

Review

Ancient Wheat Species: Biochemical Profile and Impact on Sourdough Bread Characteristics—A Review

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Abstract: In recent years, the attention of farmers, bakers and consumers towards ancient wheat species has been increasing. Low demands of pedo-climatic growth factors, the suitability for organic cultivation along with their high nutritional quality and their content in pro-health compounds make them extremely attractive for bakers and modern consumers, equally. On the other hand, in recent years, sourdough has gained attention due to its ability to produce new functionally active molecules with higher bioaccessibility and thus to produce bread with enhanced nutritional quality. This paper highlights the relevant nutritional profile of einkorn, spelt, emmer and Khorasan which could lead to bread with improved textural, sensorial, microbial and nutritional characteristics through sourdough fermentation. The ancient wheat species could be used as promising substitutes for common wheat flour for the design of innovative types of bread, even for special needs.

Keywords: einkorn; spelt; emmer; Khorasan; lactobacili; bread quality



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1. Introduction

Bread is one of the products that has always been essential for human nutrition. Initially manufactured in households, it evolved and became industrialized after the microorganisms responsible for dough fermentation were identified [1]. Nowadays, due to the increased demands from consumers for traditional, healthy and unique products, the production of bread with sourdough has increased considerably in the most recent period of time [2]. Sourdough consists of a mixture of water and flour fermented by spontaneous or added microorganisms. These three major components are the basis of the process of obtaining sourdough fermented bakery products [3]. There is a large diversity of lactic acid bacteria (LAB) and yeasts capable of fermenting flour obtained from cereals and pseudocereals [4–7]. The chemical reactions produced by these microorganisms during fermentation have an important effect on the rise of the dough and on the formation of the flavor compounds in the final product [8]. Sourdough is also used to obtain some attributes such as the extension of the shelf life, increasing the content of bioactive compounds and improving the nutritional and sensory profile of the product [9].

With respect to the raw materials, in recent years, certain ancient cereals (spelt, einkorn, emmer, Khorasan) and some soft cultivars such as *Verna*, *Andriolo*, *Gentilrosso* have become more popular among the consumers due to their superior chemical composition compared to that of modern wheat varieties [10–13] and among the farmers as a result of the increased adaptability and resistance to pedo-climatic conditions [14–16]. A number of recent studies are investigating possible starter cultures that could ferment the sourdough obtained with ancient cereals [17–19]; thus, by evaluating and characterizing these LAB strains, it is possible to improve the nutritional and technological properties of bakery products based on ancient grains [20].

This review focused on the rheological, nutritional and functional characteristics of sourdough bread obtained with flour of some ancient wheat species such as einkorn, spelt, emmer and Khorasan. Also, these ancient species are characterized in order to make a clear demarcation between their abilities and technological properties compared to those of modern varieties.

2. Ancient Wheat Species General Characteristics and Chemical Composition

At the beginning of civilization, ancient cereals represented an important source of food in the human diet. However, over the centuries their human consumption has decreased dramatically due to the appearance of domesticated species that present a higher yield and a superior technological quality given by gluten proteins [21,22]. Literally, the term ancient cereals refers to those primitive species of *Triticum* which have not undergone any modern process of selection or breeding and which have preserved certain characteristics from their wild ancestors, such as a low harvest index, individual variability, brittle rachis and ear height [23].

The ancient wheat grains category includes species such as spelt (*Triticum aestivum* L. subsp. *spelta*), einkorn (*Triticum monococcum* L. subsp. *monococcum*), emmer (*Triticum turgidum* L. subsp. *dicoccum*) and Khorasan wheat (*Triticum turgidum* L. subsp. *turanicum*) (Figure 1) [24]. These are also called hulled wheat species due to the fact that their glumes are well fixed on the grain and cannot be removed even when threshing, requiring a separate operation [25]. From the group of modern species, the most used in the bakery industry are common wheat (*Triticum aestivum* L.) and durum wheat (*Triticum turgidum* subsp. *durum*), and according to studies, their flour produces dough with superior rheological properties (elasticity, extensibility, viscosity) compared to flour of ancient species that has a lower gluten quality [26].



Figure 1. Ancient cereal grains, where: (A) einkorn, (B) emmer, (C) spelt, (D) durum, (E) Hard Red Spring wheat and (F) barley. Taken from Fujita et al. [25]. Copyright 2020 Creative Common Attribution License.

The grain of all cereals consists of three main parts: bran, endosperm and germ. The endosperm is the major component and is composed of two types of starch granules, which differ in terms of their dimension and shape [27]. Figure 2 reveals the starch granules from

the endosperm of different species of ancient wheat, as well as the degree to which this is embedded into the protein matrix [27]. Ancient wheat species show differences between the embedding of starch granules and protein matrix. On a scale from a slight enclosure to a firm one, spelt wheat showed the least firm embedding, followed by einkorn and emmer.

