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Review

Effect of Processing on *p*-Hydroxybenzoic Acid Content of Cereals-A Review

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Abstract: Cereals hold an important place in human nutrition due to their high content of numerous bioactive compounds, primarily phenolic acids. This review aims to evaluate the presence and distribution of *p*-Hydroxybenzoic Acid (*p*-HBA) in cereals and pseudo-cereals, and the effects of different food processing methods on *p*-hydroxybenzoic acid content, in light of the literature findings. Current studies indicate that *p*-hydroxybenzoic acid is predominantly found in the bran, aleurone layer, and embryo of cereals, and its content decreases significantly with refining processes. Biological processes such as germination and fermentation lead to significant increases in *p*-HBA content by releasing phenolic acids present in bound form in the grain matrix. This effect is reported to be further enhanced, and multi-fold increases are observed, particularly when supported by enzymatic treatments. The effects of heat treatments on *p*-hydroxybenzoic acid vary depending on the method and conditions applied. Long-term high-temperature treatments, such as baking, can cause *p*-hydroxybenzoic acid losses, while short-term treatments, such as microwaves, can promote *p*-hydroxybenzoic acid release by disrupting cell wall structures. In conclusion, *p*-hydroxybenzoic acid appears to be a phenolic acid sensitive to grain processing methods, and its content can be increased with appropriate processing strategies. These findings suggest that the development of *p*-hydroxybenzoic acid-enriched whole grain and pseudo-cereal products offers a significant approach to high-nutritional-quality and health-focused foods.

***Keywords*:** germination, fermentation, baking, *p*-Hydroxybenzoic Acid (*p*-HBA)

1. Introduction

Bakery products containing whole grains have many health benefits, including blood sugar management and reduced risk of obesity and cardiovascular disease. Phytochemicals, including phenolic acids with antioxidant and anti-inflammatory properties, contribute to these benefits of whole grains on human health [1-3].

Cereals and whole-grain products are rich in bioactive compounds. These bioactive compounds are components such as phenolic acids, their esters and glycosides, aventramides, flavonoids, phytoestrogens, phytosterols, tocopherols and tocotrienols, carotenoids, melatonin, inositol phosphates, glutathione, micro and macro elements. Among the phytochemicals identified in cereal grains, polyphenols, a specific group of phenolic acids, play the most important role [4]. Phenolic acids are a group of secondary metabolites that are part of a large group of phenolic compounds that are widely distributed in plants. They are considered to be very important components in foods that contribute to flavor,

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color, and nutritional properties [5]. Phenolic acids are classified according to their chemical structures as derivatives of benzoic acid (gallic, *p*-Hydroxybenzoic Acid (*p*-HBA), protocatechuic, vanillic, and syringic acids) and cinnamic acid (caffeic, ferulic, *p*-coumaric, and sinapic acids) [4, 6, 7]. These acids are found in high amounts in the bran, aleurone fractions, and embryos of cereals, while trace amounts are found in the starchy endosperm of the cereal [4]. Phenolic acids are also used as additives in foods to preserve color, delay microbial growth, and prevent lipid oxidation [8].

Among benzoic acid derivatives, *p*-HBA is attracting increasing attention due to its widespread presence in cereals and its marked sensitivity to cereal processing methods. Investigating the effects of cereal processing technologies on the *p*-HBA content in cereals is important for the development of functional cereal products. This review aims to present concise and up-to-date information on the presence of *p*-HBA in cereals and the effects of cereal processing practices on *p*-HBA content.

2. *p*-Hydroxybenzoic acid in cereals

p-Hydroxybenzoic acid (4-hydroxy benzene carboxylic acid) $C_7H_6O_3$ is an organic compound with a molecular weight of 138.13 and a melting point of 216.2 °C [9, 10]. *p*-HBA is a phenolic acid commonly found in cereals and is considered one of the main antioxidant components of cereals [11]. *p*-HBA, a monohydroxy phenolic derivative of benzoic acid, is widely used as an antioxidant, preservative, and fungicide in food, beverage, pharmaceutical production, and the cosmetics industry [12].

