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A high protein ancient wheat species: Einkorn

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ABSTRACT

The current interest for ancient hulled wheats is motivated by the increased demand for healthier foods and for more sustainable agricultural production systems. The diploid einkorn *Triticum monococcum* is an ancient, hulled wheat cultivated for thousand years and progressively replaced by free-threshing and higher yielding wheat species. Nowadays, its traditional cultivation is limited to marginal areas, but recently it has been re-introduced in different regions thank to its adaptation to poor soils and low-input agriculture, tolerance/resistance to pests and diseases, good technological and organoleptic properties and peculiar nutritional value. The better nutritional quality of einkorn respect to bread and durum wheat lies mainly in the superior content of proteins (up to 19–21% also under organic management), microelements and antioxidant compounds. The *T. monococcum* gluten content is comparable to that of modern tetraploid and hexaploid wheats, but its gliadin and glutenin allele composition displays an excess of gliadins over glutenins, leading to flours with very low gluten index and gluten strength. Nevertheless, einkorn flour is suitable for the manufacturing of cookies, unleavened foods and dry pasta. Finally, several studies are consistent with differences in einkorn gluten digestibility, resulting in a lower content of immunostimulatory peptides involved in gluten-related disorders.

1. Introduction

The limited cultivation of einkorn is related to its 30–60% lower grain yield, the presence of husks and the poor baking properties of its flour compared to common and durum wheat. Nevertheless, hulled wheat species such as einkorn, emmer and spelt have been rediscovered in the last 30 years, and a growing number of consumers associate their consumption with pleasant sensorial properties and significant health benefits, due to their higher content of bioactive compounds and protein.

2. Einkorn evolution and breeding

Einkorn (*Triticum monococcum* L. subsp. *monococcum*) is a hulled diploid wheat (2n = 2x = 14, A^mA^m genome) closely related to the widely cultivated tetraploid wheat *T. turgidum* (durum, emmer, poulard, etc.) and hexaploid wheat *T. aestivum* (bread wheat, spelt, etc.). A feral or residual subspecies, *T. monococcum* subsp. *aegilopoides* (Link) Schiem., survives in disturbed habitats of the Balkan and Western Turkey, while the ancestral wild *T. monococcum* subsp. *thaoudar* (Reut. ex Hausskn.) Grossh. (synonym *T. boeoticum* subsp. *boeoticum* Boiss.) still grows in partially undisturbed habitats of South-East Turkey, South-West Iran, North Iraq, West Syria and East Lebanon, under a broad range of altitudes (100–1600 m a.s.l.) and soils, in pure stands or in mixtures with other wild cereals and pulses (Brandolini et al., 2016).

Domestication is the process of selection that converts a wild crop into a domesticated one, fit for cultivation. For einkorn, this transition was driven by changes to very few traits. First and of foremost importance was the selection of genotypes with tough rachis and limited seed shattering; additional traits of interest were a reduction in seed dormancy, a greater seed size and number, and free-threshing seeds. Migration, crossings and recombination events contributed to modify the genetic composition of the crop; however, the strong selection pressure applied by the domestication process led to a reduction of genetic diversity in the domesticated forms.

Einkorn domestication has been the subject of intensive research during the last 30 years. Two main schools of thought developed. The first, mainly based on archaeological data, considers einkorn domestication an unconscious, slow and protracted process with three steps (gathering, cultivation and domestication), each lasting thousand years,

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that happened autonomously in several locations (Purugganan and Fuller, 2011). A modification of this hypothesis suggested by Kilian et al. (2007) was that a specific wild einkorn race, which arose without human intervention, underwent multiple independent domestication events. The second hypothesis, relying on ecological and molecular information, assumes that einkorn domestication was largely conscious, rapid, geographically localized in the Karacadağ region and monophyletic (Heun et al., 1997; Brandolini et al., 2016).

