



Determination of the health-protective effect of ancient cereals and one possibility of increasing their functionality

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Received: 7 February 2023 / Accepted: 21 April 2023 / Published online: 3 May 2023
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Abstract

This study aimed to examine the differences between the antioxidant capacity, and the total polyphenol content of the ancient grains namely Einkorn (*Triticum monococcum* L.) and Khorasan (*Triticum turanicum*) compared with wheat (*Triticum aestivum* L.), furthermore attempt to create a functional loaf enriched with rosemary (*Salvia Rosmarinus* Spenn.). To achieve this goal, 1, 3, and 5% of rosemary were added to the loafs made of Einkorn, Khorasan, and common wheat flour, and then the changes in their antioxidant and polyphenol content were examined. Based on the spectrophotometric results, it can be said that the type of wheat clearly affects the amount of the tested components. In all cases, the loafs made from common wheat contained the least amount of antioxidants (0.74 mg AAE/g) and polyphenols (0.41 mg GAE/g). On the other hand, Khorasan loaves showed outstanding values in terms of their antioxidant (0.89 mg AAE/g) and polyphenol (0.68 mg GAE/g) content. Einkorn loaves contained smaller amounts of the tested antioxidants (0.71 mg AAE/g) and polyphenols (0.51 mg GAE/g), but they were still better than common wheat loaves. The addition of rosemary can significantly increase the amount of compounds with an antioxidant effect in bakery products, but care must be taken to preserve the favorable organoleptic properties. It has been proven that ancient grains have a more positive physiological effect than common wheat, and the health-protective properties of bakery products can be increased by adding herbs.

Communicated by Bernadett Langó.

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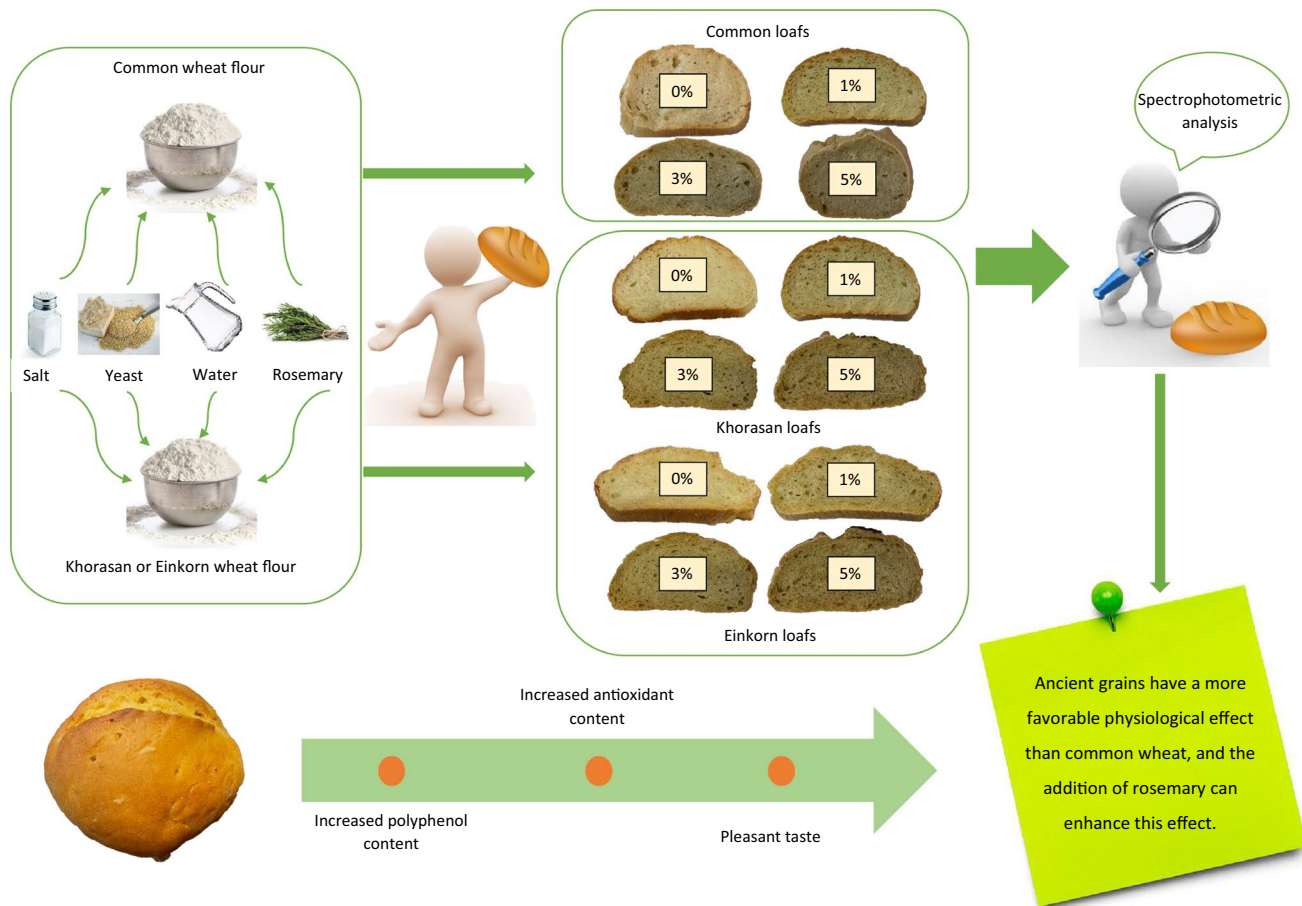
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Graphical abstract