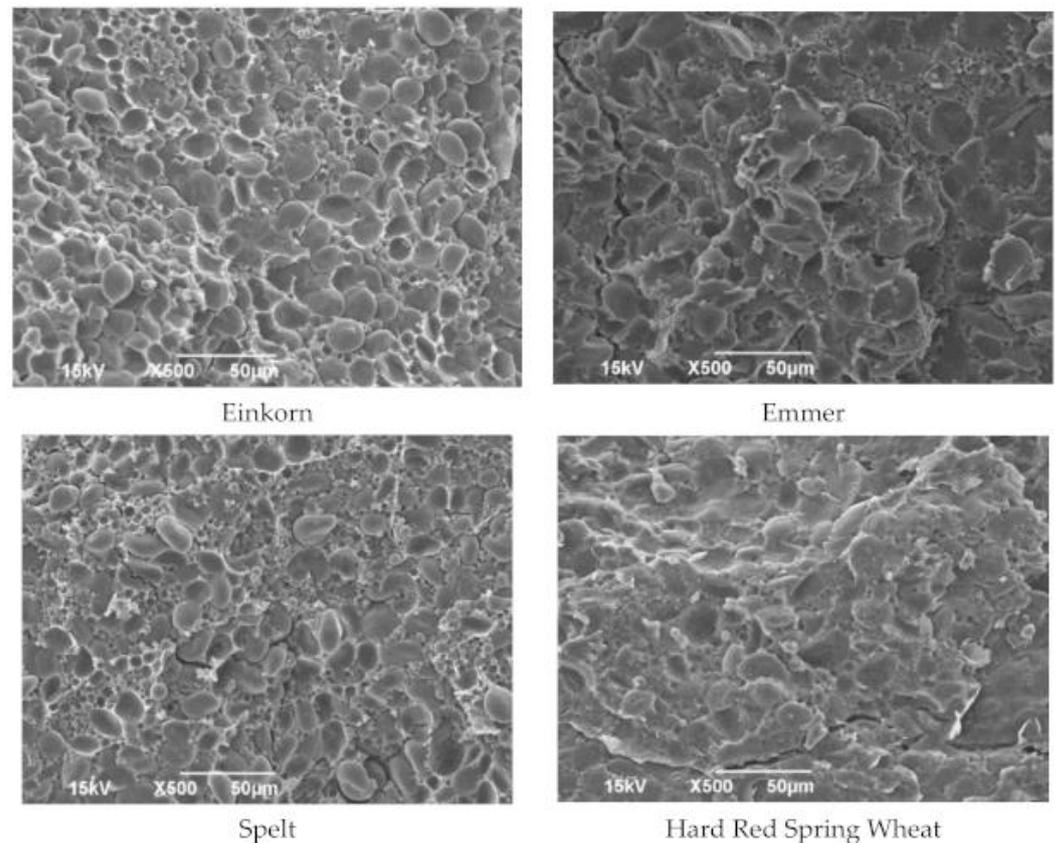


Figure 2. SEM (Scanning Electron Microscopy) images of the endosperm of different ancient wheat revealing the structure of starch granules and protein matrix. Taken from Kulathunga et al. [27]. Copyright 2020 Creative Common Attribution License.

Moreover, recent findings [27] reported that the combination between the pericarp thickness, incomplete protein matrix and the presence of air pockets in the aleurone cells of einkorn is responsible for the kernel softness. It is now well known that small starch granules and thinner cell walls confer more accessibility for amylase compared to large granules and thick cell walls. Additionally, the percentage of small starch granules significantly influences the flour water absorption capacity, the peak viscosity, the final viscosity and, overall, the breadmaking quality [28].

In recent years, the attention of farmers, bakers and consumers to these cereal species has been renewed. This is due to the low demands that these grains have on the climate conditions, soil and water, as well as due to high resistance to pests, diseases, pollution and salinity [15,29]. All these factors are important because they make ancient cereals a suitable crop for organic farming due to the non-use of chemicals, and at the same time may be able to help climate adaptability and increase biodiversity [30]. On the other hand, artisanal bakers are on the search for exclusive products that cannot be found in commercial bakeries and, for this reason, it is important for them to know certain specifications about ancient cereals such as nutritional benefits, quality parameters and agronomic yield [31]. As for consumers, they are looking for innovative, natural and healthy products. Studies have shown that ancient cereals tend to have a high potential in terms of nutrient availability and their beneficial effects on human health [32–35].

However, the popularity of the ancient grains has increased considerably due to their complex nutritional composition, being a major source of proteins, lipids (mainly unsaturated fatty acids), soluble fibers, minerals, vitamins and bioactive compounds with antioxidant activity [36–39].

Until now it has not been possible to establish the exact nutritional value and health benefits of ancient cereals compared to those of modern varieties, and further studies are needed to make a clear delimitation and to establish with certainty the superiority of ancient species [13,16]. However, based on their composition in bioactive compounds, some studies are reporting only little differences compared to modern wheat varieties [11,13], the health benefits of ancient wheat are reported, mainly due to the antioxidant activity of some compounds naturally present or formed during baking such as polyphenols and melanoidins [13,32].

As in the case of modern cereals, the main constituents of ancient cereals are proteins, carbohydrates and lipids. According to the study of Arzani and Ashraf [29], the highest protein content is found in einkorn, followed by spelt, emmer and, lastly, common wheat. On the other hand, the carbohydrate content of ancient cereals is quite similar, with einkorn wheat having the smallest content and common wheat the highest [38]. Lipids are found in the smallest amounts in cereals. However, the ancient wheat species have slightly higher content of lipids than common wheat [27]. Additionally, Hidalgo and Brandolini [40] reported in einkorn a higher level of monounsaturated fatty acids (MUFA) and a lower level of polyunsaturated (PUFA) and saturated fatty acids (SFA) than in common wheat, as could be seen in Table 1. From the point of view of the dietary fiber content of ancient wheat species, emmer wholemeal flour and einkorn wholemeal flour present values well below those offered by spelt, Kamut[®]Khorasan, durum and common wheat [29,38,41]. The mineral content of ancient and modern wheat is very variable being influenced by two major factors: environment and genetics [33]. According to Geisslitz and Scherf [42], emmer and einkorn flour are distinguished by a higher zinc content compared with wheat flour. Furthermore, einkorn and spelt contain a larger amount of iron than emmer grain [43,44]. A significant similarity in terms of the level of iron, magnesium, calcium, potassium and zinc exists between wholemeal Kamut[®]Khorasan flour and whole wheat flour [38].

Table 1. Comparative composition of certain chemical constituents of einkorn and common wheat.

Compound	Einkorn	Ref. ¹	Common Wheat	Ref.
Protein (%)	15.5–22.8	[29]	12.9–19.9	[29]
Carbohydrate (%)	67	[38]	75	[38]
Crude fat (%)	2.3	[27]	1.5	[45]
Monounsaturated fatty acids (MUFA) (%)	27.8	[40]	18.2	[40]
Polyunsaturated fatty acids (PUFA) (%)	52.8	[40]	57.2	[40]
Saturated fatty acids (SFA) (%)	19.4	[40]	24.5	[40]
Dietary fiber (%)	6.7–9.8	[29,38,41]	12.7–13.4	[29,38]
Zinc (mg/100 g)	2.24–4.7	[42,44]	2.6	[42]
Iron (mg/100 g)	4.59–4.9	[43,44]	3.75	[43]

Ref. ¹ is an abbreviation for References.

A comprehensive chemical composition of the ancient cereals is presented in Table 2. All these results support and confirm the idea which claims that ancient cereals can add value to bakery products through their exceptional nutritional content. The health effects produced by introducing them into the diet deserve to be studied in detail in the future.

Table 2. Approximate chemical composition for ancient (emmer, einkorn, spelt, Khorasan) and modern (common and durum wheat) whole grains (expressed as g or mg/100 g dry weight).