The spread of einkorn into Europe probably followed two different Neolithic migration routes, one continental and the other maritime (Brandolini et al., 2016). For several thousand years this crop, together with emmer and barley, was the staple food of European farmers, as confirmed by archaeological remains and by the analysis of the colon content of Ötzi (a.k.a. the Similaun Iceman), a frozen Copper Age (8000–5000 years Before Present) man found in 1991 in the Alps. From the Bronze Age (5000–3000 years BP), einkorn cultivation lost momentum because of the availability of higher yielding, free-threshing tetraploid and hexaploid wheats. However, it was still consumed in Roman times, as well as in the ensuing “Dark Ages”. Today, traditional einkorn crops may be found in restricted areas of Turkey, the Balkan countries, Italy, France, Spain and Morocco (Volante et al., 2020).

After the Copper-Bronze age, einkorn was of little agricultural use and, consequently, was never object of intensive breeding (Salamini et al., 2002): hence, the populations still present in restricted mountain areas of Europe, Morocco and the Near East evolved independently and adapted to specific growing conditions and utilisations. In recent years, the trends towards low-input and sustainable agriculture, along with a new interest on the nutritional aspects of food, have led to the revaluation of einkorn and its nutritional and technological quality has been assessed. At the same time, breeding programmes for the creation of new einkorn lines with high productivity, free-threshing and suitable for modern cultivation practices are underway in several Countries (e.g. Canada, Germany, Italy, France, Bulgaria, Switzerland, Austria, Denmark, Sweden and Czech Republic).

A major problem for einkorn utilisation is the requirement of an additional de-hulling process to separate the seed from the husks. However, some accessions exist with partially free-threshing spikes, such as *T. monococcum* subsp. *monococcum* var. *sinskajae*, a natural mutant for the soft glumes trait. This characteristic is intricately linked to a semi-compact spike and a short plant height, but also to partial sterility of the spikelets (Salamini et al., 2002), poor germinability and increased susceptibility to soil-borne diseases.

Among the first einkorn breeding efforts, should be mentioned those by Cultivari GmbH of Darzau, Germany, where since the 1990s the constitution of varieties with winter hardiness and resistant to bunt diseases (*Tilletia* spp.), free-threshing and with good baking characteristics has been pursued. To date, they have released three cultivars, Tifi, Terzino and Svenskaja, obtained by intra-varietal selection and recommended for organic farming. In Italy five cultivars, released by Council for Agricultural Research and Economics (CREA), have been listed in the National Voluntary Variety Register: Monlis (excellent bread-making attitude and good productivity), Antenato (early maturing, particularly suitable for spring sowing), Monili (semi-early maturing, excellent productivity), Norberto (good bread-making attitude) and Hammurabi (free-threshing and early-maturing). Similarly, in Hungary the Martonvasar Institute has created three cultivars, Mv Hegyes, Mv Alkor and Mv Menket, in Czech Republic the variety Rumona was selected by the Crop Research Institute in Prague, while in Canada the variety AC Knowles was obtained from the Eastern Cereals and Oilseeds Research Centre, Ottawa. Other breeding programmes are ongoing, and new varieties are becoming available elsewhere.

The domesticated einkorn is a low-yielding crop, producing ca 2.0–2.5 t/ha of naked seed, i.e. about one-third of bread wheat and half of durum wheat (Volante et al., 2020). This gap is due to several factors, including different morphological characteristics of the spikelets (one seed per floret *vs* 3–4 in bread wheat) and smaller kernels (1000 kernels weight range: 16–36 g *vs* 46–72 g for tetraploid wheats and 29–42 g for hexaploidy wheats; Volante et al., 2020). On the other hand, einkorn is well-known for the high protein content of its kernels, which can range from 14.4 to 23.1 g/100 g dry matter (DM) (Volante et al., 2020). Although the small kernel size, and hence the higher proportion of germ and of the protein-rich aleurone layer, favour protein concentration, a broad variation exists within the gene pool (Volante et al., 2020), stressing the relevance of the genetic component. Nitrogen fertilisation, particularly when applied at the heading stage, increases the protein content of the seeds at harvest; however, to avoid the risk of lodging, the doses applied should be relatively limited (around 80 kg/ha nitrogen). With the addition of 60 kg/ha nitrogen, the cultivars Hammurabi and Norberto grown in Central Italy showed protein contents as high as 20.4% and 20.2% DM, respectively, while *T. durum* and *T. aestivum* reached 13.9% and 13.1% DM, respectively (Gazza, unpublished results).

