphorylase b (97 kDa), albumin (66 kDa), ovalbumin (45 kDa), carbonic anhydrase (30 kDa), trypsin inhibitor (20.1 kDa) and α-lactalbumin (14.4 kDa). The protein bands were stained with Coomassie Brilliant Blue tablets (PhastGel Blue R; GE Healthcare Bio-Sciences AB, Uppsala, Sweden). Destaining was performed in an aqueous solution of 25% ethanol and 8% acetic acid. The samples were stored in a solution of 10% glycerol and 7.2% acetic acid. The gels were scanned and the molecular weights of the bands were determined using an Image Scanner III and Image Quant TL software (GE Healthcare Bio-Sciences AB, Uppsala, Sweden).

Scanning electron microscopy (SEM). Kernels and flours were freeze-dried in a Lioalfa 6 freeze-drier (Telestar, Terrassa, Spain) for 24 hours. Then they were gold-coated using a Polaron E6100 Equipment (Watford, UK) at 10^{-4} mbar, 20 mA and 80 seconds. The observation was carried out in a Jeol JSM-6300 scanning electron microscope (Jeol, Tokyo, Japan) at 15 kV and at a working distance of 15 mm.

Cryo-scanning electron microscopy (Cryo-SEM). Gluten was immersed in liquid nitrogen at –210°C and transferred to a cryostage Cryo-Trans CT-1500C (Oxford Instruments, Witney, UK) coupled to a scanning electron microscope JEOL JSM 5410, which works under freezing ($T < –130°C$) and vacuum conditions (1 kPa). The samples were fractured (–180°C) and gold-coated in the Cryo-trans (0.2 kPa and 40 mA). The observation was carried out at 15 kV and at a working distance of 15 mm.

RESULTS AND DISCUSSION

Quality and composition of grains and flours. The spring emmer which was predominantly vitreous showed a higher hardness (144 N) than the autumnal emmer (129 N). The proximate composition of emmers was analysed and compared to that of common wheat suitable for breadmaking (Table 1).

Spring emmer showed a higher protein content than autumnal emmer and common wheat. Other studies reported that Italian emmer landraces generally show very high (17–18%) protein contents (Galterio *et al*. 2001; De Vita *et al*. 2006) which were higher than those observed in this study.

As far as the flours are concerned, spring emmer showed a protein content similar to that of wheat, and autumnal emmer a much lower protein content (Table 1), which indicates that the emmer milling process

Table 1. Proximate composition of farro (emmer) grain and flours (g/100 g DW)

	Spring emmer	Autumnal emmer	Common wheat
Grain			
Moisture	10.3*	12.5*	12.0*
Protein	14.4	11.2	11.8
Fat	2.33	1.52	–
Ashes	1.61	1.10	–
Fibre (NDF)	11.8	11.5	–
Flours			
Moisture	16.3*	15.4*	14.5*
Protein	11.8	10.0	11.5
Ashes	0.85	0.59	0.55
Gluten	10.7	9.04	9.60

*g/100 g FW

carried out in a laboratory mill resulted in a reduction of protein content due to the removal of the outer parts of endosperm together with the bran. The ash content was higher in emmer flours than in wheat (00 type) and in particular in spring emmer flours. Even though spring emmer and common wheat flours showed similar protein contents, the former showed a higher gluten content than the latter. On the contrary, autumnal emmer flour showed a lower protein and gluten contents than common wheat. As far as the gliadin/glutenin ratio is concerned, common wheat showed a value of 0.70 g/g, within the range reported by Eliasson and Larsson (1993), whilst spring and autumnal emmer showed values of 0.40 and 0.36 g/g; respectively, thus confirming the low aptitude of emmer to synthetise gliadins (Galterio *et al*. 2001).