Keywords Ancient grains · Polyphenols · Antioxidants · Functional food · Spectrophotometry

Introduction

The cultivation of cereals and cereal-like crops dates back to prehistory. Wheat is one of the oldest crops grown for food, it grew presumably as early as 8000–10,000 years ago. Archaeological finds suggest that the ancient Egyptian, Babylonian, Greek, Cretan, and Roman civilizations were heavily dependent on wheat as their main food (Bordoni et al. 2016; Curtis 2002). Ancient grains are the earliest domesticated grains and ancestors of wheat grown today. They are also called “husked grains” because the seed retains its peel at harvest (Abdel-Aa et al. 2006; Feldman 2001). About 95% of the wheat grown annually worldwide is made up of major wheat varieties. These varieties are *Triticum aestivum*, also known as common wheat, or bread wheat, of which approximately 700 million tonnes are grown annually. As well as *Triticum turgidum* var. *durum*, which is well adapted to the warm, dry conditions prevailing in the Mediterranean region. It

is grown annually at 35–40 million tonnes and is mainly used as a raw material for pasta, which is why it is also known as “pasta wheat” or “durum wheat” (Shewry and Hey 2015). Nowadays, ancient grains are grown only in small areas. One of the best-known ancient grains is einkorn (*Triticum monococcum* ssp. *monococcum*), which differs from common and durum wheat in that it must be mechanically husked before processing because the glume remains tightly closed on the grain and cannot be removed by threshing (Lachman et al. 2013). Einkorn was one of the first crops to be domesticated in the Middle East about 12,000 years ago. One of the most commonly cultivated ancient wheat species, but its cultivation is limited to small areas. Compared to common wheat, it is more resistant to disease, drought, harsh environments, and nutrient-poor soils. It grows even in barren areas where other wheat varieties are unviable, but their yields are lower than common wheat (Loje et al. 2003). It is a high nutritional value cereal because it contains high amounts of protein and

antioxidants (Abdel-Aal et al. 2006). Einkorn grain has a number of nutritional benefits. The seeds contain more protein, carotenoids, and minerals (calcium, manganese, and sulfur) than common wheat (Brandolini et al. 2008). In addition, einkorn grain contains in higher concentrations soluble sugars and minerals but less insoluble dietary fiber contains than other cultivated wheat species (Abdel-Aal et al. 1995). The peeled cereals contain significantly more zinc, iron than bread wheat (Suchiwilska et al. 2012), and their protein content is also higher (Borghi et al. 1996).

Another variety of “ancient” wheat appeared under the name Kamut® which means “wheat” in the ancient Egyptian language. Rumor has it the seeds of Kamut® wheat were discovered in an Egyptian tomb in the 1940s and are therefore also called mummy wheat (Moshenska 2017); however, more likely to have been purchased from a street vendor (Shewry 2018). It is also known as Khorasan wheat (*Triticum turgidum ssp. turanicum*), this is an ancient wheat variety with a distinctive character. Its trademark is mainly the protein content between 12 and 18% and the selenium content between 400 and 1000 ppb (Bordoni et al 2016; Cameron and Quinn 1999).

