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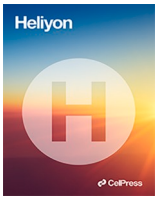

Research article

xAgrotriticum spp.: Quality properties of a potential perennial cereal candidate for sustainable agriculture

Emine Atalay

Selçuk University, Faculty of Agriculture, Department of Field Crops, Konya, Turkey

A R T I C L E I N F O

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A B S T R A C T

Perennial crop species have gained greater importance with regard to agricultural sustainability because of the ecological concerns related to annual crops. This study aimed to determine some of the primary quality traits of *xAgrotriticum* grains, a potential perennial wheat genotype, in comparison to the annual bread wheat variety Fineway.

The antioxidant activity of *xAgrotriticum* was 2.79-, 1.38-, and 2.35-fold higher than that of bread wheat according to the 2,2-diphenyl-1picrylhydrazyl (DPPH), ferric reducing antioxidant power (FRAP), and cupric ion reducing antioxidant capacity (CURPAC) methods, respectively. The free, bound, and total phenolic contents in *xAgrotriticum* were 1.34-, 1.59-, and 1.54-fold higher than those in bread wheat.

The protein content (19.58 %) of *xAgrotriticum* was 1.48-fold higher than that of bread wheat. Essential amino acids constituted 28.65 % of the total amino acids in *xAgrotriticum* and 30.38 % in Fineway. Interestingly, methionine and tryptophan were present in *xAgrotriticum* although both were below the detection limits in wheat. However, compared with wheat, the arginine content of *xAgrotriticum* was 18-fold higher with glycine and tyrosine both 8-fold more abundant. *xAgrotriticum* has significantly richer iron, zinc, and copper contents; 1.31-, 1.74-, and 2.02-fold, respectively, than wheat.

xAgrotriticum may offer potential for direct use as a foodstuff or raw material due to its nutritional elements, in addition to its potential as a genitor in hybridisation programs to improve the nutritional values of annual wheat and its prenniality which is considered one of the primary necessities for more sustainable agriculture.

1. Introduction

Prennial plants are under pressure from the monocrop-based agricultural production of annual crops [1]. Annual cereal, legume, and oilseed crops account for approximately 69 % of cultivated land worldwide [2,3]. As annual crops are not ecologically self-sustaining, practices such as soil cultivation, seed sowing, fertiliser use, and pesticide use have become indispensable elements of agriculture [1,4]. This has had negative impacts, such as water pollution, scarcity, soil erosion, increased greenhouse gas emissions, the use of large amounts of chemical inputs, and increased energy demand [5]. Owing to emerging agricultural problems, cultivation areas are decreasing [6], production patterns are changing, and significant decreases in yields are occurring [6,7], even with the increased use of polluting pesticides.

For these reasons, sustainable agricultural models that balance the environmental, social, and economic aspects of agricultural

E-mail address: eatalay@selcuk.edu.tr.

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production activities are currently among the most emphasised issues [8]. To alleviate the ecological and economic problems caused by annual production, the creation of sustainable agricultural ecosystems is recognized as key in global food security [9]. In this context, a novel approach to introducing perennial plant species, which are close relatives of annual crops, into agriculture or to strengthen annual crops in terms of sustainability by hybridizing perennial forms with existing plants has been expressed in several studies [10]. Numerous research groups are working to obtain perennial cereal varieties [11], thereby recognizing that agricultural problems can be solved if annual varieties evolve into perennial forms (https://landinstitute.org). In Argentina, Australia, China, China, India, Sweden, and the United States, breeding programs are ongoing to develop perennial forms of rice, wheat, maize, sorghum, and peas as well as oilseed crops such as sunflower, flax, and mustard [2,12]. Because most of the fruits are already perennial and are the primary source of food for living organisms, studies have focused primarily on field crops, especially cereals.

Perennial forms help reduce soil erosion [13], conserve soil moisture and nutrients, and increase soil organic matter [14]. Owing to their ability to utilise water from deep soil layers and their high water-use efficiency throughout the vegetation period [15], they can be more successful than annuals in climates with low and irregular rainfall or extreme weather conditions [16–19]. Compared with annual wheat, it is possible to produce perennial wheat with less fertiliser use, especially nitrogen fertiliser, less tillage, and less equipment use [18,19]. By incorporating more perennials, seed costs are reduced because there is no annual sowing, and weed control costs are reduced because the soil surface is covered with plants. Farmers can cultivate a larger area with fewer machines without investing in sowing, tillage, and spraying equipment [20,21] and with less soil compaction. In addition, perennial cereals have the potential to improve rural economies by reducing input requirements and labor intensity [4]. With these characteristics, they create a lower carbon footprint (approximately 200 % lower) during production [22].