The thrifty nature and the good resistance of einkorn to pests and diseases suggest its suitability for organic agriculture. In fact, as mentioned above, the three einkorn cultivars released in Germany, Tifi, Terzino and Svenskaja, are recommended for organic farming, and einkorn production according to this practice is diffused in Central and Northern Europe. A yield penalty in comparison to the modern standard cropping systems is often observed, but the flour obtained from properly managed organic einkorn can maintain high protein concentrations (Bencze et al., 2020).

3. Proteins biological value

As mentioned above, einkorn wheat has a high protein content (Table 1). The concentration is greatest in the germ (on average 47.0 g/100 g), with values 2–3 times higher than in the endosperm (18.1 g/100 g) and bran (22.2 g/100 g) (Hidalgo and Brandolini, 2008). Despite the superior protein content, the average amino acid composition is like that of the polyploid wheats (Abdel-Aal and Hucl, 2002; Acquistucci et al., 1995), with low concentrations of lysine and threonine, and high contents of cystine, glutamic acid and proline (Abdel-Aal and Hucl, 2002).

Table 1
Protein content (g/kg) and amino-acid composition (g/kg protein) of einkorn wholemeal from different references (1–6).

Reference	1	2	3	4	5	6
Number of cultivars	1	15	1	1	5	2
Protein content	17.9	16.7	14.1	18.1	17.8	20.9
Asp	47.0	51.6	47.1	45.4	58.2	60.9
Thr[a]	26.0	25.9	24.7	25.2	31.6	31.2
Ser	42.0	40.3	41.5	37.4	55.4	48.4
Glu	370.0	316.8	277.9	304.0	280.7	353.6
Gly	31.0	32.9	31.6	34.4	41.5	27.3
Ala	28.0	32.5	29.0	39.2	35.7	29.6
Val[a]	37.0	41.3	40.7	36.0	42.3	36.9
Cys	22.0	28.6	14.2	24.3	23.3	0.0
Met[a]	14.0	17.5	8.3	16.1	16.8	8.3
Ile[a]	29.0	37.9	37.5	44.1	39.7	39.0
Leu[a]	66.0	64.2	61.3	65.0	72.6	62.4
Tyr	25.0	31.3	11.3	35.1	30.3	26.0
Phe[a]	50.0	49.5	45.0	54.4	52.8	51.6
Lys[a]	19.0	26.0	23.8	29.4	32.9	20.2
His[a]	21.0	23.4	20.2	30.7	29.9	–
Arg	39.0	45.7	43.6	54.1	39.7	40.0
Pro	87.0	103.2	86.0	89.0	101.8	93.7
Trp[a]	13.0				12.2	
Total[a]	275.0	285.7	261.5	300.9	330.8	249.6
Total	966.0	968.3	843.7	963.8	997.4	929.0

[a] Essential amino acids; 1, Abdel-Aal and Hucl (2002); 2, Acquistucci et al. (1995); 3, Estivi et al. (2022); 4, Gabrovská et al. (2002); 5, Galterio et al. (1998); 6, Matuz et al. (2000).

According to Acquistucci et al. (1995), the variation in composition among accessions is quite low, except for tyrosine, arginine and proline.