Per 100 g	Emmer	Ref. ¹	Einkorn	Ref.	Spelt	Ref.	Kamut® Khorasan	Ref.	Common Wheat	Ref.	Durum Wheat	Ref.
Water (g)	9.4	[27]	9	[27]	10.4	[27]	11.07	[46]	10.42	[46]	10.94	[46]
Energy (kcal)	362	[46]	333	[46]	324	[46]	337	[46]	340	[46]	339	[46]
Protein (g)	12.77	[46]	13.33	[46]	14.71	[46]	14.54	[46]	10.69	[46]	13.68	[46]
Ash (g)	2.2	[27]	2.2	[27]	2.1	[27]	1.67	[46]	1.54	[46]	1.78	[46]
Total Lipid (g)	2.13	[46]	1.67	[46]	2.94	[46]	2.13	[46]	1.99	[46]	2.47	[46]
Saturated fatty acids (g)	n.d. ²	[46]	n.d.	[46]	n.d.	[46]	0.196	[46]	0.368	[46]	0.454	[46]
Monounsaturated fatty acids (g)	n.d.	[46]	n.d.	[46]	n.d.	[46]	0.213	[46]	0.227	[46]	0.344	[46]
Polyunsaturated fatty acids (g)	n.d.	[46]	n.d.	[46]	n.d.	[14]	0.621	[46]	0.837	[46]	0.978	[46]
Carbohydrate (g)	72.34	[46]	66.67	[46]	67.65	[46]	70.58	[46]	75.36	[46]	71.13	[46]
Fiber, total (g)	10.6	[46]	6.7	[46]	5.9	[46]	11.1	[46]	12.7	[46]	n.d.	[46]
Sugars (g)	n.d.	[46]	n.d.	[46]	2.94	[46]	7.84	[46]	0.41	[46]	n.d.	[46]
Calcium (mg)	36	[43]	42	[43]	17.6	[46]	22	[46]	38	[47]	34	[46]
Iron (mg)	3.41	[44]	3.6	[46]	3.18	[46]	3.77	[46]	3.86	[47]	4.5	[46]
Magnesium (mg)	128	[43]	200	[46]	150	[43]	130	[46]	138	[47]	144	[46]
Potassium (mg)	439	[43]	429	[43]	417	[43]	403	[46]	372	[47]	431	[46]
Phosphorus (mg)	512	[43]	520	[43]	470	[43]	364	[46]	352	[47]	508	[46]
Sodium (mg)	1.2	[43]	0.7	[43]	1	[43]	5	[46]	2	[46]	2	[46]
Zinc (mg)	5.4	[43]	5.3	[43]	4.7	[43]	3.68	[46]	3.46	[46]	4.16	[46]
Copper (mg)	0.41	[43]	0.4	[43]	0.5	[43]	0.506	[46]	0.426	[46]	0.553	[46]
Manganese (mg)	2.4	[43]	2.8	[43]	2.7	[43]	2.735	[46]	3.406	[46]	3.012	[46]
Selenium (µg)	54.3	[44]	n.d.	[46]	n.d.	[46]	81.5	[46]	33.4	[44]	89.4	[46]
Thiamin (mg)	0.38	[48]	0.34	[48]	0.30	[48]	0.566	[11]	0.41	[11]	0.419	[11]
Riboflavin (mg)	0.09	[48]	0.09	[48]	0.07	[48]	0.184	[11]	0.107	[11]	0.121	[11]
Niacin (mg)	8.511	[46]	5.7	[48]	6.6	[48]	6.375	[46]	4.766	[46]	6.738	[46]
Pantothenic acid (mg)	1.14	[48]	0.47	[48]	0.6	[48]	0.949	[46]	0.850	[46]	0.935	[46]
Vitamin B6 (mg)	0.39	[48]	0.4	[48]	0.36	[48]	0.259	[46]	0.378	[46]	0.419	[46]
Folate, total (µg)	69	[11]	58	[11]	58	[11]	n.d.	[46]	41	[46]	43	[46]
Vitamin A (µg)	n.d.	[46]	n.d.	[46]	n.d.	[46]	0.3	[46]	0	[46]	0	[46]
Total tocopherols (mg)	4.63	[11]	6.90	[11]	3.71	[11]	4.02	[49]	4.65	[11]	4.85	[11]
α-tocopherol (mg)	1.05	[11]	1.10	[11]	1.51	[11]	0.75	[49]	1.34	[11]	0.93	[11]
Total carotenoids (µg)	226	[11]	823	[11]	216	[11]	442	[49]	236	[11]	358	[11]
α-carotene + β-carotene (µg)	17.8	[11]	60.3	[11]	18	[11]	n.d.	[49]	10.1	[11]	10.7	[11]
Lutein + zeaxanthin (µg)	291.2	[11]	747.3	[11]	180.2	[11]	301	[46]	220	[46]	302.4	[11]
Vitamin K (µg)	n.d.	[46]	n.d.	[46]	n.d.	[46]	1.8	[46]	1.9	[46]	n.d.	[46]

Ref.¹ is an abbreviation for References and n.d.² for non-detectable.

Gluten and Starch Characteristics as Affecting the Baking Quality of Ancient Wheat Species

The main interest regarding the utilization of the ancient wheat flours in breadmaking is their technological performance. Thus, breadmaking with ancient wheat flours was studied from the perspective of the two key constituents, namely the gluten and the starch. Due to its superior chemical composition, spelt flour is more widely used in obtaining healthier bakery products compared to the flour of modern wheat species. Thus, the assortment of products that can be obtained from this ancient cereal is vast and includes foods such as bread, pasta, cookies and muffins. As spelt gluten is more extensible and less elastic than that of common wheat, certain processing techniques are required, such as reducing mixing times and water quantities, as well as extending the rest time of the dough [50]. The baking quality of spelt flour is obtained mainly from the gluten proteins, namely the ratio of gliadins (GLIA) and glutenins (GLUT). To obtain high-quality products, the flour of the cereals used in the recipe must have high glutenin content and a low ratio of GLIA/GLUT [51,52]. Geisslitz et al. [26] reported that although the amount of gluten and protein is higher in ancient wheat species (120.7 mg/g) than common wheat (96.1 mg/g), their glutenin level is lower. The most significant level of GLUT was found in spelt (19.0 mg/g) and common wheat (16.6 mg/g), and the poorest flours were those of durum wheat (16.0 mg/g), emmer (12.8 mg/g) and einkorn (10.3 mg/g) [26]. With respect to GLIA/GLUT ratio, an increase from 1.6–3.8 for common wheat flour to 2.8–4.0 for spelt, 2.2–5.3 for durum wheat, 3.6–6.7 for emmer and 4.2–12.0 for einkorn was reported [26]. These results show that although common wheat has the best breadmaking performance, ancient cereals can also be used successfully to obtain bakery products with good quality. Starch is another key constituent of flour that influences the dynamic properties of the dough, its gelatinization can cause significant changes in the quality of the bread [16,53]. Starch gelatinization, gelation and pasting properties are influenced by the ratio between its two constituents: amylose and amylopectin [54]. For ancient grains, a study performed by Brandolini et al. [55] established that einkorn dough has a significantly higher peak

(2426 cP), breakdown (765 cP) and final viscosity (2788 cP) compared to those of wheat dough, where cP is an abbreviation for centipoise. This fact can be explained by the lower level of amylose from einkorn (15–27%) compared to that of common wheat (26.6%), and also due to the small size of the flour's starch granules [55,56]. Regarding emmer wheat, the results show that its gelatinization process takes place at a higher temperature and lasts longer than in the case of common wheat flour, which leads to a starch paste with a viscosity up to 58% lower [57]. Wilson et al. [58] demonstrated in their study that spelt starch can contain from 2.1 up to 12% more amylose comparative with the hard red winter wheat control. In terms of gelatinization temperature, there are also differences; this being higher in spelt (87–93.2 °C) than in common wheat (84.6 °C) [36].

