Review

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Dynamic role of natural antioxidant sources on different parameters of bread quality: a review

Debasmita Pathak, Jayshree Majumdar, Utpal Raychaudhuri, Runu Chakraborty*

Correspondence to:
Runu Chakraborty
crunu@hotmail.com

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Abstract

Improved understanding of the relationship between the consumption of antioxidant-rich food and human health has resulted in the supplementation of food products with natural antioxidants. Enrichment of a staple food such as bread with natural antioxidants can provide additional health benefits. Sources of natural antioxidants include cereals, seeds, spices, herbs, green plants, fruit, vegetables and the less expensive and easily available waste products from the food industry. However, there are technological constraints to the fortification of bread, while sensory and physical properties are important for overall consumer acceptability. Synthetic antioxidants can have serious adverse effects on health, and their substitution with natural antioxidants enhances the therapeutic and functional properties of bread and has the added advantage of increasing shelf-life. This review discusses the antioxidant activity and scavenging properties of various types of breads enriched with natural antioxidants from natural sources and the associated health benefits and sensory attributes of these breads.

Introduction

In biological systems, oxidative stress is produced by an imbalance between chemical stresses caused by abnormal quantities of reactive oxygen species (ROS) and physical ability to detoxify the reactive intermediates. Oxidative stress if prolonged can damage the entire system and has been implicated in over 100 common diseases such as cancer, diabetes, heart conditions and neurological disease [1]. However, antioxidants can scavenge ROS and free radicals, thus reducing their numbers. The growing interest in foods rich in antioxidants has led to the development of a large market for antioxidant-rich products and ingredients [2]. Antioxidants can also be used to prevent the degradation of food during processing and storage, thereby increasing its shelf-life.

The importance of natural antioxidants for food applications has been highlighted in numerous studies. Natural antioxidants are found in plants which can be consumed. For example, spices, herbs and essential oils are rich in antioxidant properties [3]. The consumption of natural antioxidants including phenolic and polyphenolic compounds, chelators, antioxidant vitamins and enzymes as well as carotenoids has received considerable interest because of their presumed safety and potential nutritional and therapeutic effects including anti-inflammatory, anti-carcinogenic and anti-athero-

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*Department of Food Technology and Biochemical Engineering, Jadavpur University, Kolkata 700032, India
tel: ++91 983112 2626; fax: ++91 033 2414 6822

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sclerotic properties. Artificial antioxidants such as butylated hydroxytoluene (BHT) and butylated hydroxyanisole (BHA) are synthetically produced chemical antioxidants which are widely used for their antioxidant properties in the food industry. However, the use of these substances is restricted due to toxicity problems and fears of liver damage and carcinogenesis [4]. Consequently, there is ongoing research on natural sources of antioxidants.

Bread is a good source of calories and other nutrients and has been a staple food for centuries. It is traditionally made from wheat flour, yeast, sugar, salt and water. Wheat grain contains many bioactive compounds, including antioxidants (phenolic compounds) particularly in the bran and aleurone layer, although the processing of wheat bread also confers some antioxidant activity [5]. Many different synthetic antioxidants have been used in bread for years to increase storage stability. The current rapidly growing functional food and nutraceutical market has stimulated research on the use of natural antioxidants in bread which do not affect its textural and organoleptic properties.

Functional and therapeutic properties, basic nutritional quality and sensory and textural properties must be carefully considered when incorporating various sources of antioxidants into bread. Recent studies have shown that the addition of small amounts of natural antioxidant sources does not significantly affect the textural and sensory properties of bread.

Dziki *et al* [5] discuss enhancement of the antioxidant activity of wheat bread by the addition of different plant materials along with changes in antioxidant activity during the bread-making process. Thus, the aim of this study was to review wheat and non-wheat bread and discuss the therapeutic and nutritional benefits achieved with the addition of sources of antioxidants as well as the impact of fortification on the sensory and textural properties of the bread.

Review of antioxidant rich breads

Many consumers prefer to eat healthier foods in order to prevent non-communicable diseases. Consequently, researchers are seeking to optimize bread-making technology in order to improve the quality, taste, functionality and bioavailability of this staple food. Accordingly, supplementation with cereals and their fractions, fruits and vegetables, gums and starches, and agricultural wastes has been used to produce antioxidant-rich bread. This research is discussed below and bread characteristics are summarised in Table 1.