The nutritional value of food is strongly linked to protein quality, which depends on amino acids content, essential amino acids rate, susceptibility to hydrolysis in the digestion, absorption and utilisation of amino acids, and immune system reaction to proteins as antigens (Matuz et al., 2000). To assess the biological value of einkorn protein, the digestible indispensable amino acid scores (DIAAS) were commuted using the true ileal digestibility of individual dietary indispensable amino acid (IAA) against three amino acid scoring patterns, infant (birth to 6 months), child (6 months–3 years), and older child/-adolescent/adult (from 3 years), as recommended and outlined by Food and Agriculture Organization (FAO, 2013). To estimate the ileal digestible IAA content, the true ileal digestibility coefficients (%) of wheat flour (Rutherfurd et al., 2012) were used. Table 2 presents the DIAAS values of the einkorn protein, computed from the amino acid composition reported by several Authors (Table 1). These DIAAS values are approximations, because the true ileal digestibility coefficients for indispensable amino acids were obtained from growing rats (Rutherfurd et al., 2012), but should closely represent the true ileal digestibility in humans. From a dietary point of view, lysine is the most deficient essential amino acid in einkorn (Table 2), as well as in other wheat and cereal species. In fact, employing the infant reference pattern the scores range from 26% to 45% (lysine), with the child pattern vary from 31% to 54% while with the older child/adolescent/adult pattern span from 37% to 64%.

Table 2
Digestible indispensable amino acid (IAA) reference ratio and digestible indispensable amino acid scores (DIAAS) of einkorn proteins considering the amino acid composition from different references (1–6).

Reference	1	2	3	4	5	6
Reference pattern: infant (birth to 6 months)						
Thr	1.41	1.40	1.34	1.36	1.71	1.69
Val	0.65	0.72	0.71	0.63	0.74	0.64
SAA	1.09	1.40	0.68	1.22	1.22	0.25
Ile	0.52	0.68	0.67	0.78	0.71	0.70
Leu	0.68	0.66	0.63	0.67	0.75	0.64
AAA	0.78	0.84	0.59	0.93	0.87	0.81
Lys	0.26	0.35	0.32	0.40	0.45	0.28
His	0.99	1.10	0.95	1.45	1.41	0.00
Trp	0.70	–	–	–	0.65	–
$DIAAS_{infant}$ (Lys)	25.9	35.4	32.4	40.1	44.8	27.5
Reference pattern: child (6 months–3 years)						
Thr	0.77	0.77	0.73	0.75	0.94	0.93
Val	0.83	0.92	0.91	0.80	0.94	0.82
SAA	1.33	1.71	0.83	1.49	1.49	0.31
Ile	0.89	1.16	1.15	1.35	1.22	1.20
Leu	0.99	0.96	0.92	0.98	1.09	0.94
AAA	1.41	1.52	1.06	1.69	1.57	1.46
Lys	0.31	0.43	0.39	0.49	0.54	0.33
His	1.04	1.16	1.00	1.52	1.48	0.00
Trp	1.39	–	–	–	1.31	–
$DIAAS_{child}$ (Lys)	31.3	42.9	39.2	48.6	54.3	33.3
Reference pattern: older child/adolescent/adult (from 3 years)						
Thr	0.96	0.95	0.91	0.93	1.16	1.15
Val	0.89	0.99	0.98	0.86	1.02	0.89
SAA	1.57	2.00	0.98	1.75	1.74	0.36
Ile	0.95	1.24	1.23	1.44	1.30	1.28
Leu	1.07	1.04	0.99	1.05	1.18	1.01
AAA	1.79	1.93	1.35	2.14	1.99	1.86
Lys	0.37	0.51	0.47	0.58	0.64	0.40
His	1.30	1.45	1.25	1.90	1.85	0.00
Trp	1.79	–	–	–	1.68	–
$DIAAS_{older\ child}$ (Lys)	37.2	50.9	46.6	57.7	64.4	39.5

1, Abdel-Aal and Hucl (2002); 2, Acquistucci et al. (1995); 3, Estivi et al. (2022); 4, Gabrovská et al. (2002); 5, Galterio et al. (1998); 6, Matuz et al. (2000).