3. Ancient Wheat Species Health Benefits in Relation to Their Nutraceutical Composition

Recent studies have pointed out the health beneficial effects of ancient grains consumption in relation to their content in functional compounds such as carotenoids, tocopherols, flavonoids, isoflavones, lignans and fiber. Even if the amounts of these compounds are not significantly different from the modern cultivars, their diversity is one of the key aspects [59]. These benefits are most often associated with the nutritional content of ancient grains and with the ratio between the compounds. Thus, the whole grain of ancient wheat is distinguished by a great content of protein, lipid and bioactive compounds such as tocotrienols, phenols, tocopherols, dietary fibers, unsaturated fatty acids, vitamins and minerals [10,27,60,61]. Specific for cereals are compounds with antioxidant capacity, and the most representative are phenolic compounds. Generally, phenolic acids and flavonoids are found in larger quantities in cereals and their concentrations are influenced by the type, variety, and part of the cereal where they are located [62]. According to the study of Zrcková et al. [63], einkorn grains have the highest content of total polyphenols, 744.97 mg/kg dry matter (DM), and is followed at a considerable distance by spelt (694.99 mg/kg DM), emmer (705.28 mg/kg DM) and common wheat (702.15 mg/kg DM). In terms of flavonoids, einkorn is the ancient species with the highest content, it can have up to 3.8 times more bound flavonoids than emmer wheat [15]. With a lower antioxidant capacity than phenolic compounds, in cereals present are also vitamin E, carotenoids, tocopherols and tocotrienols [64]. According to Shewry and Hey [11], einkorn contains the higher content of tocopherols, with values ranging from 19.6 to 109.89 µg/g, followed by spelt at 8.9–69.18 µg/g, emmer at 19.7–69.85 µg/g and common wheat at 23.3–79.7 µg/g. As a confirmation of these data, the results of other studies have shown that the content of tocopherols, out of dry matter (DM), in pasta is more abundant in those made with einkorn flour (20.4 mg/kg DM) than in those with durum (8.2 mg/kg DM) and common wheat flour (5.3 mg/kg DM) [16]. From a genetic perspective, Khorasan wheat is very similar to modern durum wheat. What differentiates it from the rest of modern cereal species, is its nutraceutical value given by the high content in phytochemicals. It was reported that bread obtained from this ancient species contains ten times more selenium than that made from durum wheat [34]. The compatibility of the use of emmer flour in bakery and pastry products has been also confirmed. Medical data also back up its nutritional importance. For these reasons, it is considered to be a safe alternative for people with diabetes, with emmer having a low glycemic index compared to other cereals [65].

All these nutrients are vital for the proper activity of the human body and for preventing the occurrence of diseases [66]. Moreover, ancient cereals can be used in daily diets as an appropriate source of macro- and micronutrients since studies are reporting their capacity to deliver bioactive compounds [67–69].

One of the most relevant conclusions of several studies is that whole grains may be able to reduce the risk of type 2 diabetes [33]. Relatedly, the study performed by Thourup et al. [70] on rats showed that by feeding them for 9 weeks with whole grains of einkorn and emmer, there was a significant reduction of insulin in their blood. In another study performed on 22 healthy persons who consumed Kamut® Khorasan products for 8 weeks, it was concluded that replacing wheat in the diet with this type of flour

improves the antioxidant, inflammatory, metabolic and lipid blood profile for people with high cardiovascular risk [67]. Furthermore, another studied aspect is related to their compatibility with patients with celiac disease [68,69,71,72]. In this regard, results showed that celiac epitopes can be found in emmer, einkorn and Khorasan wheat and this suggests that these ancient grains have the ability to activate the immune response linked to celiac disease [73]. Other health benefits of ancient wheat species are related to their capacity to control obesity, to reduce the incidence of colon cancer and decrease the rate of heart disease [12,41,74].

In conclusion, with respect to their nutritional, technological and health-promoting properties, recent studies show that ancient cereals have a promising future on the world market, the progress made by these grains in recent years being significant. They offer an attractive alternative for bakers, farmers and consumers and offer a greater and diversified variety of food products, following market trends [75].

The following conclusions can be drawn based on the findings [29,38,42] of the studies mentioned above:

- ancient cereal species have a unique and superior biochemical composition compared to modern cereals, and thus it can be considered a healthier food alternative;
- even if the technological properties of ancient wheat species are slightly inferior to those of modern varieties, these grains could be used to manufacture high-quality bakery products mainly under controlled processing conditions;
- sustainable future food can be developed by using ancient cereals.

4. Ancient Wheat Species and the Sourdough Fermentation Process

Sourdough obtaining technology has a long history that is based on its use as a biological leavening agent. The uniqueness of this biotechnological process is given by the ability of the sourdough to choose spontaneously the necessary lactic acid bacteria and yeasts, which are subsequently acclimatized and form typical communities of microorganisms [76]. On the other hand, studies are reporting the successful use of several starter cultures of LAB and/or yeasts on ancient wheat flours [18,77,78]. Some specific effects that sourdough produces in bread are to improve acidification, leavening ability, dough properties, flavor, volume and texture in the final product, as well as increased resistance to microbial spoilage and bread shelf-life [79]. All of these changes have a significant impact on the final quality of the products [80]. The metabolites produced during sourdough fermentation have a positive impact on the dough and bread texture, leading to less elastic and softer dough but to harder and tougher bread crumb. Furthermore, the nutritional value of the bread is improved due to the lactic acid bacteria and yeasts, which play an important role in decreasing phytates content and postprandial glucose levels, as well as increasing mineral bioavailability and supplying some exopolysaccharides with antistaling and prebiotic properties [81]. Nowadays, it is well known that the acidification activity of lactic acid bacteria, exopolysaccharides, enzymes and organic acids are responsible for the majority of the beneficial properties attributed to sourdough [82–84]. The functional, rheological and nutritional properties of sourdough are all based on the interaction between the activity of lactic acid bacteria microbiota and the raw matrix used as a substrate of fermentation [85]. Thus, the combination between nutritious raw flour and microbial fermentation could lead to bakery products with improved quality and health-promoting characteristics.

