



Folate Analysis in Ancient Wheat Variety: A Comprehensive Review of Factors Impacting Nutritional Content and Dietary Implications

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Abstract: This comprehensive review explores the nutritional significance of ancient wheat varieties, including spelt, einkorn, emmer, and Khorasan wheat, focusing on their folate content. Wheat, a fundamental cereal grain, plays a crucial role in global diets. Despite claims about the high folic acid content in ancient wheat, there is limited research to substantiate these assertions. The review systematically compares folate levels in ancient wheat varieties, highlighting the scarcity of data in this domain. Factors influencing folate content, such as geographic and genetic variations, processing, milling, fermentation, germination, and storage, are extensively examined. The study emphasizes the potential health benefits of consuming folate-enriched ancient wheat, addressing deficiencies and offering practical strategies like crop fortification. Additionally, it discusses dietary recommendations for folate intake in India. The methodology section details challenges in folate quantification, utilizing microbiological assay and high-performance liquid chromatography. In conclusion, this review calls for a renewed focus on the exploration and validation of the nutritional profile of ancient wheat varieties, emphasizing their potential to contribute significantly to improved dietary folate intake and public health.

Key words: Ancient wheat, Folate, HPLC, Implication.

Wheat stands as one of humanity's earliest cultivated food crops, assuming a pivotal position as a dietary staple in diverse cultures around the world, each with its own characteristic wheat-based culinary traditions. Wheat holds a prominent position within the family of cereal grains, serving as a primary source of carbohydrate-rich diets that reinforce the foundations of nutrition in both ancient and modern eras across the globe (Jones *et al.*, 2015). Moreover, beyond its fundamental role as a significant source of dietary carbohydrates worldwide, wheat distinguishes itself by its capacity to yield flour that can form cohesive dough. As a result, wheat flour serves as the basis for an extensive array of leavened bread varieties and an even broader spectrum of food products, including noodles, soups, biscuits, and pasta (Morris, 2016). In the marketing year 2022-2023, global wheat production increased to exceed 781 million metric tons, representing a notable rise compared to the previous marketing season (Anonymous, 2023).

Wheat-based food items serve as essential dietary staples for a significant portion of the global population, spanning more than 100 countries (Shewry, 2018). As increasing scientific evidence emphasize the pivotal role of diet in mitigating the risk of chronic ailments, consumers exhibit a preference for grains possessing elevated levels of dietary fiber (DF) and antioxidants, as well as a lower glycemic index (GI). Within the diverse array of wheat species, ancient wheat varieties are regarded as nutritious grains. A notable example is organic khorasan wheat products, which have demonstrated a positive influence on blood insulin, glucose levels, low-density lipoprotein (LDL) cholesterol, and the prevention of vascular diseases. (Jirillo *et al.*, 2017). The term “ancient wheat” lacks a precise and universally agreed-upon definition. It generally refers to wheat species that have remained unchanged for more than a century (Dinu *et al.*, 2018).

While several studies have explored the contents and compositions of bioactive components in ancient wheat species (Shewry *et al.*, 2015), with a particular focus on spelt (Ratajczak *et al.*, 2020), erikson (Hidalgo *et al.*, 2012), emmer and khorsan (Bordoni *et al.*, 2017), there is a noticeable scarcity of comparisons between these ancient varieties and modern bread and durum wheat. This scarcity is particularly pronounced in the context of their folic acid content. Notably, despite prevalent claims in media and marketing suggesting the richness of folic acid in these ancient wheat varieties limited definitive research has been conducted to substantiate such assertions (<https://www.paigambari.com/>).

The primary purpose of this comprehensive review is to systematically assess and compare the folate content in ancient wheat varieties. In doing so, it aims to provide a comprehensive understanding of the nutritional composition of these ancient wheat grains, shedding light on their potential implications for human health and nutrition. This review serves to synthesize existing scientific knowledge and offer insights into the dietary considerations and historical significance of ancient wheat varieties, while also identifying avenues for further research in this domain.

Ancient Wheat Varieties

In the early stages of human civilization, ancient cereals played a significant role as a primary source of food in the human diet. However, their consumption by humans has significantly declined over the centuries. This decline can be attributed to the emergence of domesticated cereal species that offer higher yields and improved technological quality, mainly due to the presence of gluten proteins (Tran *et al.*, 2020). To be precise, the term “ancient cereals” refers to those primitive *Triticum* species that have not undergone modern selection or breeding processes. These ancient cereals have retained certain characteristics inherited from their wild ancestors, such as a low harvest index, significant individual variability, brittle rachis, and ear height (Giambanelli *et al.*, 2013).