4. Enzymes

The enzymes exert a key role on seed development and conservation, milling, flour storage and food manufacturing, and affect the quality and nutritional properties of the end-products. Sound einkorn seeds display low alpha-amylase (0.14–0.29 CU/g DM) and beta-amylase (8.2–15.9 B3U/g DM) activities (Hidalgo et al., 2013), thus limiting the formation of reducing sugars (in particular, maltose) and the development of heat damage during processing, but show greater polyphenol oxidase (PPO) activity (252.1–478.5 U/g DM) than the polyploid wheats. A high PPO activity in the kernels and in the flour is responsible for the darkening of many end-products. The embryo/scutellum has the highest polyphenol oxidase and alpha-amylase values, followed by the bran and the endosperm; in contrast, beta-amylase is evenly distributed in the bran and the endosperm, and is absent in the embryo/scutellum (Hidalgo et al., 2013). Einkorn also shows low lipoxygenase activity (0.45 ± 0.072 μmol/min/g DM), unlike most polyploid wheats (*T. aestivum*, 8.02 ± 0.492 μmol/min/g DM; *T. turgidum*, 3.48 ± 0.701 μmol/min/g DM; Hidalgo and Brandolini, 2012). Thus, the oxidation of polyunsaturated fatty acids (mainly linoleic and linolenic acids) that leads to the formation of fatty acid radicals is minimal and the bioactive lipophilic compounds (carotenoids and tocols) are better preserved during food processing. Lipoxygenase activity is generally higher in the germ (206 μmol/min/g DM) than in the bran (13.4 μmol/min/g DM) or in the endosperm (3.1 μmol/min/g DM).

5. Gluten proteins

Despite the high protein content, einkorn wheat flours produce soft doughs with low elasticity and high extensibility, because of poorer gluten quality than durum and common wheat (Wieser et al., 2009). Indeed, a good baking quality is not only due to the amount and the subunits composition of gluten proteins, but also to the ratio between the gliadins and glutenins fractions. Although the *T. monococcum* gluten content is similar to modern tetraploid and hexaploidy wheats, its gliadin and glutenin allele composition is characterized by an excess of gliadins over glutenins (Wieser et al., 2009; Geisslitz et al., 2018). In particular, the gliadin/glutenin ratio in common wheat ranges between 1.5 and 3.1, whereas in ancient wheat species it is much higher (spelt: 2.8–4.0; emmer: 3.6–6.7; einkorn: 4.2–12.0; Geisslitz et al., 2018). Geisslitz et al. (2019) found that the gliadin/glutenin ratios of 15 einkorn genotypes grown in four locations ranged from 3.1 to 12.1, and was influenced by growing location, environmental conditions, amount and timing of fertilization. Their concluded that this information should enable the identification of einkorn cultivars with predictable and possibly stable good baking performance. Galterio et al. (1998) found a strong correlation between the absence of ω-gliadins and good bread-making performance of einkorn genotypes. Indeed, among the *T. monococcum* constituted by CREA, cv Monlis has no ω-gliadins in its electrophoretic pattern and displays excellent bread-making quality, cv Norberto expresses a single ω-gliadin and has good baking performance, whereas cv Hammurabi shows 12–16 ω-gliadin subunits and is not suitable for leavened bread (Gazza, unpublished results). Hence, to improve dough and baking properties, einkorn breeding programs should select for low gliadin/glutenin ratio genotypes.

Nevertheless, einkorn wheat flour is suitable for the manufacturing of oven products such as cookies, pastries and unleavened bread. The gluten index (GI), a criterion defining whether the gluten is weak (GI < 30%), normal (GI = 30–80%), or strong (GI > 80%), provides a simultaneous determination of gluten quality and quantity. Gazza et al. (2022) showed that, despite a significantly higher protein content (19% *vs* 16% of durum wheat), whole wheat flour from cv Hammurabi presented an extremely weak gluten network (GI = 0%), whereas cv Norberto was of normal strength (GI = 52%). This outcome had several effects on the technological and rheological aspects of Hammurabi, including low values of SDS sedimentation volume (less than half those of Norberto) and alveographic parameters W (an index of dough strength) and P/L (the ratio between dough resistance to deformation, P, and dough extensibility, L). Indeed, the SDS sedimentation volume, a small-scale test for predicting bread-making quality and loaf volume, is highly correlated with dough strength (W).