Sourdough bread has a special place in many European countries like Italy, France, Germany or Greece but also on the American continent, considered a traditional product and widely consumed. Thus, over time, sourdough has been studied in detail and the technology of obtaining it is well documented [86]. We can currently classify sourdough into four distinct types as follows: type I is characterized by spontaneous fermentation of lactic acid bacteria and yeasts present in flour, fermentation for type II takes place as a result of inoculation of a starter culture, type III is the type II sourdough after it has been dehydrated and type IV is made on a small laboratory scale by a combination of sourdough type I and type II [87].

At present, many of researchers focus their attention on the study of sourdough fermentation and its effects on bioactive compounds from some ancient cereals (spelt, einkorn, emmer and Khorasan wheat), pseudocereals (buckwheat, quinoa, amaranth), legumes (bean, soybean, faba bean, chickpea, lentil, grass pea, cowpea, lupine) and milling by-products (germ and bran) [88,89]. However, ancient wheat sourdough gains attention due to its possibility to produce new functionally active molecules with higher bioaccessibility and thus to produce bread with enhanced nutritional quality.

4.1. Selected Lactic Acid Bacteria Used for Ancient Wheat Sourdough Bread Production

Lactic acid bacteria are one of the basic elements of sourdough fermentation which plays a crucial role as the type of microorganisms engaged in dough fermentation affecting its quality in an extended manner. On the other hand, of elemental importance is the flour used, while, depending on its type (ash content, enzymatic activity, damaged starch, etc.), the proportion of sourdough that is added to the final bread dough can vary between 10 and 40% [90]. Due to these factors, the sourdough fermentation process has undergone some changes and has become more controlled with the addition of starter cultures [91]. The biggest advantage that starter cultures have compared to bacteria from the spontaneous microflora is given by their capacity to ensure the quality and safety of fermentation. Thus, with the help of the selected bacteria, any risk of microbiological contamination is eliminated and the obtained products will not present a danger for human health [92]. Various lactic acid bacteria have been shown to have beneficial effects on the quality of bakery products. In this category are found, according to the new nomenclature starting in 2020, *Levilactobacillus brevis* and *Lactiplantibacillus plantarum*, which are codominant with heterofermentative lactic acid bacteria, and *Lacticaseibacillus casei* which is distinguished by two skills, the capacity to produce exopolysaccharides and the possibility to be used as a starter culture [83]. Therefore, in the case of ancient wheat species, LAB strains such as *Weissella confusa* 24S, *Lactobacillus brevis* 14G, *Lactobacillus alimentarius* 15M, *Lactobacillus sanfranciscensis* 7A, *Lactobacillus brevis* 20S, *Lactobacillus hilgardii* 51B and *Lactobacillus plantarum* 31S were used to produce spelt sourdough [17,77]. To obtain emmer sourdough *Lactobacillus plantarum* 6E, *Lactobacillus plantarum* 10E, *Lactobacillus plantarum* T6B10, *Weissella confusa* 12E and *Weissella confusa* BAN8 have been selected and used [17,78]. Einkorn flour was fermented with strains of *Lactobacillus brevis* 3BHI, *Lactobacillus sanfranciscensis* BB12, *Lactobacillus plantarum* 98a [32]. *Lactobacillus sanfranciscensis* BB12, *Lactobacillus plantarum* M4, *Lactobacillus plantarum* 98a, *Lactobacillus paracasei* I1, *Lactobacillus brevis* 3BHI and *Lactobacillus brevis* T4, were used for Kamut® Khorasan sourdough [18,93]. As these studies are reported, the selected LAB strains are contributing to products with improved sensorial quality, but can also serve other purposes, for example, at release of peptides with antioxidant activity [18,77,93]. It is worth mentioning that soft ancient wheat species *Verna* and *Andriolo* with non-optimal rheological characteristics also demonstrated that they could make bread with similar quality properties to the samples from modern wheat variety when sourdough type I was used [59].

Sourdough fermentation is influenced by several parameters, two of which are of essential importance. The first parameter is the temperature, an exogenous factor that plays a critical role in the metabolic activity of lactobacilli and also influences the dynamics of microbial population, while the second parameter is the flour's hydration capacity and how it impacts the fermentation process [94]. Additional exogenous factors that can disturb the sourdough microbiota are fermentation time, redox potential, oxygen tension, dough yield, storage temperature, pH and the number of propagation steps [95]. However, there are also endogenous factors that can affect the fermentation and implicitly the quality of the bread. The most relevant of these are enzymatic activity, mineral availability, nitrogen sources, lipids, carbohydrate type, and their concentration in the raw matrix [96].

4.2. Nutritional Value and Health-Promoting Compounds

The effects of sourdough fermentation on the nutritional properties of bread quality are among the most diverse and depend on the interaction between the raw flour and the microbial strain used as the fermenting agent. Thus, various studies have confirmed aspects such as: increase the bioaccessibility of vitamins and bioactive compounds, decrease glycemic index, improves the bioavailability of minerals, reduces the content of anti-nutritional factors, helps in the degradation of gluten and the solubilization of fibers [3,89,97,98].

Protein degradation via proteolysis involves the breakdown of proteins into peptides and/or amino acids [99]. This process occurs during fermentation and is initiated by endogenous proteases from cereals and, later, hydrolysis of peptides into amino acids is performed by lactic acid bacteria proteinases. Proteolysis presents great importance for the nutritional quality of bread. Thus, the products of proteolysis, as peptides and amino acids, as well as other compounds transformed by microorganisms' metabolic pathways, have an impact on the bioactive chemicals content, aromatic derivatives, and, as a result, on the bread flavor [82,100,101]. Some of the health beneficial effects of amino acids (e.g., lysine, histidine, isoleucine) and peptides (e.g., lunasin) resulting from proteolysis are: promoting muscle synthesis, reducing the risk of type 2 diabetes and cardiovascular disease, preventing cancer, lowering blood pressure, and others [3,102–104].