Table 1 - Effect of natural antioxidant sources on different quality parameters of bread

Source of fortification	Antioxidant activity (assay) value (highest shown)							Effect on sensory evaluation	Effect on physical properties	Effect on functional properties
	TPC	DPPH	FRAP	Others						
Black tea [6]	24.9±0.2 (GAE mg/g sample)		18.8±1.2 Fe(II) (mM/g sample)	ABTS 58.4±0.2 (percentage of inhibition)		Total flavonoid content 640.1±6.0 (QE mg/g sample)		Overall good acceptance	Less effect on textural and physical properties with darker colour	Antimicrobial effect
Rice bran [7]	183.9±2.9 free phenolics 110.4±8.3 bound phenolics (mg GAE/100 g dw)	400 mg TE/100 g dw (total phenolics) (approx.)	400 mg TE/100 g dw (total phenolics) (approx.)	Total flavonoid content 76.5±0.6 (free) 34.4±3.4 (bound) (mg CATE/100 g dw)	Ferulic acid 4.2±0.6 (free) 285.1±8.3 (bound) (mg/g dw)	Sinapic acid 0.5±0.1 (free) 14.0±2.1 (bound) (mg/g dw)	*p*-Coumaric acid 2.1 ± 0.5 (free) 76.8 ± 4.0 (bound) (mg/g dw)	Lower volume with increased firmness and hardness	Low overall acceptability	
Green tea extract (GTE) and microencapsulated GTE [8]	216.48±1.01 (mg/loaf)							Lower overall scores due to astringency and colour	Insignificant effect with enhanced moisture content	
Stevia rebaudiana extracts (var. Morita) [9]		51.98 IC_{50} (mg/ml)						Lower sweetness and overall likeability	Insignificant effect up to 50% replacement	Antidiabetic and antimicrobial effect

Source of fortification	Antioxidant activity (assay) value (highest shown)							Effect on sensory evaluation	Effect on physical properties	Effect on functional properties
	TPC	DPPH	FRAP	Others						
Quinoa leaves [10]	2.90±0.05		6.07±0.15	ABTS 5.14±0.14 (scavenging %)	LPO 6.04±0.20 (scavenging %)	CHP 2.52±0.20 (% inhibition)		No significant effect	Linear increase in crumb hardness, cohesiveness and gumminess	
Nejayote solids [11]				Ferulic acid 14.16 (mg/100 g)		ORAC 2.32 (µM Trolox/100 g)		Up to 9% supplementation without affecting sensory parameters	No alteration	Higher fibre and bioavailable calcium
Broccoli sprout powder [12]	2.0 (mg GAE/g dw)		56.98±1.94 (EC$_{50}$ mg dw/ml)	ABTS 12.69±0.53 (EC$_{50}$ mg dw/ml)	LPO 83.93±2.71 (EC$_{50}$ mg dw/ml)	Rich in free amino acids		Satisfactory overall consumer acceptability up to 2% supplementation		Rich in free amino acids and chemopreventive potential
Onion skin powder [13]		59.29 ± 1.88 (mg dw/ml)		ABTS 164.52±5.65 (mg dw/ml)	LPO 14.67±0.87 (mg dw/ml)	Availability of quercetin		Satisfactory acceptability up to 3% supplementation	Insignificant difference	Availability of quercetin
Chestnut flour [14]		1.04 mmol (Trolox eq./g of dw)							Heterogeneous crumb structure	A richer volatile profile
Banana pseudo-stem flour [15]	204.16±3.04 (mg GAE/100 g of sample)	241.39 ± 23.06 (µmol TEAC/100 g of sample)	849.01 ± 32.46 (µmol Fe(II)/100 g of sample)					Higher acceptability with CMC	Increases volume with decreases in other physical characters	Rich in fibre
Fennel seed powder [16]	1.7 (mg GAE/100 g)	97±2% (DPPH % inhibition)	2±0.1 (mg FeSO$_4$/100 g)					Good consumer acceptability up to 7% supplementation	Crumb moisture and firmness increased	Abundant minerals and dietary fibre
Cinnamon powder [17]	0.94 (mg GAE/100 g dw)	27.67% (DPPH % inhibition)						Maximum overall acceptability at 2% supplementation	Increase in crumb moisture. 2% Cinnamon powder improved baking and textural properties	
Citrus peel [18]	132.3±3.9 (mg GAE/g)	192.3 ± 15.2 vitamin E equiv. (mg/g)		H_2O_2 reduction method 75.9±1.3 Vitamin C equiv. (mg/g)						
Intermediated pearled wheat fraction [19]	3066 (mg/kg)	0.73 (mmol TE/kg)						Rheological and technological properties were acceptable	Acceptable rheological and technological properties	Rich in protein, dietary fibre, ash content and β-glucan
Maca flour [20]	0.39±0.07 (mg GAE/100 g dm)	21±1.7 Inhibition (%)		ABTS 40±1.7 (inhibition %)	Hydroxyl radical (•OH) 51±1.9 (inhibition %)	Peroxynitrite radicals 25±1.6 (inhibition %)	ORAC 1.621 ± 1.5 (inhibition %)			Anti-inflammatory effect and reducing glucose absorption
Micronized by-products from debranned durum wheat [21]	0.95±0.03 (mM GAE/g)	72.8±0.4 (DPPH % inhibition)						Enhanced sensory properties of breads containing bran	Weak structure, poor baking quality, decreased bread volume and elasticity, increased hardness	Free amino acids and insoluble fibre
Sorghum flour [22]	2.02±0.09 (mg GAE/g sample)	9.45±0.45 (µmol TE/g)						No reduction in sensory acceptability	Observed differences in dough farinograph properties	Remarkable amount of total dietary fibre