In general, cereals are rich in phenolic compounds. Phenolic compounds can significantly contribute to the antioxidant capacity of wheat grains (Kim et al. 2006). Cereals can be an important source of antioxidants. It has a beneficial effect on health and contains vitamins and enzymes (Ehrenbergerová et al. 2009). Wheat has an antioxidant activity caused by various antioxidant compounds, these are typically hydrophilic and lipophilic groups, polyphenols, and carotenoids (Knudsen et al. 2008).

In this study, we want to demonstrate that bakery products made from ancient grains (Einkorn, Khorasan) have a better antioxidant effect than products made from common wheat. We also investigated the effect of rosemary enrichment on the antioxidant and polyphenol content of the products.

Materials and methods

Chemicals

Chemicals for the determination of polyphenol, and antioxidant content were 97% ethanol (Reanal, Budapest, Hungary), anhydrous sodium carbonate (Riedel-de Haen, Seelze, Germany), Folin–Ciocalteu reagent (Merck), 2-4-6-tripyridyl-s-triazine (TPTZ) (Sigma-Aldrich, Budapest, Hungary), acetic acid (Reanal, Budapest, Hungary), anhydrous iron chloride (Merck, Budapest, Hungary), ascorbic acid (Sigma-Aldrich, Budapest, Hungary), and gallic acid (Sigma-Aldrich, Budapest, Hungary).

Wheat samples

The Einkorn (*Triticum monococcum* L.), Khorasan (*Triticum turanicum*) and common wheat (*Triticum aestivum* L.) flour, and also the dried, and grounded Rosemary leaves were purchased commercially.

Loaf-making process

The loaf-making process was based on the publication of Kapcsándi et al. (2021). Loafs made from both Einkorn, Khorasan, and common wheat flour, were baked from 100 to 100 g of flour mixture containing 1, 3, and 5% Rosemary. In order to prove the added Rosemary value of the loafs, we also made control loafs from 100 to 100 g of each flour. To each loaf was added 2 g of salt, 5 g of baking yeast, and 60 mL of water. Knead the dough from the ingredients, and leaven it in a baking tin until it doubled in volume. Before baking, the surface of the dough was smeared with water and baked in a preheated oven at 200 °C for 45 min.

Determination of total antioxidant and polyphenol content

Loaf sample preparation

The loafs were frozen and then ground with a hammer grinder. For extraction, 2–2 g of loaf grinds was weighed into Erlenmeyer flasks on an analytical balance and 20 mL of an extraction mixture containing methanol and water (80:20 V/V%) was added. The extraction was performed at 65° C in an ultrasonic bath for 1 h. The extracts were centrifugated at room temperature, 2500 g, 20 min, and the filtrate was further analyzed.

FRAP assay

The antioxidant content of the loaf samples was estimated according to the method described by Benzie and Strain (1996) as modified by Pulido et al. (2000) with minor modification. 200 µL of the extracted sample, 3 mL of FRAP solution, and 100 µL of water were pipetted into a test tube. The finished solutions were placed in a dark place for 5 min and then their absorbance was measured with a Spectroquant Pharo 100 spectrophotometer (Merck, Germany) at a wavelength of 593 nm against the blank. Ascorbic acid (40–500 mg/L) was used as a standard and the results were expressed as mg ascorbic acid equivalent capacity (AAE)/g dry matter.

Folin–Ciocalteu assay

Determination of total polyphenol content based on the Folin–Ciocalteu method was described by Singleton et al. (1999) with some modifications (Barba et al. 2013). To 200 μ L of loaf extract, 1.5 mL of high purity water was pipetted and the reagents were added. First 2.5 mL of Folin–Ciocalteu reagent, then 2 mL of Na_2CO_3 . The tubes containing the mixture were placed in a dark place for 90 min, and then the absorbance was measured at 725 nm versus the blank. Gallic acid was used as a standard (25–1000 mg/L).