The *p*-HBA content in whole grain flours was determined to be 7.4 µg/g in triticale, 9.2 µg/g in wheat, 215.0 µg/g in barley, and 11.6 µg/g in corn [13]. When looking at both cereals and pseudo-cereals, it is seen that the bran fraction of the grain is richer in *p*-HBA (Table 1). It is also understood that whole-grain flours contain higher levels of *p*-HBA than refined flours. In cereals, phenolic acids are found in the largest amounts in the outer part of the aleurone layer, the seed coat, and the embryo. This is because phenolic acids are most frequently bound to cell wall polymers by covalent bonds, which is very important for the immune mechanism in plants [4]. A study conducted with four different wheat varieties determined that *Triticum turanicum* was richer in *p*-HBA compared to other wheat varieties [14]. In this study conducted by Suchowilska et al. [14], it was found that all four different wheat varieties used contained higher levels of *p*-HBA in the bran portion of the grain compared to the flour and whole grain. This result also explains why whole-grain flours contain more *p*-HBA than refined flours. Moreover, while the *p*-HBA content in grains varies greatly depending on the fraction type (bran-flour) and genotype, pseudo-cereals appear to contain higher levels of *p*-HBA (Table 1). Buckwheat, in particular, has higher *p*-HBA values in its whole grain form compared to many other grain types. This suggests that pseudo-cereals have significant potential in terms of *p*-HBA content for evaluation as a functional food component.

Table 1. *p*-Hydroxybenzoic acid in grains and grain botanical fractions

Samples	*p*-Hydroxybenzoic acid		References
Rye flour	6.8	mg/kg fw	[15]
Whole rye flour	10		
Rye bran	24		
Rye bread	4.6		
Whole wheat flour	7.4		
White wheat flour	2.3		
White wheat bread	1.6		
Barley flour	3.1		
Oat bran	22		
Corn flour	5.7		

Table 1. (cont.)

Samples	*p*-Hydroxybenzoic acid		References
Oat	4.48	mg/kg	[16]
Whole corn flour	11.6	µg/g	[13]
Whole triticale flour	7.4		
Whole barley flour	215		
Buckwheat flour	110	mg/kg dm	[4]
White rice	13		
Brown rice	17		
Millet groats	3		
Rye	14.1	µg/g dm	[17]
Sorghum	36.2		[18]
Quinoa (pseudo-cereal)	21.7		[19]

p-Hydroxybenzoic acid (mg/kg)				
Wheat Varieties	Flour	Bran	Grain	
T. polonicum	41.2	59.4	65.2	[14]
T. durum	64.5	276.4	104.2	
T. aestivum	31.8	109.1	49.1	
T. turanicum	88.4	413.7	176.7	

3. Effect of germination

Cereal germination is considered a simple, low-cost, and highly effective method of enriching the nutritional content of cereals and pseudo-cereals. Germination is widely applied to improve cereal quality, soften kernel structure, and enrich the bioactive compound content of cereal seeds [20-24]. This process not only reduces anti-nutritional compounds but also improves the overall nutritional value of cereals by increasing the amount of nutrients and physiologically active compounds. Enzyme synthesis and metabolic modifications occurring during germination significantly contribute to the increase in phenolic compound content and antioxidant activity [25-28].

For example, it has been reported that the levels of free, bound, and total phenolic compounds in Chinese wild rice increased by 46.60%, 93.58%, and 57.85%, respectively, compared to raw seeds at the end of germination [26]. In a study conducted on Chinese wild rice (*Zizania latifolia*), Chu et al. [27] showed that the germination process statistically significantly increased the *p*-HBA content. In a study with foxtail millet, it was determined that the *p*-HBA content, which was 2.03 mg/kg dry weight before germination, increased to 2.20 mg/kg dry weight on the second day of germination and to 7.20 mg/kg dry weight on the third day. The highest *p*-HBA concentration reached at the end of germination was found to be approximately 2.5 times higher compared to ungerminated seeds [29]. In a study conducted on quinoa, it was determined that the *p*-HBA content increased 1.5 times at 24 hours of germination, 2 times at 48 hours, and 4 times at 72 hours when compared with the initial unsprouted grains (Table 2) [30]. In addition, it has been reported that *p*-HBA is one of the main components contributing to the increase in antioxidant activity observed during germination, together with *p*-hydroxybenzaldehyde, *p*-coumaric acid, vanillic acid, ferulic acid, and sinapic acid [28].