The category of ancient wheat grains encompasses varieties such as spelt (*Triticum aestivum* L. *spelta*), einkorn (*Triticum monococcum* L. *monococcum*), emmer (*Triticum turgidum* L. *dicoccum*), and Khorsan wheat (*Triticum turgidum* L. *turanicum*) (see Fig. 1) (Matsuoka *et*



Fig. 1. Ancient cereal grains, where: (A) einkorn, (B) emmer, (C) spelt, (D) durum, (E) Hard Red Spring wheat and (F) barley. Photograph from Fujita *et al.* (2020) with permission.

al., 2011). These are often referred to as “hulled wheat species” because their glumes are firmly attached to the grain and cannot be separated during threshing. Thus, an additional step is required to remove the hulls (Fujita *et al.*, 2020).

Folate (Vitamin B9) overview: Folate, specifically tetrahydrofolate (H4F), and its glutamated derivatives belong to the category of water-soluble B-group vitamins. They serve a crucial role in several bodily functions, including cell proliferation, gene activity regulation, the production of red and white blood cells, skin and intestinal health, and the synthesis of specific chemicals that govern brain functions (Gazzali *et al.*, 2016). Folate is obtainable through both natural sources and synthetic means. Folic acid, the synthetic form of folates, is utilized in food fortification. Foliates encompass the different tetrahydrofolate derivatives naturally occurring in food. (Boz, 2021)

Vitamin B9, commonly known as folate, holds significant importance in supporting the regular functions of the human body. It actively participates in a range of metabolic and biochemical processes (Obeid, 2013). This compound is notably involved in the synthesis of purines and pyrimidines, the metabolism of homocysteine, as well as in DNA replication and methylation. Moreover, it plays a vital role in ensuring the normal development of tissues. (Ducker and Rabinowitz, 2017)

Inadequate levels of folate in the human body can lead to a range of health complications, including conditions such as cancer in newborns, cardiovascular diseases, growth impairments, megaloblastic anemia, congenital abnormalities, and neural tube defects, (Kanmani *et al.*, 2013).

Folate in Ancient Wheat Varieties

Folate in various ancient wheats: Folate within wheat encompasses various vitamers, and their bioavailabilities are likely similar, but differences in their stability can impact their ability to facilitate and contribute to vitamin activity (Ohrvik *et al.*, 2011). The majority of folate in cereal grains predominantly appears as formyl and methyl derivatives (Konings *et al.*, 2001). Folate content is not uniform within the grain, with higher concentrations typically present in the outer layers of the kernel and the germ. For example, aleurone flour has been

documented to contain 5150 ng g⁻¹ of folate (Fenech *et al.*, 1999), and wheat bran had more than double the folate content (1600 ng g⁻¹ of dry matter) in comparison to the grain. Different wheat bran types from various classes were reported to exhibit elevated folate levels, ranging from 1820 to 4140 ng g⁻¹ of dry matter (Arcot *et al.*, 2002).

To analyze the folate vitamer distribution using high-performance liquid chromatography (HPLC), the process involved the use of hog kidney conjugase to enzymatically break down folate polyglutamates into folate monoglutamates. Due to the limited number of genotypes studied, consisting of only five each for spelt, diploid einkorn, and tetraploid emmer, the current studies offer only preliminary insights into these cereals as sources of folate. The average total folate content for both spelt and diploid wheat genotypes was approximately 577 ng g⁻¹ of dry matter, closely resembling the mean values for winter and spring wheat. A slightly higher mean folate content of 694 ng g⁻¹ of dry matter was observed in tetraploid wheats. The ranges of folate content were notably broad, particularly for diploid and tetraploid wheats. Among the studied genotypes, the highest folate content, measuring 975 ng g⁻¹ of dry matter, was identified in one of the tetraploid variants. To date, there is a dearth of published data on folate content in these early cultivated cereals. The substantial variation observed in this limited sample set implies the necessity for further exploration of folate content in ancient diploid and tetraploid varieties (Kariluoto *et al.*, 2010).

Geographic and Genetic Variations

The total folate content varies more significantly among the growing locations than it does among the harvesting years. Environmental variability plays a crucial role in determining folate content in wheat. A study involving 24 winter wheat and 2 spring wheat genotypes grown in Martonvásár, Hungary, for three years and at four locations across Europe (Hungary, France, the United Kingdom, and Poland) demonstrated significant differences in folate content due to harvesting years and growing locations. Hungarian-grown grains exhibited the highest average folate content, while those from Poland had the lowest.