Only a few studies have been conducted on assessing the influence of ancient wheat sourdough on the nutritional characteristics of baking products and the most recent are performing metabolomics approaches for this aim [3,105]. Colosimo et al. [105] reported that the proteolysis of spelt flour protein led to enhanced content of essential amino acids like isoleucine, leucine, valine and methionine became potentially bioavailable. Leucine, isoleucine and valine are branched amino acids (BCAA), known for their health benefits such as lowering the insulin response but also for the capacity to contribute to the production of bioactive peptides which have antioxidant, anti-inflammatory and anti-hypertensive effects [3,105]. Another advantage of sourdough fermentation is given by its potential to eliminate the peptides responsible for the occurrence of intolerance to cereals [79,106–108], this result could help for the development and expansion of the range of products destined for people suffering from celiac disease. Additionally, as a result of proteolysis, in vivo and ex vitro tests performed by Colosimo et al. [105], showed an important increase in antioxidant and total phenolics activities in the case of spelt sourdough; the health benefits that these compounds could bring are major due to their capacity to reduce the risk of diabetes, systemic attack, leukemia, asthma or depression. These results were obtained on a sourdough made from water, spelt from Garfagnana (Lucca, Italy) and sourdough; the mixture was fermented at 38 °C for 4 days in which it was continuously mixed.

According to the study conducted by Coda et al. [77], certain selected lactic acid bacteria such as *Lactobacillus franciscensis* 7A, *Lactobacillus brevis* 14G, *Lactobacillus alimentarius* 15M and *Lactobacillus hilgardii* 51B were able to release antioxidant peptides during sourdough fermentation obtained from Kamut®Khorasan, spelt, rye and wholemeal flour. The sourdough subjected to the experiment was obtained from flour and water fermented together with the selected microorganisms in stirring conditions (200 rpm) for 24 h at 37 °C. Unlike chemically fermented doughs, the radical scavenging activity of water/salt-soluble extracts from sourdough obtained from these cereals is considerably higher. In the active fraction of sourdough water-soluble extracts, twenty-five peptides with amino acid residues from 8 to 57 were discovered with the help of nanoliquid chromatography–electrospray ionization tandem mass spectrometry.

It has also been shown that einkorn sourdough bread contains a higher level of carotenoids compared to wheat sourdough bread; and the sourdough fermentation helped to maintain these compounds even if they were exposed to oxygen for a long time [32]. Another study revealed a decrease in phytate content, *Lactobacillus plantarum* AAS3 and *Lactobacillus paraplantarum* 2815 proving the highest phytase activity and consequently,

the bioavailability of minerals was increased. However, other species of LAB, such as *Lactobacillus brevis* qp 109 and *Lactobacillus plantarum* GM 1403 had the lowest phytase activity, a fact that indicated that the phytase activity investigation could help to enhance the bioavailability of the minerals in bread sourdough [19].

Moreover, Kamut®Khorasan wheat flour fermented with a specific strain of *Lactobacillus plantarum* revealed the capacity to determine the metabolic features of sourdough due to the specific flour's compounds involved both in nutritional and aromatic quality. A comparative study between Kamut®Khorasan and durum sourdough highlighted that Kamut flour resulted in a higher content of acid compounds, a large range of volatile compounds with a high correlation between volatiles and polyphenolic compounds and total polyphenols [109]. In addition, Kamut®Khorasan sourdough bread was found to be richer in fibers like arabinoxylans and fructans than durum wheat, revealing the role of specific interaction between the raw matrix and the microbial strain in the accumulation of the bioactive compounds [93].

4.3. Textural Properties of Dough and Bread as Affected by Ancient Wheat Sourdough

The decrease of pH by organic acids produced by LAB has a major impact on the swelling and solubility of gluten proteins. This process led to softer dough that can present better gas retention and a higher loaf volume upon baking. As well, CO₂ has a similar mechanism of action on textures as organic acids [110]. Last but not least, endogenous proteolytic enzymes are also responsible for softening the dough by growing the content of hydrolyzed products [111].

According to Zamaratskaia et al. [16], emmer sourdough bread is characterized by a hearty crumb, a medium rough surface, and grainy and low dryness. Spring emmer wheat varieties present a greater sensory variation than winter ones, having a great influence on the textural characteristics of the bread. Coda et al. [17] reported that emmer and spelt sourdough bread presents a concentration of free amino acids, a phytase activity and a titratable acidity significantly higher than common wheat sourdough bread; per contra, it presents a similarity in terms of pH values. The authors stated that volume and crumb grain were positively influenced by bread acidity. The bread volume was evaluated after 4 h of storage, while the bread crumb was determined after 24 h with the help of image analysis technology. Sensorial analysis showed that there is a greater resemblance between the values of textural parameters of spelt and common wheat sourdough bread than in the case of emmer sourdough bread, but both ancient kinds of cereals are able to be used in bakery products manufacturing.

Sourdough bread obtained from three varieties of *T. aestivum* ssp. *vulgare* L. and two varieties of *T. aestivum* ssp. *spelta* L. were analyzed from a sensory point of view (crumb firmness and elasticity, cohesiveness, moistness bread crumb, crumb cells homogeneity, crumb cells number, aroma and flavor) in a study by Callejo et al. [112]. The sensory profiling method was used to perform the evaluation. The results revealed major differences in terms of crumb elasticity, which normally is a parameter that indicates a good baking performance and crumb cell homogeneity between spelt and wheat bread. The authors concluded that the higher crumb elasticity of the bread indicates a good baking performance and is associated with the rheological characteristics of spelt dough. These characteristics are determined by the high ratio of gliadin to glutenin subunits in spelt dough which generates low extensibility. Another cause could be the pre-gelatinization process of the spelt flour which could lower the probability of premature membrane rupture between gas cells, and as a result prolonged and variable oven rise is possible. This process can also explain why the spelt breads had received poor scores for "crumb cell homogeneity".

For sourdough bread made with 100% einkorn flour, a study by Piasecka-Józwiak et al. [113] concluded that it has an irregular porosity, a small volume and thick walls of the crumb pores. These results were predicted by previous tests to which einkorn flour was subjected. For example, the Zeleny test had shown that the proteins of the einkorn have poor quality, also the gluten is in a lower amount than in wheat flour and has high plasticity and viscosity which makes it

difficult to wash out. Examination of einkorn dough with the farinograph revealed that it has low stability, low water absorption, a brief development time of the dough, and a high softening degree; all of these indicating the einkorn flour as having low rheological properties. Thus, by adding 3% gluten, all these parameters (volume, bread crumb and texture) are improved significantly, the same result is possible by replacing 30–50% of einkorn flour with wheat flour. However, these results were contradicted by Çakır et al. [19] who reported that the einkorn sourdough bread (with 100% einkorn flour) does not present a thick wall structure in the crust or in the crumb and the panelists scored the texture of the bread as acceptable. Differences in the rheological behavior of ancient cereal could be related to the stage of maturation, climatic conditions, soil properties, while metabolomics approaches could be a tool for better understanding the influencing factors and predicting the cereal quality.