Wieser et al. (2009) investigated white flours from 23 einkorn breeding lines and wholemeal flours from 24 einkorn lines for their qualitative and quantitative protein composition and characterised by RP/HPLC the albumin/globulin, gliadin and glutenin fractions. The analysis of the albumin/globulin fractions did not reveal any significant differences between the lines, whereas the HPLC chromatograms of the gliadin fractions enabled the diversification between einkorn and other wheat species as well as among single einkorn lines. In particular, the absence of a group of γ-gliadins was unique for einkorn compared to all other wheat species, while the differences in the γ-gliadins patterns allowed the classification of einkorn genotypes into four groups; a further subdivision was possible through the number of ω5-gliadins and the different patterns of α-gliadins and low-molecular-weight glutenin subunits. The total gluten protein contents of the einkorn flours were similar to or higher than those of common wheat and spelt, as a consequence of their higher protein content. As already mentioned, an excess of gliadins over glutenins characterised the einkorn genotypes: the α-gliadins being the most abundant and the high-molecular weight glutenins being the rarest subunits. Micro-farinograph tests on mixing properties and baking performance of einkorn wholemeal revealed that dough development time was negatively correlated with gliadins/glutenins ratio and positively with glutenins content, while bread volume was mainly dependent on glutenins content. The authors concluded that einkorn lines with high glutenin content and low gliadin/glutenin ratio were more suitable for bread-making (Wieser et al., 2009). Therefore, the determination of the gluten protein ratios is a reliable predictor of baking quality during the initial stages of breeding activity, when limited seeds and flour availability is a constraint, preventing the use of standard technological and rheological tests.

In relation to einkorn pasta-making quality, Brandolini et al. (2018) found that macaroni and spaghetti obtained from refined flour of four einkorn genotypes (Monlis, ID331, ID1395 and SAL98-32) exhibited lower firmness and cooking loss, along with higher nutritional value (mainly in terms of lutein content) than durum wheat pasta. The lower water absorption and the reduced loss of soluble solids from the pasta into the cooking water were likely due to the very high protein content of einkorn, which led to the formation of a more compact protein network, better resistant to water penetration over cooking and able to contain the swelling and breaking starch granules. Despite the very weak einkorn gluten network, Gazza et al. (2022) reported that the sensory and instrumental assessment of wholemeal einkorn spaghetti from cultivars Hammurabi and Norberto, compared with durum semolina pasta, demonstrated good sensorial properties related to texture (mainly overall acceptability and firmness), likely a consequence of the large amount of proteins in einkorn pasta from cv Hammurabi (19% DM on average), almost one percentual point higher than pasta samples from cv Norberto. Noteworthy, these einkorn cultivars were grown in organic management, confirming the great protein content of *T. monococcum* also on low-input agriculture (Bencze et al., 2020).

6. Celiac disease and gluten-related disorders

As discussed before and contrary to the expectations of the consumers, who typically think that ancient wheats have lower gluten content, common wheat has the lowest protein and gluten contents among wheat species. The consumption of gluten-based foods has been linked with a range of clinical disorders such as celiac disease (CD), wheat allergy and non-celiac wheat sensitivity (NCWS), triggered by the proteins of several cereals. Among these emerging food disorders, CD is the best defined in terms of pathogenesis, clinical manifestations and treatment. The toxicity of wheat proteins is mostly attributed to several gliadin amino acid sequences which reach intact the gut mucosa because of their high resistance to gastric, pancreatic and brush border peptidases during the digestion process, and therefore act as the part of antigen (epitope) recognised by the immune system. These hydrolysis-resistant gliadin peptides trigger both adaptive and innate immune response in the gut mucosa that, in full-blown CD patients, lead to intestinal villous atrophy (Molberg et al., 2005).

