Identification of a commercial emmer (*Triticum dicoccum* Schübl.) by a proteomic approach

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**Introduction**

Emmer (*Triticum dicoccum* Schübl.) is a cereal crop. Ground emmer is used primarily as an alternative feed grain to oats and barley in some restricted regions. Its nutritional value is close to that of oats. The protein content of emmer is about 11.7%, compared to 12% to 13% for oats (Stallknecht *et al*., 1996). Emmer nutrients have high water solubility, so the body easily absorbs the nutrients. Emmer contains special carbohydrates, which are important factors in blood clotting and stimulating the body’s immune system. It is also a superb fibre resource and has a large amount of B-complex vitamins.

Emmer hull has nearly as much feeding value as kernel. Emmer can be used as a food grain after the removal of the hull (Stallknecht *et al*., 1996).

Globalisation, the opening of international markets, consumer demands and clear differences between quality/price in different markets, permit the intervention of opportunistic elements and the falsification of the documentation that must accompany foodstuffs, usually in order to increase profit. The consumer is entitled to information about the species and the origin of the foodstuffs. Traceability is the ability to trace a product batch and its history through the whole, or part, of a production chain from harvest through transport, storage, processing, distribution and

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sales or internally in one of the steps in the chain, for example the production step (Moe, 1998). Traceability is a generic issue, as its fundamentals are independent from the type of product, production and control system it serves (Kim et al., 1995). We adopt the basic definition of the term traceability found in the quality management and quality assurance standard from ISO [European Standard (195)]: traceability is the ability to trace the history, application or location of an entity, by means of recorded identifications.

The identification of molecular markers being able to discriminate among species and among species varieties could be an important tool for the traceability. Some molecular markers have been used to investigate the genetic diversity and structure of the emmer gene pool (Peng et al., 2000).

We applied a proteomic approach to underline the differences between three commercial emmer varieties. Proteomics is the systematic analysis and documentation of all the protein species of an organism or a specific type of tissue (Blackstock, Weir, 1999; Cahill et al., 2000; Anderson et al., 2001). With the accumulation of vast amounts of DNA sequences in database, researches are realizing that merely having complete sequences of genomes is not sufficient to elucidate biological function. A cell is normally dependent upon a multitude of metabolic and regulatory pathways for its survival. There is no strict linear relationship between genes and the protein complement or ‘proteome’ of a cell. Proteomics is complementary to genomics because it focuses on the gene products, which are the active agents in cells (Pandey, Mann, 2000).

In our experiments we used three commercial varieties of emmer: an organic one (produced by “I cereali Ki”, Torino, Italy), an Austrian one (produced by Perlinger, Austria), and the Garfagnana (an area of high hills and mountains in the Lucca Province, Tuscany, Italy) emmer (produced by Pierantoni, Lucca, Italy), which obtained European recognition for Protected Geographical Indication (PGI) in 1996. In particular we focused our attention on the differences in the protein pattern and especially on the specific spots of each gel to use them as markers to estimate the origin of the product. Moreover, we have analysed the amylotic pool of the caryopses and of the endosperms of the three-day germinated caryopses. Our aim was to test a simple method that could be useful to check differences, using as molecular markers as possible, between the tested varieties.

**Materials and methods**

For the 2D-electrophoresis, the caryopsis were grounded in a mortar with a pestle and the powder was transferred in 1.5 mL eppendhorf. The emmer proteins were solubilized in 8M urea, 2% (w/v) CHAPS (3-[3-Cholamidopropyl] dimethylammonio]-1 propanesulfonate), 2% IPG buffer 4-7 (Amersham Biosciences, Sweden), 20 mM dithiothreitol and trace of bromophenol blue. The precipitation of the proteins was performed with 2-D Clean-Up Kit (Amersham Biosciences, Sweden). Protein quantification was carried out according to Bradford (Bradford, 1976) using bovine serum albumin as a standard (Bio-Rad). The IPG gel strips (pH 4-7, 11 cm, Amersham Biosciences, Sweden) were rehydrated with the extraction solution containing 30 μg of sample. The IPG gel strips were allowed to rehydrate overnight at 20°C. The isoelectrofocusing (IEF, first dimension run) was performed using Pharmacia Multiphor II (Amersham Biosciences, Sweden). The running conditions for the IEF were: 1 min at 300 V, 2 mA and 5 W; 1 h and 30 min at 3500 V, 2 mA, 5 W; 3 h and 30 min at 3500 V, 2 mA, 5 W. After IEF, the IPG gel strips which are not used immediately for the second dimension run (SDS-PAGE) are stored between two sheets of plastic film at -80°C. Before SDS-PAGE, the IEF gel strips were equilibrated first for 20 min in 50 mM Tris-HCl, pH 6.8, 6 M urea; 30% (v/v) glycerol, 2% (w/v) SDS (sodium dodecyl sulphate) and 1% (w/v) dithiothreitol, followed by 20 min in the same buffer without dithiotreitol but with 2.5% (w/v) iodoacetamide and a trace of bromophenol blue. The SDS-PAGE was performed using pre-cast gel 8%-18%, 11 x 24 cm (Amersham Biosciences, Sweden) and run on a Pharmacia Multiphor II according to manufacture’s recommendations. Gels were stained with silver nitrate in a gel stainer according to Heukeshoven and Dernick (1985). The two-dimensional gels were scanned using an image scanner and evaluated with the PDQuest 7.1.1 software (Bio-Rad, USA).