Perennial forms have been determined to be richer than annual forms in terms of proteins, fats, and carbohydrates, which are considered the primary components that determine nutritional value [23]; they have higher rates of dietary fibre and antioxidant content [19] and superior amino acid content and amount [24,25]. In addition, owing to the high protein and mineral content in the green parts of these plants, their value as feed was found to be better than that of annuals [26].

Reducing the use of energy-intensive inputs in agriculture is necessary to meet the carbon emission reduction targets of most signatories to the European Union Green Deal Memorandum Protocol. The transition to perennial crops is one of the most suitable solutions for this purpose, and the world has entered an intensive research and development process.

In this study, quality-related chemical and functional properties of a potential perennial wheat genotype *xAgrotriticum*, were determined and compared with those of an annual bread wheat genotype. To the best of our knowledge, this is the first *xAgrotriticum* study in Anatolia, Türkiye, where wheat was first cultivated, grown, and bred. We hope that this study will provide inspiration to add *xAgrotriticum* both as a genitor in wheat improvement programs and as an alternative food source/feed material through direct cultivation for more sustainable agriculture.

2. Material and methods

2.1. Material

xAgrotriticum seeds, known as “Salish Blue” with accession number PI 676253, and *Triticum aestivum* seeds, known as “Fineway” with accession number PI 653509, were obtained from the United States Department of Agriculture Germplasm Resources Information Network (GRIN) (Fig. 1).

xAgrotriticum (2n = 56, *Triticeae* tribe of the genus *Poaceae*) is a perennial hybrid of *Triticum aestivum* “Chinese Spring”/*Thinopyrum ponticum*/*Triticum aestivum* “Madsen” [13,27]. It resembles tall, awnless wheat with a blue-green seed colour, abundant tillering, moderate susceptibility to stripe rust, and a soft cooking quality.

Fineway is a hard red winter wheat (*Triticum aestivum* L.) variety developed by the Washington State University Agricultural

Fig. 1. Seed materials (left; *xAgrotriticum*, right; Fineway-*Triticum aestivum* L.).

Research Center for semi-arid and arid regions based on high yield and stripe rust resistance [28].

xAgrotriticum and Fineway seeds were sown and multiplied in the Research and Application Field of Selçuk University Agricultural Faculty, Konya, Türkiye, where annual wheat cultivation is intensively practiced and is therefore called the "granary of Türkiye". During vegetation period, the average precipitation was 292 mm, the average relative humidity was 54.6 %, and the average air temperature was 13.96 °C [29]. The lowest temperature values were recorded as −28.2 °C, and the highest temperature value was 37.1 °C [30]. The soil is a typical Central Anatolian soil with a clayey loamy texture, is alkaline, and rich in calcium. No salinity problems were observed (Table 1). During the tillering and heading periods, the plants were irrigated twice with drip irrigation without any fertilisation, and the pecticide applied to the weeds was mechanically controlled.

2.2. Methods

2.2.1. Seed and flour colour

The colour values of the seeds and flours of both *xAgrotriticum* and Fineway were assessed using a Hunter Lab Chroma Meter (Minolta CR-400, Osaka, Japan), with measurements provided in terms of L^* value, which signifies brightness (0 for dark and 100 for light); a^* value, representing redness/greenness (+ for red and - for green); and b^* value, denoting yellowness/blueness (+ for yellow and - for blue) [31].

2.2.2. Antioxidant activity and phenolic content

xAgrotriticum and Fineway seeds were milled to a particle size of less than 0.5 mm and flour samples (1 g) were mixed with 10 mL methanol:water solution (80:20, v/v). Extraction was carried out by shaking the mixture at room temperature (24 ± 1 °C) for 2 h. After extraction, the mixture was centrifuged at 3000 rpm to obtain the supernatant for analysis, and the separated supernatant was stored at −20 °C for analysis.