Table 2. Effect of germination on *p*-hydroxybenzoic acid content

Germinated seeds	Time	Germination				Unit	References
		Free *p*-HBA		Bound *p*-HBA			
		Before	After	Before	After		
Wheat, 25 ℃	24-96 h	0.19	0.38-4.70	0.29	0.27-0.85	mg/100 g DW	[31]
Wheat (Jimai22) 25 ℃	12-96 h	5.34	4.07-27.23	2.83	2.49-5.20	μg/g DW	[32]
Highland barley 30 ℃, 90% RH	60 h	61.30	254.42	5.36	1.51	μg/g DW	[33]
Highland barley 28 ℃	60 h	2.87	13.61	1.29	1.28	mg/100 g DW	[34]
Barley 12.1-19.9 ℃	1.6-6.2 days	ND	ND	0.45	0.12-0.41	μg/mg extract	[35]
Spelt, 25 ℃	144 h	1.07	5.35	13.64	16.21	μg/g DW	[36]
Spelt, 25 ℃ (Enzymatic treatment)			40.60		51.59		
Brown rice 30 ℃, 90% RH	18-72 h	1.59	1.60-1.70	1.76	1.91-1.65	μg/g DW	[37]
White quinoa 24 ℃, 95% RH	24-96 h	1.98	2.18-3.38	7.23	7,35-6.54	mg/100 g DW	[38]
Black quinoa 24 ℃, 95% RH		3.17	4.63-6.29	12.82	13.85-11.1		
Sorghum, 30 ℃	12-48 h	6.72	7.32-8.21	6.02	6.48-6.25	μg/g DW	[39]

Ceccaroni et al. [40] revealed that free and bound polyphenols in wheat exhibited different bioavailability profiles at different germination temperatures. It was reported that at low germination temperatures (15 °C), the bioavailability of free polyphenols in wheat increased, while the bound polyphenol fractions were significantly affected by the wheat genotype. High temperatures (20 °C) were reported to have a positive effect on the bioavailability of free and bound polyphenols in millet samples. Overall, it was stated that the free phenolic compound content was positively affected by low germination temperatures, while the opposite trend was observed for bound phenolic compounds.

4. Effect of fermentation

Food processing methods are processes that enable the transformation of food raw materials into edible and sensorially acceptable products. Among these methods, fermentation, which involves the controlled transformation of a substrate through the metabolic activities of microorganisms, is one of the oldest and most widespread applications historically. The fermentation process can improve the nutritional composition, aroma, and textural properties of foods while also reducing the level of anti-nutritional factors [36, 41].

Fermentation can lead to significant changes in the amount and form of phenolic compounds found in cereals. These changes occur either through the release of naturally occurring phenolic compounds in the cereal matrix or the formation of new phenolic structures [42]. For example, it has been reported that the *p*-HBA content of quinoa flour increased approximately eightfold compared to raw pseudo-cereal after fermentation with bread and brewer's yeast. This increase has been attributed to the ability of *Saccharomyces cerevisiae* strains to produce hydrolytic enzymes that can release soluble conjugated and bound phenolic acids [30].

It has been determined that fermentation carried out with a mixed culture of *Monascus anka* Guangdong Institute of Microbiology (GIM) 3.592, *Saccharomyces cerevisiae* GIM 2.139, and *Bacillus subtilis* 784 in maize seeds causes significant increases in the content of various phenolic acids, including *p*-HBA [43]. Similarly, while an increase in *p*-HBA content was observed as a result of fermentation in whole wheat breads produced from different wheat varieties,

a limited decrease was reported in the Turkish red wheat variety [42]. These findings show that microorganism activity during fermentation can partially break down phenolic acid-carbohydrate complexes, increasing the amount of soluble phenolic acid, but in some cases, phenolic acids can be consumed by microbial metabolism.