SDS-PAGE of gluten proteins. Figure 1A shows the SDS-PAGE electrophoregrams corresponding to the gliadin fractions of emmer and common wheat. Emmer wheat samples show similar electrophoretic profiles. A protein band appears at 53 kDa in both spring emmer and autumn emmer, in contrast to common wheat. This protein band was previously observed in spelt wheat cultivars by Dvořáček and Čurn (2003) and could be attributable to ω-gliadins which have a molecular weight ranging from 44 kDa to 74 kDa (Kasarda *et al*. 1998). ω-Gliadins were classified by Shewry *et al*. (1986) as compounds poor in sulphur content with scarce tendency to form disulphide bounds. Moreover, common wheat shows a band at 90 kDa that is not shown in either type of emmer. Figure 1B shows the electrophoretic patterns of glutenin fractions. Spring emmer (vitreous) and common wheat showed similar electrophoretic profiles while autumn
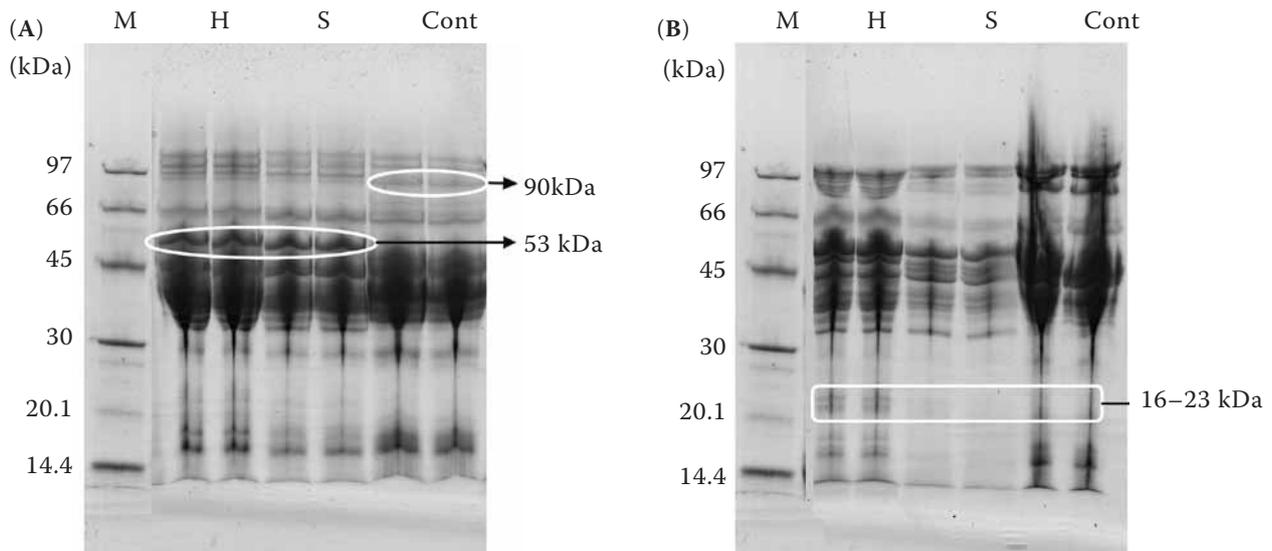
Figure 1. SDS-PAGE: (A) gliadin fraction and (B) glutenin fraction

M – low molecular weight standard; H – vitreous spring emmer wheat; S – non-vitreous autumnal emmer wheat; Cont – common wheat

emmer (non-vitreous) did not show the low molecular weight (LMW) protein bands (16–23 kDa). The LMW fraction is rich in inter- and intra-molecular disulphide bonds which participate in the formation of the gluten polymer. According to De Vita *et al.* (2006), the poor gluten quality of emmer could be related to its storage protein composition characterised by a low content in LMW glutenin subunits.

In general, the gluten protein profile of spring emmer resembles much more that of common wheat than that of autumn emmer. On the other hand, Barcaccia *et al.* (2002) studied Italian Farro of the central Italy group and observed a higher genetic diversity between spring emmer wheat and wheat (either *T. durum* or *T. aestivum*), higher than that observed between autumn emmer wheat and common wheat.

Kernel and flour microstructure. Figure 2 shows SEM micrographs of the common and emmer wheat kernels. The endosperm of vitreous spring emmer (Figure 2A) showed a compact structure of densely packed cells filled with starch granules firmly cemented by a protein matrix. Two typical distinct populations of starch granule sizes were observed. The larger granules were lenticularly shaped and the dimensions of these larger granules manifested a wide dispersion, while the smaller granules had a spherical appearance and were more uniform in size. When the non-vitreous kernel is observed (Figure 2B), the starch granules, similar to those observed in the vitreous kernel, seem to be less embedded in the matrix. The common wheat structure (Figure 2C) is more similar to that of the vitreous emmer than to the floury emmer, as it exhibits an intimate contact between the starch granules and the cementing protein. This typical structure of hard wheat kernel has also been described by other authors (Anjum & Walker 1991; Rojas *et al.* 2000; Jackowiak *et al.* 2005).
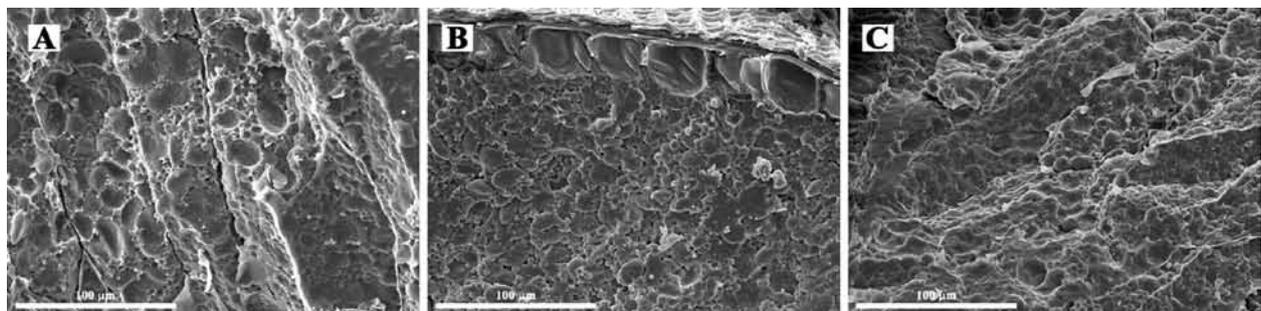