In this experiment, the 2,2-diphenyl-1picrylhydrazyl (DPPH) radical scavenging capacity of *xAgrotriticum* and Fineway flour extracts was determined according to a previously described method [32], with minor modifications. Both sample extract (0.1 mL) was added to 3.9 mL of 6×10^{-5} mol/L methanolic solution of DPPH. The absorbance was measured at 517 nm after the solution was allowed to stand in the dark for 30 min. Lower absorbance of the reaction mixture indicated higher free radical scavenging activity. The Trolox calibration curve was plotted as a function of DPPH radical-scavenging activity. The results were expressed as micromoles of Trolox equivalents (TE) per kilogram of dry matter (μmol TE/kg dm).

Ferric reducing antioxidant power (FRAP) was measured as previously described [33]. Briefly, freshly prepared FRAP reagent consisted of 300 mM acetate buffer (pH 3.6), 10 mM 2, 4, 6-tripyridyl-s-triazine (TPTZ), and 20 mM $FeCl_3$ at a ratio of 10:1:1 (v/v/v). Wheat flour extract (50 μL) was mixed with 700 μL of the FRAP reagent, and after 5 min of incubation at 37 °C, absorption was measured at 593 nm using a spectrophotometre (Hitachi-U1800, Japan). Methanolic solutions with known Trolox concentrations were used to calibrate the FRAP assay. FRAP values, expressed as μmol of Trolox equivalent per g dry matter, were obtained by comparing the absorption change in the test mixture with doses received from the Trolox standard concentrations curve.

The cupric ion reducing antioxidant capacity (CUPRAC) of the extract was determined according to the method of [34]. A mixture of 1 mL of 10 mM $CuCl_2$ solution, 7.5 mM neocuproine alcoholic solution, and 1 M ammonium acetate (pH 7.0) buffer solution, and the extract (x mL) and H_2O [(1.1-x) mL] were added to make a final volume of 4.1 mL. The mixture was 5 min of incubation at 37 °C. Absorption was measured at 450 nm using a spectrophotometre and is expressed as Trolox equivalents (μmol TE/g).

Free and bound phenolic compounds were extracted as previously described [35]. For the free phenolic extraction, *xAgrotriticum* and Fineway seeds were milled to a particle size of less than 0.5 mm and flour samples (1 g) were mixed with 10 mL of 1 % acidified (HCl) methanol:water solution (80:20, v/v). Extraction was carried out by shaking the mixture at room temperature (24 ± 1 °C) for 2 h. After extraction, the mixture was centrifuged at 3000 rpm, and the separated supernatant was stored at −20 °C for analysis.

For bound phenolic extraction, 20 mL of methanol/H_2SO_4 (10:1) was added to the residue remaining after free phenolic extraction, and the mixture was incubated in a shaking water bath for 20 h at 85 °C, then the cooled supernatant was separated by centrifugation was stored at −20 °C until analysis.

Table 1
Soil characteristics of the research fields.

Soil Dept (cm)	0–30	30–60	Mean
pH	8.00	7.95	7.98
Electrical conductivity $EC^{25}x10^3$	0.80	0.75	0.78
Organic material (%)	2.20	1.24	1.72
CaCO3 (%)	37.5	34.2	35.85
P_2O_5 (kg/da)	1.80	1.35	1.58
Fe (ppm)	14.70	8.70	11.70
Zn (ppm)	0.30	0.34	0.32
Cu (ppm)	1.67	1.76	1.72
Mn (ppm)	7.00	5.73	6.37
Saturity (%)	63	61	62.00
Texture	Clayey/Loamy	Clayey/Loamy	

The free and bound phenolic contents of each extract were determined according to the Folin-Ciocalteu colorimetric method, as previously described [36]. Total phenolic content was determined by summing the free and bound phenolic contents. Phenolic content was expressed as gallic acid equivalents (mg GAE/kg).

2.2.3. Protein content

The nitrogen content of the grains was determined using a LECO C/N analyser according to the dumas combustion method in the American Association for Clinical Chemistry (AACC) method 46–30 [37]. The crude protein percentage was obtained by multiplying the nitrogen percentage by this factor using both the standard 6.25 and a conversion factor (Jones factor) of 5.70 [17,38].