The interest to find cereals with few or no immunogenic sequences for celiac disease patients is high. *T. monococcum,* because of its diploid genome, contains a reduced number of epitopes and toxic peptides and completely lacks the D genome that encodes for **α**-gliadin proteins containing the highly T-cells immunostimulatory 33-mer peptide in CD patients (Molberg et al., 2005). Furthermore, the T-cell epitopes naturally occurring in the gliadin proteins of two einkorn varieties (Norberto and Monlis) are more susceptible to the digestion of gastro-pancreatic and brush border membrane (BBM) enzymes than those of bread wheat and, as a consequence, show a reduction of immune stimulatory properties in *ex vivo* and *in vitro* experiments (Gianfrani et al., 2015). Recently, Picascia et al. (2020) evaluated the *in vivo* immunological properties of einkorn cv Norberto in CD patients on a gluten-free diet. To investigate the immunogenicity of gluten-containing products, the comparison between *T. monococcum* and *T. aestivum* was conducted by short (3 days) oral gluten challenge, a non-invasive and relatively rapid *in vivo* approach. Compared to *T. aestivum*, *T. monococcum* showed a reduced capability to recruit immune-dominant epitopes from **α**-, **ω**-, and **γ**-gliadin-specific T-cell response in the peripheral blood of volunteer celiac patients that consumed 3–4 little breads for a few days. In detail, the patients eating einkorn bread had a lower frequency of IFN-**γ** secreting cells reactive to DQ2.5-glia **α**1a/b/**α**2 epitopes, harboured in the well-known immunogenic 33-mer peptide, in contrast to patients eating common wheat bread. This peptide, the most immunogenic **α**-gliadins fraction, is present in truncated form in einkorn cv Norberto (Gianfrani et al., 2015). Analogously, they observed substantially fewer activated T cells in response to DQ2.5-glia-**ω**1/2 epitopes in individuals eating einkorn, compared to those eating common wheat bread. However, the residual immunological activity confirms that einkorn is not suitable for the diet of CD patients, as reported in previous studies (Zanini et al., 2013). Nevertheless, compared to hexaploid wheats einkorn has a reduced *in vivo* immunogenicity in CD patients. Indeed, according to the gluten-threshold model, the amount of gluten immunogenic peptides is the primary factor for reaching the inflammatory response threshold. Therefore, the consumption of cereals with a reduced gluten epitopes load may be a viable approach to keep the pathogenic T cells below the limit of the inflammatory cascade, thus preventing, or at least delaying, the detrimental auto-immune response in at-risk individuals. Moreover, some einkorn genotypes have a **ω**-gliadin, and the gastrointestinal-resistant ω (105–123) peptide prevents the "toxic" effects induced by wheat gluten proteins *in vitro* cellular and mucosal models (Iacomino et al., 2016). This protective ω-gliadin sequence is absent in genotypes lacking ω-gliadins in their prolamin patterns, suggesting that variation in toxicity may exist within the *T. monococcum* germplasm.

Additional protein molecules are involved in gluten disorders, including wheat amylase/trypsin inhibitors (ATI), major stimulators of innate immune cell response. Until recently they were known to contribute only to the two IgE-mediated wheat allergy forms: inhalant or contact occupational allergy (baker's asthma) and classical food allergy triggered by ingestion of wheat proteins, including several ATIs (Geisslitz et al., 2021). In celiac disease, it has been suggested that the role of ATIs is to reduce the digestion of gliadins, leading to higher levels of digestion-resistant immunogenic gliadin epitopes crossing the small intestinal gut epithelium, and also to potentiate the CD outbreak by enhancing the release of pro-inflammatory cytokines and chemokines, which may act synergistically to gluten in causing CD (Geisslitz et al., 2021). ATIs may also play a role in non-celiac wheat sensitivity (NCWS), which has an estimated prevalence between 1 and 10% of the population, and is mainly self-diagnosed. Indeed, NCWS is a pathological condition characterized by intestinal and extraintestinal symptoms related to the ingestion of wheat and other "gluten-containing" cereals in patients in whom the diagnosis of CD and wheat allergy had been excluded but that show symptoms improvement on the elimination of these cereals from their diet. ATI comprise about 2–4% of total wheat grain proteins, even if very low ATI concentrations were determined in einkorn by mass spectrometry analysis, confirming the low contribution of ATIs from the A genome in hexaploid bread wheat (Geisslitz et al., 2021). ATI proteins play a key role in the resistance against pests and pathogens, which fits with the observation that, compared to other wheat species, einkorn shows no inhibition of Coleopteran insects or mammalian α-amylases and it presents the lowest Toll-Like Receptor 4 (TLR4) activating potential, a very important signal pathway in initiating the innate immune response, compared to other wheat species. However, einkorn inhibits α-amylase from Lepidopteran insects and contains a trypsin inhibitor similar to those of rye and barley, which may explain its high trypsin inhibitory activity.