Enzymes produced by microorganisms during fermentation can support the release of phenolic compounds from the grain matrix. Significant increases in *p*-HBA content have been reported in fermentations using *S. cerevisiae* alone or in combination with *Lactobacillus plantarum* [44]. In particular, *L. plantarum* fermentation resulted in the highest level of *p*-HBA content in hulled wheat seeds. Similar trends were observed in germinated hulled wheat samples, and the highest increase in *p*-HBA was obtained under fermentation conditions where *S. cerevisiae* and *L. plantarum* were applied together. In enzymatically treated hulled wheat samples (cellulase, xylanase, feruloyl esterase, protease, and amylase), the highest increase in *p*-HBA content was reported to have occurred during *L. plantarum* fermentation. Table 3 shows that germination treatment applied before fermentation resulted in higher *p*-HBA formation compared to enzymatic treatment alone. Changes in pH during fermentation may have provided an optimum pH for various cell wall-degrading enzymes present in germinated seeds, thus increasing *p*-HBA content.

Table 3. Effect of fermentation on *p*-hydroxybenzoic acid content

Cereals	Fermentation		Fermentation		Unit	References
			Before	After		
Spelt (*Triticum spelta* L) raw	*L. plantarum*	72 h, 30 °C	14.71	23.1	µg/g DW	[44]
	S. cerevisiae			34.48		
	L. plantarum + *S. cerevisiae*			30.40		
Spelt (*Triticum spelta*) germinated (144 h, 25 °C)	*L. plantarum*	72 h, 30 °C	21.56	103.46		
	S. cerevisiae			116.75		
	L. plantarum + *S. cerevisiae*			144.84		
Spelt (*Triticum spelta*) enzymatic treated (4 h, 40 °C)	*L. plantarum*	72 h, 30 °C	37.03	90.25		
	S. cerevisiae			57.80		
	L. plantarum + *S. cerevisiae*			87.34		
Wheat bran	*S. cerevisiae*	144 h, 30 °C	1.6	3.62	µg/g	[45]
	S. fibuligera			15.17		
	A. niger			3.31		
	R. oryzae			10.1		
Wheat bran	*L. rhamnosus* 1,473	24 h, 37 °C	7.1	9.6	µg/g DW	[46]
		48 h, 37 °C		9.8		
Oat bran	*L. fermentum* NB02	96 h, 37 °C	38.63	144.46	µg/g	[47]
Rice bran	*R. oryzae*	120 h, 30 °C	6.2	19.1-30.3	mg/g DW	[48]
Barley industry by-products	*P. acidilactici* LUHS29	72 h, 32 °C	4.26	6.18-27.03	µg/g	[49]
Quinoa (pseudo-cereal)	*S. cerevisiae* (baker's yeast)	24 h, 30 °C	100	831	% area relative to raw grain	[30]
	S. cerevisiae (brewer's yeast)			868		

In recent years, the application of enzymes during germination and fermentation processes has become increasingly common in order to further enrich the nutritional content of cereals in terms of phenolic acids. Enzymatic treatments are used as an effective approach in the release of phenolic acids bound to the cell wall matrix [50]. Cell wall hydrolyzing enzymes (cellulase, xylanase, etc.) have been shown to be effective in releasing bound phenolics by breaking down cell wall components in wheat bran [51]. Enzymatic treatment (cellulase + xylanase + esterase + protease) applied during spelt germination resulted in a much higher increase in *p*-HBA content. While the free *p*-HBA content increased from 1.07 μg/g dry weight to 5.35 μg/g dry weight during germination without enzymatic treatment, this value reached 40.60 μg/g dry weight when enzymatic treatment was applied during germination [51].