2.2.4. Amino acid content

For total amino acid analysis, 0.2–0.5 g of homogenised sample was weighed into tubes with screw septum caps, and 5 mL of 6 N HCl and 250 μL of 2 mM phenol were added to prevent oxidation. To optimise the recovery of cystine, methionine, and tyrosine, 0.1 g Na_2SO_3 was added, and heated in an oven at 110 °C for 24 h. At the end of hydrolysis, the pH of the sample was adjusted to near neutral (pH 6.7–7.3). The volume of the sample was made up to 50 mL with ultrapure water according to the expected total amount of amino acids, centrifuged at 4000 rpm for 5 min, the supernatant was transferred to a vial, and the total amino acids in the sample were determined using ultra-performance liquid chromatography-tandem mass spectrometry (UPLC-MS/MS; Düzen Norwest Laboratory, Ankara, Türkiye).

2.2.5. Mineral content

For mineral content analysis, 0.2 g of the ground sample was weighed and dissolved in 5 mL concentrated HNO_3 and 2 mL H_2O_2 (30 % w/v) in a microwave device (MarsExpress, CEM Corp., USA) at high temperature (210 °C) and pressure (200 PSI). The dissolved samples, which were made up to 20 mL with deionised water, were filtered with filter paper (Whatman No. 42), and the mineral content was determined using an inductively coupled plasma optical emission spectrometry (ICP-OES) (Agilent 5110). Mineral concentrations were checked against certified values of the respective minerals in reference samples from the National Institute of Standards and Technology (NIST; Gaithersburg, MD, USA) [39].

2.2.6. Statistical analyses

Each measurement was conducted based on independent replicate trials (at least in duplicate). Data processing was performed using Excel 2016 software to obtain the mean value and standard deviation of each measurement. The results were analysed using one-way variance factor (ANOVA) followed by Tukey's test at a 5 % significance level ($P < 0.05$). One-way ANOVA and least significant difference (LSD) test was performed using the JMP® Pro 16 software (JMP Statistical Discovery, LLC).

3. Result and discussion

3.1. Seed and flour colours

The differences between the colour values obtained from the seeds and flours of *xAgrotriticum* and Fineway were statistically significant ($P < 0.05$) (Table 2).

The L^* value of the seeds of *xAgrotriticum* was 49.25, whereas it was 47.56 Fineway. The a^* and b^* values, as the defining factors of seed color were 1.60 and 18.64 in *xAgrotriticum* and 4.03 and 18.96 in Fineway, respectively (Table 2).

Seed colour in wheat is determined by substances such as anthocyanins, carotenoids, flavonoids, and other phenolic compounds present in the seeds. Anthocyanins, which are predominant in the inner part of the pericarp and the aleurone layer of the seed, create a colour in the blue-violet range; the yellow colour is caused by carotenoids in the endosperm, and the red colour is caused by flobaphenes, a phenolic substance in the outer layer of the pericarp [40].

The environment had a significant effect on the L^* values (67.9 %). In contrast, the genotype had a^* more significant and higher impact on the b^* value (86.6 %) than other factors [41,42]. Researchers have reported that colour-related values are highly heritable traits that are controlled by additive gene effect [43]. The b^* value, which indicates the carotenoid group pigment that gives yellow colour to the grain, is accepted as a quality criterion that determines the durum quality of wheat [42,44].

For whole flour colour, the L^* value was 88.33, a^* value was −4.17, and b^* value was 11.67 *xAgrotriticum*, whereas these values were 87.44, −2.55, and 13.21 Fineway, respectively (Table 2). Accordingly, *xAgrotriticum* is richer in terms of b^* value than bread

Table 2
Colour values of *xAgrotriticum* and bread wheat Fineaway.

	Material/Colour	L^*	a^*	b^*
xAgrotriticum	**Seed**	49.25 ± 0.27 c	1.60 ± 0.48 ı	18.64 ± 0.99 e
	Whole Flour	88.33 ± 0.41 a	−4.17 ± 0.06 k	11.67 ± 0.18 g
Fineway	**Seed**	47.56 ± 0.49 d	4.03 ± 0.23 h	18.96 ± 0.54 e
	Whole Flour	87.44 ± 0.32 b	−2.55 ± 0.11 j	13.21 ± 0.18 f

The difference between two means shown with different lowercase letters in the same column is statistically significant ($P < 0.05$).
± Standard deviation of the mean value of replicates.

wheat Fireway. However, no correlation exists between the colour parameters measured in the seed and those measured in the milled material, and it would be more reliable for those working on this subject, including breeders, to use the colour parameters obtained from the milled material [45].