Figure 2. Kernels observed by SEM: (A) vitreous spring emmer wheat, (B) non-vitreous autumnal emmer wheat, and (C) common wheat

As regards the observation of the flours by SEM technique, spring vitreous emmer flour (Figure 3A) and common wheat flour (Figure 3C) showed a coat of protein covering the starch granules in some areas. However, in autumn or non-vitreous emmer flour (Figure 3B), the starch granules are more loose in the matrix than it was observed in the corresponding kernel. Less damaged starch is observed in non-vitreous emmer wheat kernels while vitreous kernels, which are harder than the floury ones, are difficult to crush and grind and produce coarser-textured flour with higher levels of starch damage (Figure 3A*). This fact was also observed by Jolly *et al.* (1993) and Ikeda *et al.* (2005) in wheat kernel quality estimation. In general, vitreous emmer wheat kernels and flour appeared to have more similarities with the common wheat used as control (which also has vitreous tendency) than non-vitreous samples.

Gluten microstructure. Figure 4 shows the structure of gluten observed by Cryo-SEM. The cells matrix formed after the sublimation of superficial frost was observed; this phenomenon is an artifact inherent in the Cryo-SEM technique and is known as eutectic artifact or solute aggregation phenomenon (Llorca *et al.* 2005). The size of the cells is related to the interaction between the system components: the bigger the cell, the weaker the components interaction and the more free water available in the system (Guardeño *et al.* 2010). The structure of gluten extracted from control wheat (Figures 4C and F) revealed a porous matrix, where the proteins mutually interacted forming a continuous and homogeneous network. In the vitreous emmer gluten (Figures 4A and D), the network structure presented bigger cells than the control, but a homogeneous structure with intimate protein-protein interactions, similar to that of the control, was still observed. Besides, in the non-vitreous gluten (Figures 4B and E) two different areas with different protein density could be observed, the cells formed during the etching of the sample being the biggest, indicating a smaller extent of protein-protein interactions; this gluten network was the most heterogeneous if the three samples were compared.

The homogeneity of the gluten network is determined by the bonds which are formed when the protein polymers cross-link. When the cross-links are predominantly formed by disulphide bonds, occurring both within single polypeptide chains and between polypeptides, the gluten shows good technological characteristics (Bache & Donald 1998). Thus, the
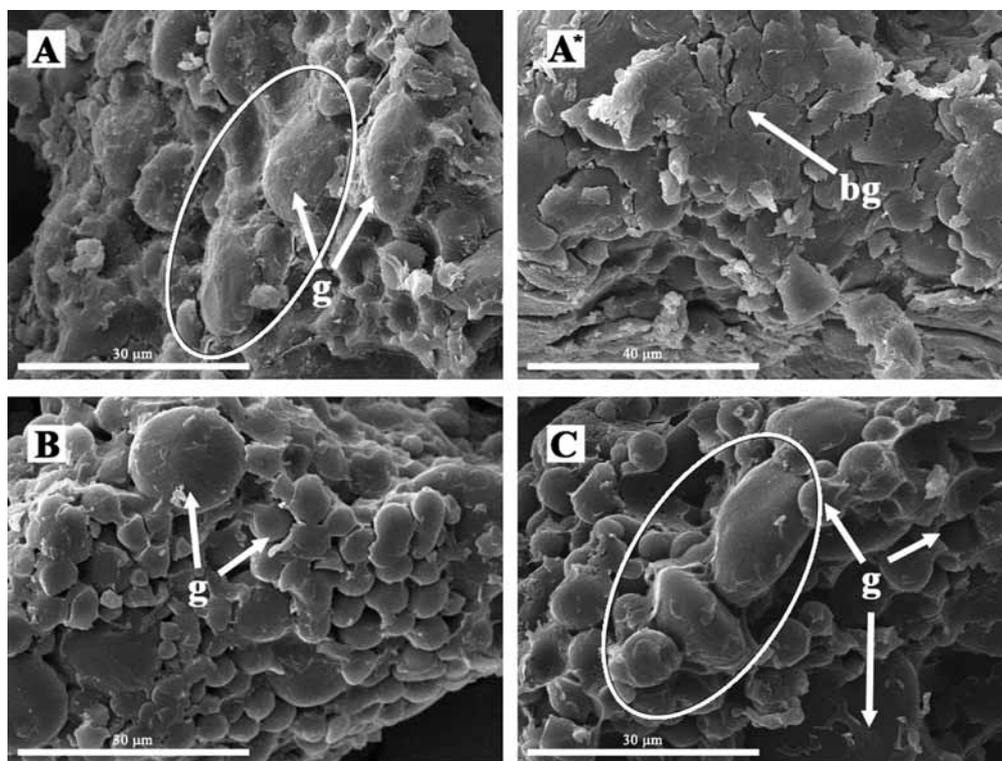