5. Effect of thermal treatments

It is reported that increasing temperatures lead to catabolism in phenolic compounds. Phenolic acids are generally able to withstand food processing conditions, but high temperatures and pH values are among the most important factors negatively affecting their activity. It is stated that processing can alter the ratio between various phenolic compounds due to thermal decomposition [52]. Studies show that during heating, esters and glycosides of phenolic acids bound to the cell walls of grain kernels are released, and these compounds can be converted into more bioavailable forms through heating. It has also been noted that changes can occur in phenolic acids during thermal processes [53].

On the other hand, there are also studies showing inconsistencies in the effects of baking on total phenolic acids. Abdel-Aal and Rabalski [53] reported a decrease in *p*-HBA content during baking in bread and cake samples made from different cereals, while Gelinas and McKinnon [54] observed an increase in total phenolic acid content. In contrast, Leenhardt et al. [55] reported that phenolic compounds were destroyed during baking.

Table 4. Effect of thermal treatment on *p*-hydroxybenzoic acid content

	Samples	Treatment	*p*-HBA	Unit	References
Wheat	Flour	Baking 220 °C, 30 min	32.53	μg/g	[56]
	Bread		22.74		
	Flour with 10% bran		114		
	Bread with 10% bran		61.43		
Wheat Ag Gallant	Flour	Baking 215 °C, 24 min	9.25	μg/g	[50]
	Fermentation		10.46		
	Bread		10.03		
	Breadcrumb		9.61		
	Bread crust		9.26		
Wheat Zenda	Flour	Baking 215 °C, 24 min	9.34		
	Fermentation		10.43		
	Bread		10.63		
	Breadcrumb		11.18		
	Bread crust		11.04		

Table 4. (cont.)

	Samples	Treatment	*p*-HBA	Unit	References
Wheat	Flour		2.73	µg/g DW	[58]
	Yeast fermented bread	Baking (230 °C, 30 min)	2.42		
	Sourdough fermented bread		4.06		
Spelt	Flour		3.24		
	Yeast fermented bread	Baking (230 °C, 30 min)	2.24		
	Sourdough fermented bread		3.56		
Rye	Flour		2.79		
	Yeast fermented bread	Baking (230 °C, 30 min)	2.61		
	Sourdough fermented bread		6.03		
Wheat bran	HUW-234	Raw	53.49	µg/g	[59]
		Autu-clave (121 °C, 90 min)	55.31		
		Hot air oven 105 °C, 40 min	58.28		
	PBW-373	Raw	50.99		
		Autu-clave (121 °C, 90 min)	61.01		
		Hot air oven 105 °C, 40 min	58.47		
	WH-1105	Raw	58.61		
		Autu-clave (121 °C, 90 min)	64.41		
		Hot air oven 105 °C, 40 min	63.55		

During bread baking, it was determined that the free phenolic acid content decreased by 22.5% in control samples, while in breads enriched with 6% and 10% wheat bran, this decrease reached 21% and 42%, respectively. It was stated that this decrease was mainly due to the decrease in *p*-HBA content (Table 4) [56]. In another study, it was found that the *p*-HBA content decreased significantly in the crust of wheat bread, while the decrease in the bread crumb was more limited. This situation was explained by the fact that *p*-HBA is heat-sensitive, and the bread crust is exposed to higher temperatures compared to the crumb [57]. However, due to the large differences in the methods used, it is difficult to make a direct comparison between the studies.

Skrajda-Brdak et al. [58] stated that the type of fermentation applied in the production of bread before baking can affect the phenolic acid content differently. They found that bread produced using sourdough fermentation had an increase in phenolic acid content after baking, while bread produced using yeast fermentation had a decrease in phenolic acid content. This situation may be due to the release of bound phenolic acids because of the pH reached during sourdough fermentation, and the enzymes produced by sourdough microorganisms. However, the authors noted that the type of fermentation applied before cooking did not have a consistent effect on the free phenolic acid content.

6. Effect of microwave heating

Microwave heat treatment is gradually replacing some traditional heat treatments in food processing due to shorter

processing times, greater penetration depth, higher efficiency, and potentially better product quality, but it requires further investigation and optimization in relation to experimental conditions [60]. Reliable and practical methods for releasing many phenolic compounds, which are generally found in covalently bound forms in plant products, are of great importance. Microwave energy can increase the bioavailability of polyphenols by preventing their binding to the plant matrix [61].