The flour colour of *xAgrotriticum* was brighter and greenish-yellow than that of bread wheat. The mixture of blue and yellow colours after grinding was measured as green, which may be due to the higher phenolic and anthocyanin content in *xAgrotriticum*. As the health benefits of foods rich in colour pigments have been discovered, interest in coloured products has increased [46]. Seed pigments affect grain appearance and nutritional quality, but also protect the seed against pathogens, insects, and ultraviolet (UV) light [47]. Thus, in recent years, colour-bearing wheat genotypes have been valued and attracted the attention of breeders and the industry [48]. Several studies have shown that colour is associated with substances such as anthocyanins and phenolic compounds which are known to support the immune system and have protective effects on human health. In this context, *xAgrotriticum* grains have the potential to improve bread wheat quality when used in breeding programs.

3.2. Antioxidant activity and phenolic contents

Testing methods for antioxidant activity are variable, because different reactions, method-specific standards, and parameters are used in each method. Although this prevents healthy comparisons [49], using the most popular methods together can produce healthier interpretation of results [50–52] to avoid inadequate and erroneous evaluations owing to the complex structure of cereals [53]. Therefore, we included all three methods in our analysis: DPPH, cupric ion reducing antioxidant capacity (CURPAC), and FRAP.

The differences between the means were significant when evaluated using standard deviation values (Tables 3 and 4). *xAgrotriticum* showed higher antioxidant activity than the Fineway bread wheat variety using all three methods. The antioxidant activity of *xAgrotriticum* was 2.79-fold higher based on DPPH, 1.38-fold higher based on FRAP, and 2.35-fold higher based on CURPAC, compared to bread wheat (Table 3).

The antioxidant activity values obtained from the CUPRAC and FRAP tests were similar, whereas the DPPH test produced significantly different values, consistent with previous reports [54]. A positive relationship between pigmentation and antioxidant activity [55] is evident and is a valuable nutritional trait [56], since antioxidants reduce oxidative stress; antioxidant-rich foods are effective elements in the protection of human health, so it is essential to know their antioxidant activities [57]. Some studies have indicated that the antioxidant properties of genotypes used in breeding programs should be considered [58,59]. Compared with wheat, the higher antioxidant activity of *xAgrotriticum* suggests that it can be incorporated as a genitor in breeding programs aimed at wheat varieties with higher nutritional value. In addition, the plant can be used directly as feed and food, and the flour and germ can be mixed with products from other cereal crops.

xAgrotriticum had higher free (1.34-fold), bound (1.59-fold), and total (1.54-fold) phenolic content than Fineway (Table 4). The variation was lower in the free form, whereas *xAgrotriticum* was approximately 50 % more bound to the total phenolic content of Fineaway.

Phenolic compounds are the most important compounds that affect antioxidant activity, and several studies have recently drawn attention to this issue [60]. Generally, a strong positive correlation between total phenolic content and antioxidant activity has been reported [61]. In wheat, there is a positive correlation between antioxidant capacity and total phenolic compounds, and it has been emphasised that those with high antioxidant activity are richer in total phenolic compounds [62].

In *xAgrotriticum*, free and bound phenolic compounds accounted for 17.39 % and 82.61 % of the total phenolic compounds, respectively, whereas these percentages were 20.40 % and 79.88 %, respectively, in Fineway. Bound phenolic compounds are found more frequently in primary plant materials such as cereals, oilseeds, and legumes than in the free form. The biological usefulness of phenolic compounds in foods depends on their release and absorption in the food matrix by digestive processes [63]. For this reason, the phenolic compound content in numerous foods is rearranged to include both free and bound phenolic compounds [64]. Because phenolic compounds with antioxidant activity in cereals are generally found largely in an insoluble form, phenolics bound to biomolecules such as carbohydrates, proteins, and lipids in the cell wall can withstand gastrointestinal digestion, reach the entire colon, and provide an antioxidant environment, thereby protecting against factors such as colon cancer [60,65].

Perennial wheat has a higher bound and free phenolic content than bread wheat [66–68]. In the present study, we report similar results from *xAgrotriticum* also contaning a higher antioxidant content and phenolic activity than bread wheat.