Sereti et al. [114] also studied the physical and chemical characteristics of some breads obtained from ancient cereals such as einkorn and spelt by comparing them to common wheat. These flours were obtained by imitating the prehistoric grinding tools, while the bread samples were obtained by the sourdough method except the control (Figure 3). As a result, it was discovered that the sample made with common wheat flour has the best crumb microstructure. The huge pores in the other bread samples could be attributable to a variety of factors, including a high amount of bran due to the grinding procedure, which weakens the gluten network, as well as gas cell coalescence caused by a weak protein gel network. In terms of crust hardness, there are no significant variations between sourdough einkorn bread and wheat flour-based bread. Figures 3 and 4 are relevant for a better understanding of the effect that ancient flours can have on the rheological properties of samples of bread. The optical micrographs revealed for the einkorn dough without sourdough that part of the starch granules was aggregated. Studies have also shown that the free spaces resulting from starch gathering were filled by the gluten network. Contrarily, the sample with sourdough revealed a uniform distribution of the starch granules, probably caused by the sourdough capacity to break the gluten gel.

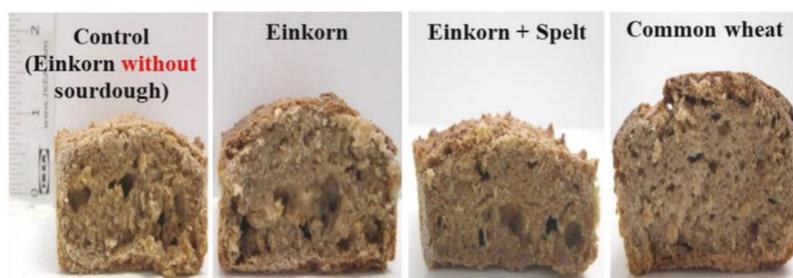


Figure 3. Section aspect of the bread samples made from ancient grains flour using sourdough method. Adapted from Sereti et al. [114]. Copyright 2020 Creative Common Attribution License.

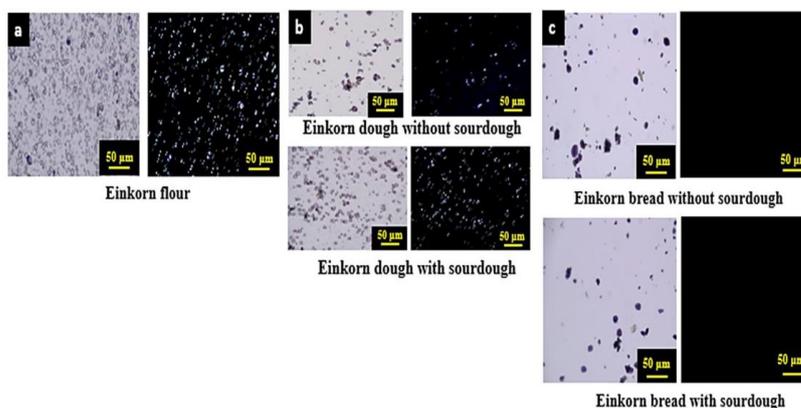


Figure 4. Starch granules from einkorn flour and bread samples with or without sourdough. Adapted from Sereti et al. [114]. Copyright 2020 Creative Common Attribution License.

4.4. Flavor Development in Ancient Wheat Sourdough Bread

One of the great advantages of bread made with sourdough is the specific flavor. Its formation in the product is mainly related to several factors such as the volatile profile of the raw flour and specific interaction between the flour and the microbial strains used in fermentation. Additionally, the aroma of the bread is influenced by the amount of sourdough added to the dough. For example, in sourdough wheat bread has been identified 82 volatile compounds, with 10 more than in wheat bread fermented only with bakery yeast [115,116]. This significant difference in the concentration of compounds between the two assortments is because sourdough bread requires a longer fermentation period but it is also due to the different metabolic pathways of LAB and bakery yeast [117]. The formation of volatile compounds in sourdough bread is caused by several processes, such as caramelization and Maillard reactions, lipid oxidation and fermentation [118]. Mainly, volatile compounds belong to the class of alcohols, esters, aldehydes, lactones, acids, ketones, hydrocarbons, furans, with a total of over 500 compounds identified in bread [119]. Depending on their role as a substrate for microorganism fermentation, different types of cereal flours can generate different volatile profiles. An example of this case is Kamut® Khorasan flour, which, due to the large amounts of protein content compared with common wheat, leads to a greater differentiation of sulphur compounds [120,121]. In a study by Di Renzo et al. [18], the doughs made from Kamut® Khorasan and fermented with *Lactobacillus plantarum* and *Lactobacillus paracasei* stood out after 48 h by the presence of some compounds such as pentanal, hexanal, nonanal, trans (E)2-heptanal, 2-octenal, 2,4-nonadienal and 2,4-decadienal. Furthermore, 2,6-dimethyl-4-heptanone, 5-methyl-3-hexanone, 4-methyl-3-penten-2-one were produced in higher quantities in sourdough fermented with *Lactobacillus paracasei* than in those fermented by *Lactobacillus plantarum* and *Lactobacillus brevis*. The sensory analysis conducted by Coda et al. [17] on emmer sourdough bread, spelt sourdough bread and wheat sourdough bread has shown that the flavor of the products is influenced by three major factors, namely fermentation, the mix of flour and the bakery process. Thus, sensory attributes such as acid flavor, acid taste, sweetness, color, elasticity, dryness and taste were scored using a 10-point scale. The analysis was performed by 10 untrained panelists who concluded that spelt sourdough bread has the highest value for the acid test (8.0 ± 0.2) compared to wheat sourdough bread (6.5 ± 0.2) and emmer sourdough bread (7.5 ± 0.3). Moreover, the sensory analysis showed that the acid flavor was higher in the bread with spelt and white wheat flour (7.5 ± 0.3) than in the emmer sourdough bread (6.5 ± 0.3). These combined results demonstrate consumers' preference for spelt sourdough bread. A sourdough made from 100% einkorn flour was used to obtain 5 samples of bread where wheat/einkorn flour ratios were 0/100, 25/75, 50/50, 75/25 and 100/0. For these products, 12 trained specialists evaluated attributes such as smell, taste, chewability, crust and bread crumb color, pore structure and general acceptance, using a 7-point scale. The result of the analysis established that as the proportion of einkorn flour increases (75–100% einkorn flour), consumer preference decreases, the best taste scores being received by products with 25 and 50% einkorn flour [19]. Starr et al. [122] and Starr et al. [123] have analyzed the sensory profile of cooked grains, flour, bread and porridge made from some species and varieties of the genus *Triticum*. In their research they used terms like cocoa, honey, vanilla, nutty, cooked malt and oat porridge for odor descriptors; and for flavor descriptors were used sweet, salt, bitter, oat porridge and cooked malt. Thus, ancient species like einkorn, emmer and spelt were appreciated as having flavor and odor of oat porridge and a mild aroma, while Kamut® wheat was distinguished by a sweet flavor and an intense aroma. Eighty-eight compounds were identified during the gas chromatographic investigation, and twenty-two of them can be associated with odor and flavor descriptions, including 2-methylbutanal, 3-methyl-1-butanol, 6-methyl-5-hepten-2-one, 2-methyl-1-butanol, 2-pentylfuran, benzaldehyde, 2-nonenal, hexanal and hexanol.