Data analysis

The total antioxidant and polyphenol contents of loafs were determined in Microsoft Office Excel from the absorbance values measured for loafs using the equation of the second-order least-squares analytical curve fitted to the measurement solutions using the nonlinear least-squares method. All the results are expressed as means ($n=3$) $\pm$ standard deviation.

Results

Antioxidant content of loafs

The results of the antioxidant contents obtained by the FRAP assay for the examined loaf varieties are shown in Fig. 1. Based on the results, the examined loaf varieties showed significant differences in terms of antioxidant content. 'Khorasan' loaf had the highest amount of antioxidants (0.89 mg AAE/g), followed by 'Einkorn' (0.71 mg AAE/g), and finally, loaf made from common wheat (0.47 mg AAE/g), these are significant differences in each case.

Loafs enriched with 1%, 3%, and 5% rosemary showed a significant increase in antioxidant content observed at all doses. When 1% rosemary was added, Khorasan loaf showed the greatest antioxidant increase at 2.13 mg AAE/g, the second-highest value was (1.49 mg AAE/g) detected in the case of Einkorn loaf, and the last one was common wheat loaf with the lowest antioxidant content (0.94 mg AAE/g). When 3% rosemary was added, the highest value was detected in the Einkorn loaf with 5.71 mg AAE/g value, followed by the Khorasan loaf with 4.94, and finally the common wheat loaf with 3.91 mg AAE/g antioxidant content. For 5% rosemary,

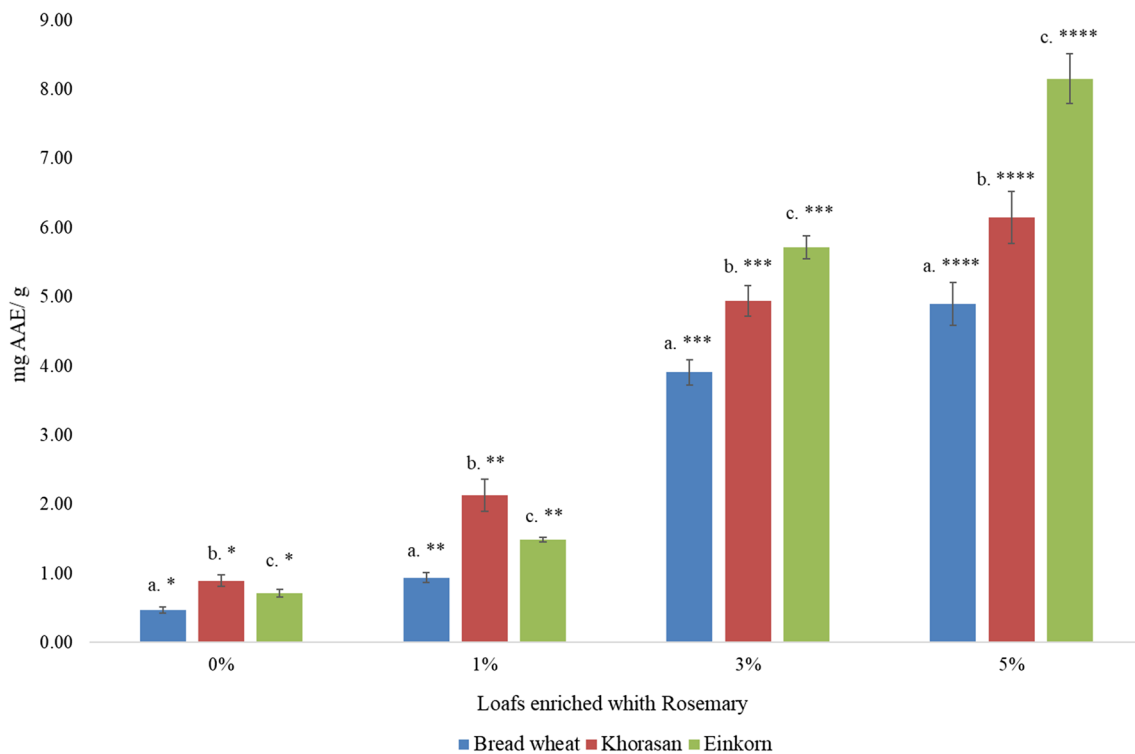


Fig. 1 Total antioxidant content of tested loaves depending on the Rosemary addition. Different letters (a, b, c, d) denote significant differences between the wheat species ($p \leq 0.05$). Different numbers

of stars (*, **, ***) denote significant differences in the degree of enrichment of each wheat species ($p \leq 0.05$)

the order of antioxidant content of wheat is the same for 3%, Einkorn (8.15 mg AAE/g), Khorasan (6.15 mg AAE/g), and common wheat (4.90 mg AAE/g).