Figure 3. Flours observed by SEM: (A) and (A*) vitreous spring emmer wheat, (B) non-vitreous autumnal emmer wheat, and (C) common wheat
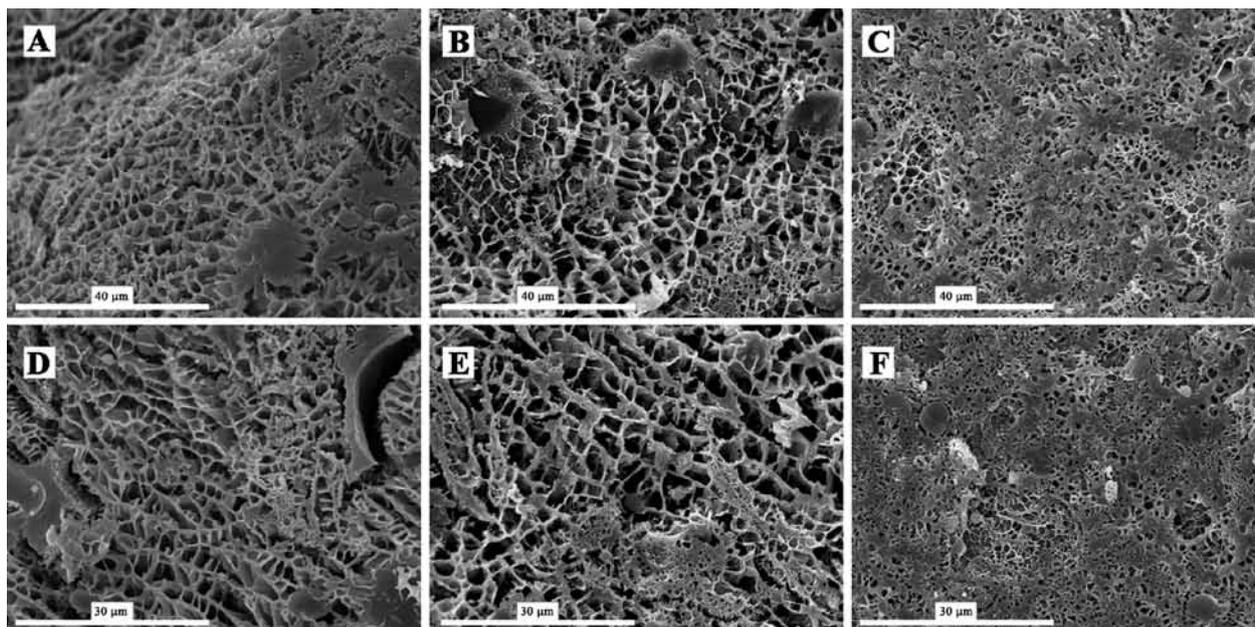
g – starch granule; bg – broken granule

Figure 4. Gluten observed by Cryo-SEM: (A) and (D) vitreous spring emmer wheat, (B) and (E) non-vitreous utumnal emmer wheat, and (C) and (F) common wheat

technological characteristics of the vitreous emmer gluten, which exhibits intimate protein-protein interactions, resemble more those of common wheat than those of non-vitreous emmer wheat.

CONCLUSION

The chemical and microstructural analyses helped to understand the functional and technological characteristics of different landraces of emmer wheat as regards the ability to form the gluten network. In general, spring emmer, which showed a much more vitreous tendency and possessed a higher gluten content, seems to have also a gluten protein profile different from that of autumnal emmer, which appears to be more similar to that of common wheat. Microstructural analyses demonstrated that spring emmer showed a more structured gluten network in comparison to autumnal emmer. Spring emmer landraces in central Italy are worth testing for their bread making properties in the perspective of the development of new bakery products.

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