When microwave-treated food, both the treatment time and the microwave power used are of great importance. Hong et al. [62] observed a significant decrease in *p*-HBA content in hulled barley after five minutes of treatment at 700 W power, while Li et al. [63] determined a significant increase in *p*-HBA content in red sorghum after 40 and 60 seconds of treatment at 600 W power (Table 5). Similarly, in rice bran, an increase in *p*-HBA content was observed at 260, 440, and 880 W power for 90 seconds, while a decrease was observed at 120 seconds of processing time [64]. In general, although high temperatures can lead to the breakdown of phenolic compounds, they can also enable the release of bound phenolic acids by disrupting cell wall structures. Therefore, the amount of phenolic acids in heat-treated foods depends on the balance between the compounds that are broken down and released, and in some cases can reach higher levels compared to raw foods [65].

Table 5. Effect of microwave heating on *p*-hydroxybenzoic acid content

Samples	Microwave power (W)	Time (s)	Total *p*-HBA	Unit	References
White sorghum	600	0	7.12	µg/g dw	[63]
		20	7.01		
		40	6.68		
		60	6.87		
Red sorghum	600	0	9.87		
		20	8.94		
		40	12.19		
		60	12.50		
Wheat bran HUW-234	800	0	53.49	µg/g	[58]
		50	63.72		
Wheat bran PBW-373		0	50.99		
		50	65.47		
Wheat bran WH-1105		0	58.61		
		50	75.88		
Wheat bran PBW- 50		0	55.42		
		50	73.54		
Wheat bran HD- 2967		0	48.14		
		50	53.99		
Wheat bran PBW-343		0	51.03		
		50	54.65		
Qingke (hull-less barley)	700	0	94.26	µg/g	[62]
		300	28.96		

Table 5. (cont.)

Samples	Microwave power (W)	Time (s)	Total *p*-HBA	Unit	References
Rice bran	260	0	2.79	µg/g	[64]
		30	4.20		
		60	2.70		
		90	3.24		
		180	1.70		
	440	0	2.79		
		30	2.23		
		60	3.85		
		90	6.00		
		120	1.29		
	880	0	2.79		
		30	0.28		
		60	0.44		
		90	3.71		
		120	0.48		

In a study investigating the effects of different heat treatments (autoclave, hot air oven, microwave, and toasting) on the bioactive components of wheat bran, a general increase in phenolic acid content was reported as a result of heat treatments. The *p*-HBA content of wheat bran increased in all wheat varieties studied, with the highest increase observed in microwave treatment. For example, the *p*-HBA content in WH-1105 wheat bran increased by 29.46%. This increase was attributed to microwave treatment disrupting cell membranes and vacuoles more effectively, thereby increasing the release of bioactive compounds. Furthermore, it was stated that high temperature hydrolyzed the ester bonds between phenolic acids and polysaccharides, thus promoting the release of phenolic acids [58].

7. Conclusion

This review comprehensively evaluates the distribution of *p*-HBA in cereals and pseudo-cereals and the effects of different food processing methods on this compound. Literature findings indicate that *p*-HBA is particularly concentrated in the bran, aleurone layer, and embryo, and is significantly reduced by refining. Biological processes such as germination and fermentation can significantly increase *p*-HBA content by releasing bound phenolic acids; this effect can be further enhanced by enzymatic processes. The effects of heat treatments on *p*-HBA can vary depending on the method applied. Long-term, high-temperature treatments such as baking can lead to *p*-HBA loss, while short-term heat treatments such as microwaves can promote *p*-HBA release by disrupting cell wall structures. This review demonstrates that *p*-HBA is not only a phenolic acid but may also be a biomarker reflecting the efficiency of cereal processing processes. In conclusion, the development of whole-grain and pseudo-cereal products enriched with *p*-HBA through appropriate processing strategies offers a significant approach to nutrient-rich and health-focused foods.

Conflict of interest

The author declares no conflicts of interest.

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