Polyphenol content of loafs

Significant differences in the total polyphenol content in the case of investigated wheat species, and the addition of rosemary supplementation were observed (Fig. 2). The results of the total polyphenol contents were obtained by the Folin–Ciocalteu assay.

Similar to the antioxidant contents, Khorasan was the most prominent in the polyphenol content with 0.68 mg GAE/g, followed by the Einkorn loaf with 0.51 mg GAE/g and the common wheat loaf with 0.41 mg GAE/g polyphenol.

After the addition of different amounts of rosemary, not all wheat varieties showed a significant difference in polyphenol content. The addition of 1% rosemary resulted in a significant increase in polyphenol contents for all cultivars compared to control loafs, but there were no significant differences between 1% rosemary common wheat loaves (0.58 mg GAE/g) and Einkorn loaves (0.63 mg GAE/g). In contrast, Khorasan wheat loaf showed a significant increase (1.24 mg GAE/g) compared to both control and

1% rosemary added loafs. For loaves containing 3% rosemary, common wheat loaves (2.08 mg GAE/g) and Khorasan loaves (2.27 mg GAE/g) had outstanding polyphenol content. Einkorn loaf contained significantly fewer phenolic compounds (1.62 mg GAE/g). The addition of 5% rosemary for ancient wheat flours resulted in a significant increase in polyphenol content compared to loafs containing 3% rosemary. In the case of common wheat, the addition of 5% rosemary did not result in a significant increase in polyphenols. In this case, Khorasan wheat loaf had a remarkably high polyphenol content (3.19 mg GAE/g), while Einkorn (2.16 mg GAE/g) and common wheat loaf (2.28 mg GAE/g) containing 5% rosemary did not show significant differences.

Discussion

Fogarasi et al. (2015) examined the antioxidant content of common wheat, einkorn, and barley, they also concluded that the antioxidant content of common wheat was the lowest.

The antioxidant content for each loaf variety was successfully increased with the added herb. Numerous scientific publications report that the antioxidant content of bakery products can be increased with plant additives (Dziki et al.