The total folate content showed a 2.8-fold variation, ranging from 323 ng g⁻¹ of dry matter in Chinese Spring wheat grown in Hungary in 2005 to 889 ng g⁻¹ in Riband wheat grown in Hungary in 2007. The variation was more pronounced among different locations than across harvesting years, highlighting that environmental factors exert a stronger influence on folate accumulation than genetic differences. The dominant vitamer in wheat genotypes is presently 5-Hydroxy-H4-folate, with other formylated folates and 5,10-CH₂-H4-folate also existing in substantial quantities. Variations in the proportions of 5-Hydroxy-H4-folate and 5-Methyltetrahydrofolate (are mainly responsible for the differences in total folate content. Samples with high total folate content are currently characterized by a high proportion of 5-CH₃-H4folate and a low proportion of 5-Formyltetrahydrofolate (Kariluoto *et al.*, 2010). It was also shown that the cereal genotypes were found to be good sources of methyl donors, but there was significant variability in folate concentrations among different cereal types (Hefni *et al.*, 2018).

Studies reveal significant folate content variation among wheat accessions, with genotype identified as the primary factor. Total folate levels correlate with four key derivatives: tetrahydrofolate (THF), 5-methyl-tetrahydrofolate (5-CH₃-THF), 5-formyl-tetrahydrofolate (5-CHO-THF), and 5,10-methenyl-tetrahydrofolate (5,10-CH+THF). Among these, 5-CH₃-THF and 5-CHO-THF are the most abundant and strongly influence total folate content. (Zheng *et al.*, 2022).

Processing and Milling Variations

Effect of milling on folate content: Refined flour, as is commonly understood, is the product of milling cereal grains after separating their outer layers. In the food industry, approximately 70% of extracted flours are typically utilized. It's worth noting that folates are not uniformly distributed across the various grain fractions. When considering grains, it's established that the outer layers of the grain tend to contain higher concentrations of folate, much like many other essential nutritional components (Fenech *et al.*, 1999), it was reported that folate levels were in the range of 94-100 µg 100 g⁻¹ in wheat bran flour and 515 µg 100 g⁻¹ in aleurone flour (Arcot *et al.*, 2002).

Studies have indicated that the highest folate content is found in the germ and outer layers of grains, and during the milling process of barley and wheat, there is a significant reduction in folate content, typically ranging from 63% to 86%, (Giordano *et al.*, 2016). In a research endeavor involving the production of flour with 70% extraction from six distinct wheat varieties, an average folate loss of approximately 71% was observed in these wheat varieties due to the milling process, (Liang *et al.*, 2020).

Effect of fermentation on folate content: The process of fermentation, an ancient method for preserving food and enhancing its health and nutritional value, involves the production of numerous beneficial and antimicrobial substances in food through the actions of microorganisms (Nout, 2009). This practice is recognized for its ability to boost the folate content of food products, as both yeast and lactic acid bacteria have the capacity to synthesize folate (Moslehi-Jenabian *et al.*, 2010; Saubade *et al.*, 2017; Tamene *et al.*, 2019; Bationo *et al.*, 2020). Previous research has demonstrated the isolation of folate-producing strains from various grains (Salvucci *et al.*, 2016). Moreover, studies focusing on fermented grain-based foods, particularly those utilizing rye, wheat, and oat sourdough, have revealed that fermentation can contribute to an enhancement in folate content (Kariluoto *et al.*, 2006; Laino *et al.*, 2013). Arcot *et al.* (2002) reported a notable 68% increase in the overall folate content during dough fermentation.

Effect of germination in folate content: An alternative approach to increasing the folate content in cereal products is through biological processing methods such as germination and fermentation, as highlighted by various studies (Kariluoto *et al.*, 2006; Katina *et al.*, 2007; Hefni and Witthoft, 2012). Germination, in particular, has been shown to be an effective method for enhancing folate levels in cereals like barley (Walker *et al.*, 2002). For instance, when wheat and rye grains were subjected to 96 hours of germination at 25°C, their folate content increased from 45% to 75% in both cases. Furthermore, it has been found that subsequent drying at 50°C for 48-72 hours after germination does not result in folate loss (Koehler *et al.*, 2007). As a result, the germination process has been suggested as a means to boost the folate

content in bakery products (Hefni and Witthoft, 2012).