A decrease in the total phenolic content of wheat during its evolution or improvement may have occurred, as wheat has a higher total phenolic content than cultivated wheat [69]. Similarly, the protein, fibre, and antioxidant capacity of perennial wheat are higher

Table 3
Antioxidant activities of *xAgrotriticum* and bread wheat Fineway.

	Antioxidant activity		
	DPPH (mg TE/kg)	FRAP (umol TE/g)	CUPRAC (umol TE/g)
xAgrotriticum	557.02 ± 6.99	3.20 ± 0.09	9.16 ± 0.75
Fineway	199.38 ± 9.12	2.32 ± 0.02	3.90 ± 0.82
***xAgrotriticum*/Fineway**	2.79	1.38	2.35

DPPH: 1,1-diphenyl2-pikrilhidrazil method, FRAP: Ferric Reducing Antioxidant Power method, CURPAC: Cupric Ion Reducing Antioxidant Capacity method.
± Standard deviation of the mean value of replicates.

Table 4
Phenolic contents of *xAgrotriticum* and bread wheat.

	Phenolic content		
	FPC (mg GAE/kg)	BPC (mg GAE/kg)	TPC (mg GAE/kg)
xAgrotriticum	3396.74 ± 60.47	16137.97 ± 40.43	19534.71 ± 111.45
Bread wheat cv. Fineway	2584.82 ± 71.67	10121.25 ± 71.93	12670.07 ± 132.59
***xAgrotriticum*/Fineway**	1.34	1.59	1.54

FPC: Free phenolic content, BPC: Bound phenolic content, TPC: Total phenolic content.
± Standard deviation of the mean value of replicates.

than those of bread wheats [70] and pigmented wheat is rich in phenolic compounds and antioxidants [71]. Because coloured wheat contains higher antioxidant and phenolic compounds than normal wheat, and phenolic compounds have positive effects on health, the development of varieties rich in these bioactive compounds in wheat breeding programs has gained importance as a potential target [72,73]. It is considered that *xAgrotriticum*, with its high phenolic content and antioxidant activity, would directly benefit from the creation of healthy diet programs and the avoidance of free radicals in the faulty living conditions, and indirectly, it has the potential to increase its quality by crossing with cultivated wheat.

3.3. Protein contents

Protein content is one of the most important criteria for determining grain quality. xAgrotriticum had considerably higher crude protein contents (32.32 % and 32.28 %) than Fineway bread wheat according to the both factors (6.25 and 5.70, respectively) (Table 5). When evaluated using standard deviation values, the differences between the means were significant.

Various reports [74–76] have indicated that hybrid wheat with perennial parents has smaller grains than annual forms, and therefore, has higher protein levels (>20 %). Although they produce lower flour yields, they offer more bran per seed and higher fibre levels. Wild relatives of wheat serve as alternative protein sources [20]. Small seed size may be a reason for the higher protein content in *xAgrotriticum*; however, by selection, grain size and yield can be increased in perennial forms. It has been reported that perennial wheat *Trititrigia* contains an average of 17.5–18.4 % crude protein and is rich in nutrients, including vitamins, which increase the feed value of the non-grain part of the plant [23]. Perennial wheat hybrids have also been shown to have higher protein, dietary fibre, and antioxidant contents than wheat [19].

3.4. Amino acid contents

Quality, protein content, and amino acid composition were the two significant parameters. The differences between the amino acid contents of *xAgrotriticum* and Fineway were statistically significant ($P < 0.05$) (Table 6). Although the total amount of essential amino acids in Fineway (30.38 %) was slightly higher than *xAgrotriticum* (28.65 %), the latter contained methionine and tryptophan, and both amino acids were below detectable levels in Fineway. In addition, arginine (0.72 g/100 g) was 18-fold higher, glycine (0.82 g/100 g), and tyrocine (0.49 g/100 g) levels were 8-fold higher in *xAgrotriticum* than in Fineway (Table 6).

Amino acids are essential for nitrogen metabolism and growth, even when other amino acids are present at adequate levels. Essential amino acids, which are also defined as “limiting” or “indispensable” amino acids due to these properties, cannot be synthesised in the vertebrate class, including humans, as they do not have the necessary metabolic pathways/processes, and must be consumed with nutrients for metabolic activities to function correctly [78].

