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Review

DNA-based methods for identification and quantification of small grain cereal mixtures and fingerprinting of varieties

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Abstract

The composition of cereal-based foods is a key factor in determining the quality and safety of the final product while the reliable identification of cereal species and cultivars are essential for the handling, marketing and processing of grain and for the protection of plant breeders' rights. Analytical methods have therefore been developed and applied to identify and quantify cereal species in food products and also to fingerprint and identify grain at the genotype and variety levels. DNA-based methods for the detection and quantification of mixtures of small grain cereals are reviewed, together with the recent development of molecular markers for varietal fingerprinting.

Keywords: Variety; Detection methods; Fingerprinting; Real time PCR; Molecular markers

1. Introduction

Five major cereal species (bread and durum wheats, maize, rice and barley) satisfy two thirds of the human food requirements, either directly or indirectly, with total annual yields exceeding 2000 million tonnes (FAO, 1999). Cereal crops are therefore essential for human welfare. The end use quality of cereals for food and feed is determined by their composition. In general, cereal grains contain high levels of carbohydrates (65–75%), mainly represented by starch, low lipid contents (2–9%) and protein contents, ranging from about 7–15%. Nevertheless, they currently provide over 200 million tonnes of protein for human and livestock nutrition. Furthermore, the functional properties of the grain proteins are key determinants of the final utilisation of the grain in food processing. In addition, other classes of grain component, such as arabinoxylans (pentosans), (1→3,1→4)-β-D-glucans, antioxidants and vitamins, have potential importance for the production of functional foods and have been shown to vary in amount and composition between species, genotypes and, to a lesser extent, with growth environment. Consequently, the species composition of a cereal-based food is a key factor in determining the quality and safety of the final product and the reliable identification of species and cultivars is essential for the handling, marketing and processing of grain (Ko et al., 1994).

Two strategies have been developed in response to the need to maintain and strengthen consumers' trust in the quality and origin of foods introduced into the market. One is to control all the production steps, with the application of Hazard Analysis Critical Control Point (HACCP) systems from field to table. The second is to develop analytical tools to monitor the composition of raw materials and processed food and feed. With this aim, DNA-based analytical methods have been developed and applied to identify and quantify cereal species and to fingerprint and identify genotypes and varieties to determine authenticity (Popping, 2002).

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2. DNA-based detection and quantification of mixtures of small grain cereals

DNA profiling is widely applied to identify small grain cereals in raw materials and in processed food and feed. In fact, despite a high level of gene conservation between grasses (Devos and Gale, 2000), cultivated cereal species show different end-use characteristics which are mainly related to their storage protein fractions. For example, the high content of globulin storage proteins in oat grain contributes to its high nutritional value and explains the widespread use of oats for livestock feed (Cuddeford, 1995). Similarly, the malting quality of barley is related to differences in grain composition and structure that affect the modification of the grain and digestion of starch during malting and brewing, including storage protein composition (Shewry and Darlington, 2002). In wheats, the prolamin storage proteins are the major components of the gluten protein fraction that forms a viscoelastic network in doughs and is largely responsible for the ability to process wheat to form bread, pasta and many other food products (Shewry and Halford, 2002). As a consequence, pasta made from durum wheat is considered superior in several qualitative aspects to that manufactured from bread wheat or from a mixture of the two species. Several regulations, including Italian law, currently prohibit the manufacture for sale of pasta containing more than 3% bread wheat, and require the correct labelling of imported pasta products to show the proportions of each wheat species in the final product. The incentive for the adulteration of pasta with bread wheat comes from the lower price at which bread wheat is usually traded compared with durum wheat.

Several analytical procedures are currently available to detect the presence of wheat in food and to test pasta for authenticity, as summarized by Autran et al. (1994). Most of these methods are based on the detection of specific proteins, separated by electrophoresis or high-performance liquid chromatography or identified immunochemically (Durotest, Rhône Diagnostic Technologies, France). The main limitation to the use of these analytical methods is the occurrence of protein denaturation during the technological processes involved in manufacturing food and pasta. In particular, for pasta, the use of faster drying processes characterised by high temperatures can lead to extensive protein denaturation. In contrast, the thermostability of DNA molecules, together with the use of PCR techniques, can provide molecular markers to identify raw materials and trace them through the manufacturing steps. Analytical tools have been developed to detect the presence of wheat species in flour and pasta based on end-point and real-time PCR. A common feature of this PCR approach is the use of sequences related to the D genome of bread wheat. Bread and durum wheats are in fact polyploid species containing three (AABBDD) and two (AABB) related genomes, respectively. Bryan et al. (1998) exploited this difference to design a set of PCR primers based on the 2.2 kb Dgas44 sequence (McNeil et al., 1994) and determined their specificity for a range of samples including wheat species, bread and durum wheat cultivars and chromosome substitution lines. Four primers (Table 1) showed very strong PCR amplification and a very high degree of genome specificity when used in any of the possible forward–reverse combinations. An alternative end point PCR method for detection of bread wheat recently been developed by Arlorio et al. (2003) using the sequence of the the puroindoline b gene (which is present on chromosome 5D) as a target for primer design (Table 1). Universal ribosomal primers based on the Internal Transcribed Spacers region (ITS) were also used to monitor the quality of genomic DNA extracted from food. The limit of sensitivity of the detection method was given as 0.2% bread wheat contamination. Alary et al. (2002) used the same puroindoline b sequence to develop a real-time PCR system (Table 1) for the detection and quantification of bread wheat adulteration of durum wheat pasta. They analysed a range of pasta types (dried at low, high and very high temperatures) containing 3% bread wheat and determined a mean value of $3\% \pm 0.4$ at a 95% confidence limit. The work of Terzi et al. (2003) was directed toward the development of analytical systems for the qualitative and quantitative detection of specific cereals in food. More specifically, the primary aim of the work was to develop analytical tools based on end-point and real-time PCR to detect the presence of *Triticum* species in flour and food. Furthermore, qualitative and quantitative PCR-based methods were evaluated to detect hexaploid wheat adulteration in pasta. Seed storage protein sequences were used to design primer pairs and probes and the genus and species-specificity of the two systems were tested on a panel of grass genotypes, on hexaploid and tetraploid wheats and on standard spaghetti made with 100, 98, 96, 93 and 85% of durum wheat. GLUD primers/probe (based on consensus sequences of low molecular weight glutenin storage proteins) detected *Triticum* and *Triticosecale* (triticale) species, whereas GLIA primers/probe (based on gliadin sequences) detected only *Triticum aestivum* (Table 1).

This type of analysis can be used not only to determine food authenticity but also to determine food safety for those who need to avoid the consumption of wheat and related cereal products. In particular, several cereal species (wheat, barley, rye and triticale) contain proteins that are toxic to individuals affected by coeliac disease (Kasarda, 2000; Shan et al., 2002). Consequently, analytical methods are required to check that products are free from gluten and related proteins from other species. Various immunochemical methods are available for prolamin identification and their specificity and sensitivity have been evaluated, including the effects of heat treatments of products (Denery-Papini et al., 1999; Ellis et al., 1998; Sorell et al., 1998). These methods are widely used and have the advantages that they often target the actual proteins that are toxic to individuals affected by coeliac disease