4.5. Shelf Life of Sourdough Bread

The two most common factors which affect the shelf life of bread are firming texture and microbiological spoilage which is a result of cross-contamination that occurs after the baking process [81]. Lactic acid bacteria have been proven to have significant antibacterial activity, which aids in the inhibition of certain saprophytic bacteria species (*Aspergillus niger*, *Penicillium* sp., *Rhizopus* sp., *Fusarium* sp., *Mucor* sp., *Bacillus subtilis*, *Bacillus mesentericus*, *Bacillus licheniformis*, *Bacillus pseudomesenteroides*) [124]. Thus, to prevent the appearance of bacterial spoilage it is necessary to add 10% sourdough in breadmaking and to increase the amount to 15–20% if the prevention of mold spoilage is desired [125]. Lactic microorganisms produce a series of inhibitory substances (acetic acid, lactic acid, diacetyl, hydrogen peroxide, bacteriocins) which help to prevent the appearance of pathogenic bacteria [126]. The lactic bacteria from spontaneous einkorn sourdough have significant antimicrobial activity against pathogenic bacteria like *Bacillus cereus* and *Escherichia coli*, and especially against *Bacillus subtilis*, which causes rope spoilage in bread [19]. For spelt sourdough bread, a study by Korcari et al. [127] showed that *Weissella cibaria* demonstrates a strong antifungal activity against *Fusarium verticillioides* and *Aspergillus flavus*; whereas, *Pediococcus pentosaceus* inhibits the growth of fungus *Mucor circinelloides*, *Aspergillus flavus* and *Fusarium verticillioides*. These results led to the conclusion that both strains are valid to increase the shelf life of spelt sourdough bread. In another study performed on spelt, an increase in organic acids was observed during the sourdough fermentation and this fact, according to the researchers, can be associated with the increase of the shelf life of the bread [105]. According to an opinion written by Calvert et al. [128], the shelf life may be longer for products obtained from flours that have a higher amylase content, because sourdough starters with high levels of this enzyme acidify faster. Obviously, this is only a hypothesis and detailed studies are needed to determine whether the shelf life of bread obtained from ancient cereals differs from that obtained from common wheat.

5. Glycemic Index of Ancient Wheat Species Bread

The number of patients who suffer from type 2 diabetes is constantly increasing and by 2040, the number of cases is predicted to reach 415 million [129]. Previous research has concluded that carbohydrates have a major role in the onset of this disease, but also in blood glucose control and the risk of cardiovascular disease [130].

The glycemic index (GI) is a tool with the help of which we can classify foods in terms of their carbohydrate content and the effect they have on blood glucose [131]. In this way, foods are divided according to GI into three classes: high ($GI \geq 70$), medium (56–69) and low ($GI \leq 55$) [132].

Wholemeal and white bread fall into the food group with a high glycemic index while sourdough bread belongs to the class of those with a medium or even a low index [133]. In recent years, various strategies have been tried to reduce this index such as the use of high-fiber flours (rye, oat, barley) or the addition of dietary fiber (β -glucans) [134]. Another approach is the use of flours obtained from some legumes (lupine, chickpea, guar bean) and fruits [135].

A study by Marques et al. [136] was compared wheat bread and spelt bread and came to the conclusion that there is no significant difference between their glycemic index, both values being around 93 ± 9 . Instead, another study conducted by Thorup et al. [70] studied the effects that a diet composed of flour of some ancient cereals (emmer, einkorn, spelt) and rye has on a group of 40 rats for a period of 9 weeks. The results showed that a diet based on emmer, einkorn and spelt can slow down or prevent the risk of developing diabetes. This is possible due to the downregulation that these flours make on the key regulatory genes involved in the metabolism of fats and glucose.

Regarding Kamut[®] Khorasan flour, an in vivo study was performed on 30 healthy subjects who consumed for 16 weeks products (flour, crisp toast, crackers, fusilli, penne) from this type of ancient cereal. Finally, it was possible to conclude that these types of

foods are able to decrease certain markers associated with the risk of developing type 2 diabetes [34].

According to Atkinson et al. [137], the GI for 30 g of sourdough wheat bread is 54 and its GL (glycemic load) is 8, for the same amount of the white bread GI is 71 and the GL is 9, and finally, at the opposite end is the wholemeal wheat bread which has GI 71 and GL 10. The glycemic load (GL) is determined as the sum of the product of the GI for each foodstuff and its available carbohydrate content divided by 100. This index is used to estimate the quantity of carbohydrates ingested as well the effect on the insulin content.

Studies performed so far on this subject have shown promising results, but to establish the exact effect that sourdough and, especially, ancient cereals have in controlling and lowering blood sugar, more detailed research is needed.

6. Conclusions

The market for functional food products is constantly growing and changing. This fierce expansion is based on two major causes: the evolution of scientific interest and the consumer belief in the ability of nutrition to prevent and reduce the risk of chronic diseases [138].

Thus, a special interest in recent years has been given to the changes that sourdoughs can produce on the nutrients in grains and cereal flour. Traditionally, wheat or rye flour is used to obtain sourdough, but recently there has been growing interest in the use of other types of cereals. For this reason, ancient wheat varieties such as einkorn, emmer, spelt and Khorasan have begun to hold the attention of consumers, processors and farmers. They could be used as a substitute for common flour due to their higher functional qualities, allowing the design of innovative products destined for specific dietary needs. Nowadays, spelt flour is widely used in breadmaking due to its superior nutritional composition and baking performance. However, recent studies have been conducted on assessing the baking quality of Kamut® Khorasan, emmer and einkorn flours with encouraging results, especially when the sourdough method was used. Sourdough has the great potential to level the baking performance differences between the ancient and modern wheat flours.

The results of the studies carried out so far on these ancient varieties are promising, and the benefits that bioactive compounds have on the organism are among the most diverse. Their use in sourdough further enhances and emphasizes their advantages to improve the flavor, shelf life and texture of bread, but also to prevent chronic diseases such as cancer, diabetes, obesity and cardiovascular disease.

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