The metabolic activities are determined by the least available. When the most significant limiting factor improves, the next most important limiting factor also becomes important. This process is repeated with gradual improvements until no limiting factor remains [79]. Liebig’s law, which applies to all biological pathways, states that successful functioning is limited by a deficiency of essential nutrients, and essential substances must be present in sufficient quantities for healthy functioning [80]. The fact that *xAgrotriticum* contains all essential and other amino acids in higher amounts than bread wheat makes *xAgrotriticum* a valuable food, feed, and/or breeding material.

Similarly, another perennial wheat (*Thinopyrum intermedium*) contains significantly higher levels of nutritional elements, including essential amino acids, than whole wheat flour [67]. When the amino acid values of perennial wheat were compared with both reference wheat and literature information, it was shown that all essential and non-essential amino acid values were high. Researchers have emphasised that kernza, a perennial wheat candidate, contains 16.42 % (w/w) (2.88 % N) protein, whereas most commercial

Table 5
Protein contents of *xAgrotriticum* and Fineway bread wheat.

	xAgrotriticum	Fineway	*xAgrotriticum*/Fineway
(N%)	3.13 ± 0.06	2.12 ± 0.03	1.48
Crude protein (%)(factor: 6.25)	19.58 ± 0.38	13.25 ± 0.20	1.48
Crude protein (%)(factor: 5.70)	17.84 ± 0.35	12.08 ± 0.18	1.48

± Standard deviation of the mean value of replicates.

Table 6
Amino acid contents of *xAgrotriticum* and Fineway bread wheat varieties.

Amino acids	*xAgrotriticum* g/100 g	Fineway g/100 g	*xAgrotriticum*/Fineway
Alanine	0.75 ± 0.02 fgh	0.37 ± 0.08 jkl	2.03
Arginine	0.72 ± 0.06 gh	0.04 ± 0.03 o	18.00
Aspartic acid	1.08 ± 0.03 e	0.42 ± 0.08 jkl	2.57
Cystine	0.35 ± 0.02 jkl	–	100
Glutamic acid	6.58 ± 0.11 a	2.63 ± 0.33 b	2.50
Glycine	0.82 ± 0.06 fgh	0.10 ± 0.04 mno	8.20
Histidine	*0.34 ± 0.00* jkl	*0.06 ± 0.04 no*	*5.67*
Isoleucine	*0.65 ± 0.01* ghı	*0.14 ± 0.05 mno*	*4.64*
Leucine	*1.29 ± 0.05* d	*0.64 ± 0.27 ıjk*	*2.02*
Lysine	*0.49 ± 0.01* ıj	*0.69* ± 0.09 fgh	*0.71*
Methionine	*0.27 ± 0.*02 klm	–	*100*
Phenlyalanine	*0.82 ± 0.*00 fgh	*0.35 ± 0.06 jkl*	*2.34*
Prolin	1.78 ± 0.01 c	1.21 ± 0.15 de	1.47
Serine	0.96 ± 0.06 ef	0.28 ± 0.09 lmn	3.43
Threonine	*0.64 ± 0.01* hı	*0.10 ± 0.01* mno	*6.40*
Tryptophan	*0.05 ± 0.00* o	–	*100*
Tyrosine	0.49 ± 0.02 ıj	0.06 ± 0.01 no	8.17
Valine	*0.86 ± 0.*05 fg	*0.25 ± 0.07* lmno	*3.44*
Total Aminoasid	**18.88**	**7.34**	**2.57**

Essential amino acids are shown in italic. –, not dedectable. Levels not connected by same letter are significantly different. ($P < 0.05$).
± Standard deviation of the mean value of replicates.

varieties of wheat contain between 8 % and 16 % protein [17]. Lysine, threonine, and isoleucine are limiting amino acids in wheat, that protein and amino acid composition must be improved to close the nutritional gap, and that the amino acid composition can be improved by hybridisation [77]. Accordingly, *xAgrotriticum* is a promising cereal that can be used directly to meet food and feed needs and has the potential to contribute to breeding programs.

3.5. Mineral contents

Minerals are essential for human health. Cereals, especially wheat, are an important food group with rich mineral contents [81].

According to the results, *xAgrotriticum* was richer in Fe, Zn, and Cu micronutrients than Fineway. The 1.31-, 1.74-, and 2.02-fold higher Fe, Zn, and Cu contents, respectively, were significant. Fireway was found to have higher K-Mg-Mn values (Table 7). Differences in mineral content between *xAgrotriticum* and Fineway were statistically significant at $P < 0.05$.