Effect of storage on the content of folate content: The study revealed that folate degradation in wheat grains started after the fourth month of storage. On average, folate loss was measured at 17% by the sixth month and increased to 26% by the eighth month of storage. In comparison, when whole flours made from the same wheat varieties were stored at room temperature, folate loss was more significant, reaching 21% by the fourth month, 25% by the sixth month, and a substantial 65-73% by the eighth month (Liang *et al.*, 2020). These findings suggest that folate degradation occurs at a lower rate when wheat is stored as whole grains compared to when it is stored as whole wheat flour.

Nutritional Implications

Health benefits of consuming ancient wheat rich in folates: To address folate deficiency, common strategies include supplementing with folic acid via pills and processed foods, as well as obtaining natural folate from a diverse range of dietary sources. However, it's worth noting that excessive consumption of synthetic folic acid has been associated with alterations in DNA methylation levels, which have been linked to an increased risk of colorectal and prostate cancers in men, as well as cognitive impairment in individuals with vitamin B12 deficiency (Crider *et al.*, 2012 and Rakszegi *et al.*, 2008). Unfortunately, many people around the world face economic limitations, dietary restrictions, or the absence of stable social policies and sustained financial support, making it challenging for them to consume a variety of foods beyond basic staples (Botto *et al.*, 2005).

Hence, the fortification of staple crops with folate emerges as a practical and cost-effective strategy to combat folate deficiency on a global scale. Wheat, being one of the most extensively cultivated and vital food crops worldwide, holds significant potential in this regard. Moreover, whole-grain flours are recommended by dietary guidelines due to their rich nutrient content in the seed coat and germ (Paesani *et al.*, 2021). Cereal-based products are commonly recognized as significant contributors to dietary folate intake. The folate content in wheat-based products is contingent on both

the folate levels present in the original grains and the process of separating and selecting the grain fractions used in the final product. By promoting folate-enriched wheat varieties, we can directly enhance folate intake through daily diets, thereby contributing to the well-being of individuals, particularly in economically disadvantaged regions. (Ashokkumar *et al.*, 2020).

Dietary recommendations of folate for Indians: Recent data from India, encompassing dietary intake alongside plasma folate and homocysteine levels as functional markers, has helped establish the folate requirements for the population. With the focus on maintaining plasma folate levels exceeding 10 nmol L⁻¹, the Estimated Average Requirement (EAR) was determined. Based on this analysis, the Recommended Dietary Allowance (RDA) was set at 300 µg for adult men and 220 µg for adult women. To cater to the increased demands during pregnancy and lactation, an additional 300 µg day⁻¹ and 100 µg day⁻¹, respectively, were included to fulfill the factorial extra needs (Anonymous, 2020).

Methodology for Analysis of Folates in Cereals

Assessing the folate content in cereals presents a challenging task due to the relatively low concentrations of folate, and the likelihood of folate being physically trapped within the cereal matrix. Folate compounds are sensitive to environmental factors such as heat, light, and oxygen. Consequently, the crucial stages in the folate quantification process might occur prior to the actual measurement, specifically during steps like sampling, extraction, and purification. (Zheng *et al.*, 2022).

The assessment of the overall folate content in wheat grains was carried out through two distinct techniques, specifically microbiological assay (MA) and high-performance liquid chromatography (HPLC) (Upadhyaya *et al.*, 2017). Microbiological assay (MA) offers a straightforward and cost-effective approach for determining total folate levels. In contrast, high-performance liquid chromatography (HPLC) is a more precise method that has been employed for folate content quantification but comes with the drawbacks of being time-consuming and expensive (Riaz *et al.*, 2019).

Conclusion

Ancient wheat varieties, such as spelt, einkorn, emmer, and Khorasan wheat, have shown promise as sources of folate, a vital B-group vitamin with significant implications for human health. While these grains have not undergone modern breeding processes, their folate content can vary depending on factors like geography, genetics, processing, and storage. Fermentation and germination processes have been shown to enhance folate levels in wheat-based products. The fortification of staple crops with folate, particularly in economically disadvantaged regions, holds potential to combat folate deficiency. Dietary recommendations for folate intake in India emphasize its importance for overall health. Analyzing folate content in cereals is a challenging task due to the sensitivity of folate compounds to environmental factors. Microbiological assay and high-performance liquid chromatography are two commonly used methods for folate quantification. Overall, this review provides valuable insights into the nutritional value of ancient wheat varieties and highlights the need for further research in this area to optimize their potential health benefits.

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