ATIs belong to the 2S super-family of seed proteins, low molecular weight polypeptides with a conserved skeleton of cysteine residues, which occur in the starchy endosperm of the *Triticeae* and *Aveneae* tribes and contribute also to determining the kernel texture (Taddei et al., 2009).

7. Proteins and kernel texture

Kernel texture exerts a strong impact on several quality traits such as flour yield and granules size, starch damage, water absorption, rheological and baking properties. Single Kernel Characterization System (SKCS) is a tool for the assessment of grain texture, where a sample of 300 intact kernels are individually weighed and then crushed; the seed diameter and moisture content are also recorded to determine the hardness index (HI). Einkorn accessions have extra-soft kernels, with HI values as low as −5 to 11, very uncommon among cultivated polyploid wheats. Consequently, einkorn kernels are quite prone to damage during mechanical harvesting, leading to reduced seed germination and viability. The principal determinant factors of kernel texture are puroindoline A (Pin-A) and puroindoline B (Pin-B), two helical, tryptophan- and cysteine-rich isoforms, which belong to the 2S super-family of seed proteins. Puroindolines occur at high amounts on the surface of starch granules from soft wheat compared with those from hard wheat and affect starch granule integrity during milling as well as dough rheological properties and crumb structure. Taddei et al. (2009) compared 113 einkorn accessions of different geographic origin for their kernel texture characteristics, starch bound protein patterns and gene sequencing. The kernel texture mechanism is similar in both einkorn and common wheat and involves specific puroindoline genes. The major difference between einkorn and common wheat was the relative abundance of Pin-A and Pin-B on starch granules: the einkorn accessions showed a 3.2 and 2.7-fold increase of Pin-A and Pin-B levels, respectively, when compared with cv Bolero (soft-textured common wheat). Another distinctive trait was the presence of the Einkorn Trypsin Inhibitor (ETI), which interacts tightly with starch granules and displays some common features with puroindolines, in particular the conserved skeleton of cysteine residues. It has been suggested that ETI and puroindolines could interact in reducing the adhesion strength between starch granule surface and the surrounding matrix, and work as the causal agents of the extra-soft texture of einkorn kernels.

8. Conclusions

The kernels of the diploid *T. monococcum*, the first domesticated wheat species, are characterised by their high protein content maintained also under organic management even if their amino acid composition is like that of polyploid wheats, i.e. it is poor in the essential amino acids lysine and threonine. The enzymatic activity, especially the activity of lipoxygenases and amylases, is lower than in polyploid wheats, indicating a better shelf life of some bioactive compounds and an inferior heat damage during the food manufacturing processes. Despite the high protein content, einkorn gluten is very weakly structured due to an excess of gliadins over glutenins. Hence, apart from selected varieties, einkorn flours are not suitable for transformation in leavened breads; nevertheless, thanks to the high proteins content, it is possible to produce valuable unleavened oven and dry pasta products. Furthermore, reported studies are consistent with differences in einkorn gluten digestibility, resulting in a lower content of immunostimulatory peptides involved in gluten-related disorders.

Therefore, future breeding activities should focus on the constitution of higher-yielding einkorn varieties, free-threshing and with bigger kernels, to enhance their agronomic performance, as well as with a low gliadin/glutenin ratio to improve dough and baking properties.

Declaration of competing interest

The authors declare the following financial interests/personal relationships which may be considered as potential competing interests: Laura Gazza and Andrea Brandolini are the breeders of 4 einkorn cultivars.

Data availability

No data was used for the research described in the article.

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