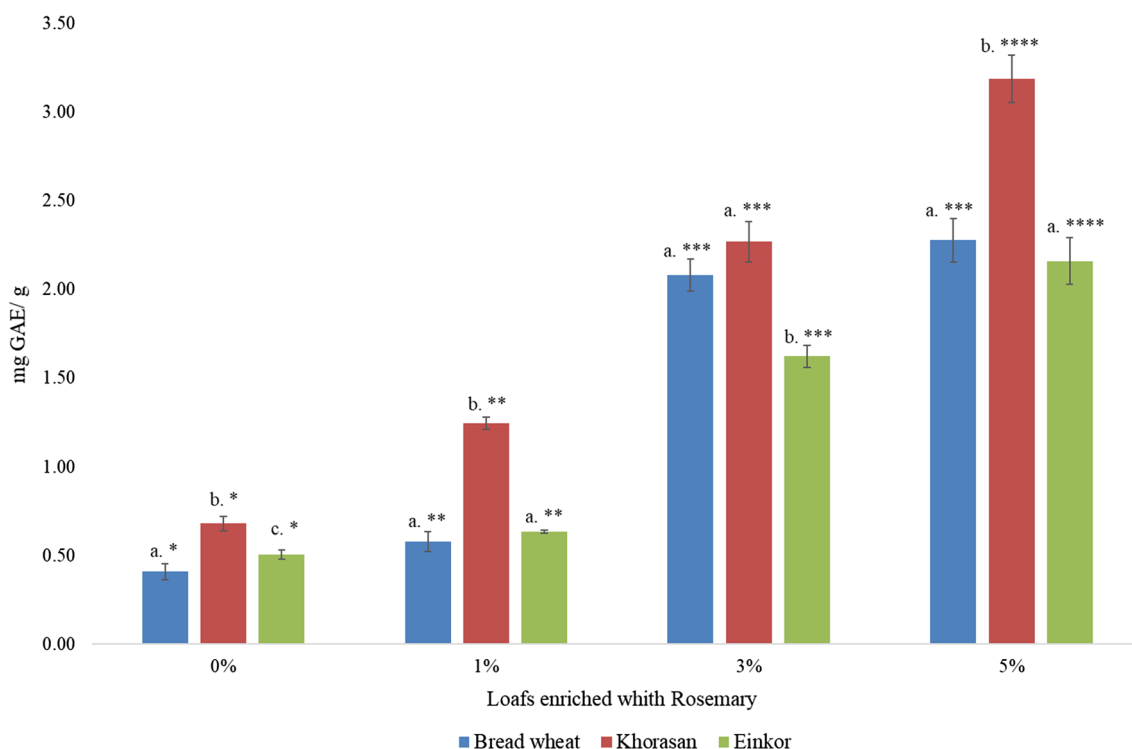


Fig. 2 Total polyphenol content of tested loaves depending on the Rosemary addition. Different letters (a, b, c, d) denote significant differences between the wheat species ($p \leq 0.05$). Different numbers

of stars (*, **, ***) denote significant differences in the degree of enrichment of each wheat species ($p \leq 0.05$)

2014), such as turmeric (Lim et al. 2011), fennel seed (Das et al. 2013), nettle (Maietti et al. 2021), or grape seed (Kapsándi et al. 2021).

Barański et al. (2020) also examined different ancient grains and found that the type of wheat was significantly influenced by the amount of phenolic compounds. Zrcková et al. (2019) determined the polyphenol content of einkorn, emmer, spelled, and common wheat grain, it was found that common wheat contains the least phenolic compound. Rosemary enrichment of loafs resulted in a further increase in antioxidant and polyphenol content for all types of grain loafs. There are no studies that enrich bakery products made from ancient grains with the addition of rosemary; however, several successful attempts have been made to increase the bakery product's polyphenol content with ginger powder (Özcan 2022), quinoa flour (Gil et al. 2022), *Cistus incanus* L. (Cacak-Pietrzak et al. 2019), plant extracts (Czubaszek et al. 2021) or fruit and tea extracts (Sik et al. 2022).

Based on the results, the antioxidant content of the studied wheat cultivars ranged from 0.47 to 0.89 mg AAE/g (Khorasan > Einkorn > common wheat), just like their polyphenol content which was between 0.41 and 0.68 mg GAE/g (Khorasan > Einkorn > common wheat). In the case of Khorasan loafs control and 1% rosemary addition resulted in the highest total antioxidant content, but in 3 and 5% loafs Einkorn has the highest amount. The reason for this may be that there are compounds in Einkorn wheat that interact with its active ingredients when a larger amount of rosemary is added, thereby increasing the antioxidant content. An individual analysis of each component could provide an answer to this phenomenon. As the results show, ancient grains have better antioxidant and polyphenol sources than common wheat and the plant variety affects the amount of these compounds. The added rosemary increased the antioxidant and polyphenol content of the products in all cases; however, our own organoleptic studies show that the 1% dosage produced favorable properties, and the 3% rosemary addition already divisive results. The addition of 5% rosemary resulted in an unfavorable, bitter taste.

Author contributions The authors confirm contribution to the paper as follows: study conception and design: EL; data collection: BS; analysis and interpretation of results: AM; draft manuscript preparation: RS. All authors reviewed the results and approved the final version of the manuscript.

Funding Open access funding provided by Széchenyi István University (SZE). The authors have not disclosed any funding.

Data availability Authors can confirm that all relevant data are included in the article and/or its supplementary information files.

Declarations

Conflict of interest All authors certify that they have participated sufficiently in the work to take public responsibility for the content,

including participation in the concept, design, analysis, writing, or revision of the manuscript. Furthermore, each author certifies that this material or similar material has not been and will not be submitted to or published in any other publication before its appearance in Cereal Research Communications.

Consent to participate Not applicable.

Consent for publication Not applicable.

Ethical approval Not applicable.

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