We have also compared amylotic isoforms of the three-emmer varieties, using zymogram
of amylase activity. We performed the activity staining on endosperms from germinated and dry caryopsis. The caryopses are placed in Petri dishes and imbibed with distilled water at 23°C in the dark. The endosperms from germinated caryopses are harvested after three days. Samples were extracted in 400 μl of 100 mM Hepes-KOH, pH 7.5; 1mM EDTA (ethylenediaminetetraacetic acid disodium salt); 5 mM MgCl₂; 5 mM dithiothreitol and 10 mM NaHSO₃. Protein quantification was carried out according to Bradford.

IEF (pH range 3.5-9.5, Amersham Biosciences, Sweden) and activity staining of equal amounts of proteins (4 μg) were performed as previously described (Perata et al., 1992, 1993).

**Results and conclusions**

Both Garfagnana and organic emmer have shown a good germinability; instead the Austrian

![Fig. 1 - Zymogram of amylase activity. The specific amylase isoforms of each emmer are reported in the white rectangular. A: Austrian dry caryopses; Ag: Austrian germinated caryopses; G: Garfagnana dry caryopses; Gg: Garfagnana germinated caryopses; O: organic dry caryopses; Og: organic germinated caryopses.](image)

Tab. 1 - Number and percentage values of specific spots of each kind of emmer after 2DE analysis.

<table>
<thead>
<tr>
<th>Emmer Name</th>
<th>Total Spot</th>
<th>Specific Spot</th>
<th>% Specific Spot</th>
</tr>
</thead>
<tbody>
<tr>
<td>Garfagnana</td>
<td>455</td>
<td>60</td>
<td>13.2%</td>
</tr>
<tr>
<td>Organic</td>
<td>432</td>
<td>56</td>
<td>12.9%</td>
</tr>
<tr>
<td>Austrian</td>
<td>422</td>
<td>53</td>
<td>12.5%</td>
</tr>
</tbody>
</table>

Fig. 1 - Zimogramma dell’attività amilasica. Le specifiche isoforme amilasiche di ciascun farro sono evidenziate dai rettangoli bianchi. A: cariossidi austriache; Ag: cariossidi austriache germinate; G cariossidi della Garfagnana; Gg cariossidi della Garfagnana germinate; O: cariossidi biologiche; Og: cariossidi biologiche germinate.

one has manifested a reduced germination capability. Probably the reduced germination rate of the Austrian emmer is due to the harvesting time (milky maturation stage). This could explain the lower presence of amylolitic isoforms in samples prepared from Austrian than in the other two kinds of emmer (Fig. 1). In the activity staining analysis, we have verified that there are some differences in the amylolitic isoform patterns between the three types of emmer, but there aren’t any differences between the amylolitic pattern of the dry and of the three-day germinated caryopses of the same customer. In the Austrian emmer we have pointed out the presence at least of different amylolitic isoforms, which have a pI between 6.0 and 6.7. In the organic emmer we have found three isoforms, which are not present in the other two kinds of emmer that we have analysed. Two of these amylolitic isoforms have a pI around at 6.1-6.5, and the other has a pI more acidic of nearly 4.5. In the Garfagnana emmer we have highlighted two amylolitic isoforms, whose one has a pI at nearly 4.7 and the other at 5.6. Using our results, we could postulate that this technique may be used to make a rapid preliminary screening, followed by more accurate analysis.

Using the 2D electrophoresis, we have analysed the total protein pattern of the three kinds of emmer in the pH range between 4 to 7. Analysing the protein pattern of each gel we obtained that in the Austrian sample there are 455 total spots, in the organic one there are 432 total spots, and in the Garfagnana emmer there are 422 total spots (table I). Our results have showed that each variety of emmer has some specific spots. In
Fig. 2 - Protein pattern of each kind of emmer. The specific spots of each gel are numbered. All the analysis was performed with PDQuest 7.1.1 BioRad, USA. A: Austrian emmer; G: Garfagnana emmer; O: organic emmer.

Fig. 2 - Profilo proteico per ciascun tipo di farro. Gli spots specifici sono numerati su ogni gel. Tutte le analisi sono state condotte con PDQuest 7.1.1 BioRad, USA. A: farro austriaco; G: farro della Garfagnana; O: farro biologico.
particular, we have found 60 specific spots in the Austrian emmer, 56 specific spots in the organic emmer, and 53 specific spots in the Garfagnana variety. These results are reported in the table 1. The specific spots of each emmer variety are localized in different regions of the gels (Fig. 2). We have calculated molecular weight and isoelectric point of each spot. Then we have reported these data in a graph (Fig. 3). In this graph the different distribution of the specific spots of each kind of emmer is evident. The results of the analysis of the protein pattern allow to obtain potential molecular markers, which can be used to identify the origin of the emmer analysed in an unambiguous way.

Acknowledgments

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References


EUROPEAN STANDARD (195) [EN ISO 402:1995, Point
3.16], European Committee for Standardization (CEN).