The fact that Mn, an essential micronutrient, is less abundant in *xAgrotriticum* can be explained by elemental interactions. Generally, an antagonistic relationship exists between Zn, Fe, and Mn [82,83].

Micronutrient deficiency in food is recognized as a global problem with severe consequences [84]. Therefore, the enrichment of mineral content in cereals to improve nutritional quality has gained significant importance worldwide, especially because wheat is one of the primary food sources in human nutrition. Numerous breeding programs have been conducted to obtain wheat varieties enriched in minerals and other bioactive components, such as antioxidants [81]. The mineral content of cultivated wheat varieties has been decreasing for more than 50 years, with Cu, Fe, Mn, and Zn concentrations 40 %, 24 %, 32 %, and 33 % higher, respectively, in perennial plants, indicating that improvement can be achieved by including perennial wheat with rich mineral content in hybridisation programs [85,86].

Table 7
Mineral content of *xAgrotriticum* and Fineway bread wheat.

Macro nutrients (%)	*xAgrotriticum*	Fineway	*xAgrotriticum*/Fineway
N	3.13 ± 0.06 hı	2.12 ± 0.06 hı	1.48
P	0.49 ± 0.03 hı	0.47 ± 0.02 hı	1.04
K	0.18 ± 0.01 ı	0.30 ± 0.01 ı	0.60
Ca	0.07 ± 0.00 ı	0.04 ± 0.00 ı	1.75
Mg	0.11 ± 0.00 ı	0.12 ± 0.01 ı	0.92
Na	0.03 ± 0.00 ı	0.03 ± 0.00 ı	1.00
Micro nutrients (mg kg^{-1})	***xAgrotriticum***	**Fineway**	***xAgrotriticum*/Fineway**
Fe	15.05 ± 1.15 de	11.53 ± 0.68 ef	1.31
Zn	58.48 ± 3.98 a	33.64 ± 1.46 c	1.74
Cu	9.45 ± 2.10 f	4.67 ± 0.12 gh	2.02
Mn	35.01 ± 0.57 c	42.67 ± 1.91 b	0.82
B	19.22 ± 3.21 d	8.10 ± 1.97 fg	2.37

Levels not connected by same letter are significantly different. ($P < 0.05$).
± Standard deviation of the mean value of replicates.

As with other annual crops, annual cereal production has become unsustainable because of its negative impacts on the environment. In addition, it is known that the mineral concentration of wheat varieties has shown a general decline over the last 50 years due to changing environmental factors [85]. Collectively, *xAgrotriticum* has the potential to be used as a new agricultural crop or as a parent in hybridization programs due to its advantages of being a perennial and its rich content of quality elements.

4. Conclusion

This study showed that *xAgrotriticum* has significantly higher colour content, antioxidant activity, phenolic substances, protein, and total amino acid content. *xAgrotriticum* also provides higher Cu, Fe, and Zn contents, which are involved in multiple enzyme systems with great physiological importance, than Fineway. The perennial condidate *xAgrotriticum* can be used directly as a foodstuff/raw material owing to its nutritional elements, as well as its potential for use as a genitor in hybridisation programs to improve the nutritional value of wheat.

This is the first preliminary study of *xAgrotriticum* in Türkiye, the gene centre of wheat. This species could also contribute to global agriculture. Further agronomic and genetic studies are needed to make *xAgrotriticum* a new perennial crop or to include it in breeding programs. Widespread cultivation of *xAgrotriticum* may contribute to improved human health and a more sustainable environment.

Data availability statement

All data analysed in this study are included in the tables. Data supporting the findings of this study are available upon reasonable request from the author.

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Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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Abbreviations

FRAP	ferric reducing antioxidant power
CURPAC	cupric ion reducing antioxidant capacity
DPPH	2,2-diphenyl-1picrylhydrazyl
GRIN	United States Department of Agriculture Germplasm Resources Information Network
GAE	gallic acid equivalents
TE	trolox equivalents
TPTZ	2, 4, 6-tripyridyl-s-triazine
AACC	American Association for Clinical Chemistry
UPLC MS/MS	ultra performance liquid chromatography-tandem mass spectrometry
ICP-OES	inductively coupled plasma optical emission spectrometer

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