Source of fortification	Antioxidant activity (assay) value (highest shown)				Effect on sensory evaluation	Effect on physical properties	Effect on functional properties
	TPC	DPPH	FRAP	Others			
Barley hull and flaxseed hull [23]	4.05±0.31 (mg ferulic acid equivalent/g bread sample)	12.87 ± 2.02 (mg Trolox equivalent / g sample)		ORAC 36.44±14.01 (mg Trolox equivalent/g sample) (barley and flaxseed hulls)			
Barley flour and barley protein isolate [24]	369.60 (mg/100 g)	63.30% (DPPH % inhibition)		ACE inhibitory activity 75.30% α-Amylase inhibitory activity 77.80			Increased the content of essential amino acids
Coriander leaf powder [25]	33 (mg GAE/100 g of sample)	28 (DPPH % inhibition)	37.5 (mg $FeSO_4$/100 g)		Highest overall acceptability at high percentage of supplementation	Affected physical parameters, increase in crumb firmness and loaf weight	
Ginger powder [26]	0.710±0.009 (mg GAE/g dw)	0.242 ± 0.009 (mmol DPPH / mg dw/ml)			Negative effect on bread acceptability	Rheology of dough and texture of bread affected	Concentration of gingerols with formation of shogaols
Turmeric powder [27]	160 (mg GAE/ 100 g)			β-Carotene-linoleate bleaching assay 79.8%±0.6% antioxidant activity	Satisfactory overall consumer acceptability up to 4% supplementation	Hardness and crumb colour a* and b* values increased	K, P and Ca with high curcumin content
Wholegrain flours and fibres [28]	580 (µg/g) (bound phenolics) 180 µg/g (free phenolics)	5% (bound phenolics) 38% (free phenolics) (% inhibition)					High total dietary fibre and protein content
Anka (fermented rice) [29]	3.86±0.05 (mg extract/ml)	7.78 ± 0.03 (mg extract/ml) (at 3% supplementation)			No significant differences with effect on colour and mouth feel	Decrease in specific volume	High monacolin K and GABA
Grape by-product [30]	613.77±1.57 (mg GAE/100 g of sample)	15.02 (mm TRE/g dm)	48 (mm Fe(II)/g dm)		Acceptable products	Affected hardness, gumminess and sharpness	Improved dietary fraction contents
Pseudocereal flour [31]	7.25±0.23 (mg/g dw)				Improves acceptability		Higher presence of dietary fibre and β-glucans
Grape seed extract [32]	TEAC 4.8±0.1 (nmol Trolox/mg bread sample)			55% Inhibition of the formation of CML in bread crust	Little effect on the quality attributes	No significant difference in hardness	Lower CML-associated health risks, but stronger antioxidant activity
Sugar beet molasses-based ingredients [33]		1677 (µmol TE/100 g)			No interference at lower levels of supplementation	Significant effect on specific volume	Rich mineral content with high protein and crude fibre
Barley flour [34]	280 (mg GAE/100 g dm)		2.5 (mmol Fe/100 g dm)		Good consistency between sensory evaluation and the amount of phenolics		
Garlic and basil [35]	0.278 (mM GAE/g)		0.368 (mM Fe^{2+}/100 g)				
Auricularia auricula flour [36]		57% (DPPH % inhibition)			No significant effect except at very high supplementation levels	Little effect on loaf weight, loaf height and loaf volume up to 9% supplementation	High crude fibre and minerals
Yam flour [37]		79% (DPPH % inhibition)		ABTS 88% (total antioxidant property %)	No interference with bread acceptability	No significant effect at low levels of supplementation	Considerable amount of dietary fibre, ash and starch

ABTS 2,20-azino-bis(3-ethylbenzthiazoline-6-sulphonic acid) radical scavenging activity, *CHP* metal chelating activity, *CMC* carboxy methyl cellulose, *CML* ne-(carboxymethyl)lysine, *dm* dry matter, *DPPH* (di(phenyl)-(2,4,6-trinitrophenyl) iminoazanium radical scavenging activity, *dw* dry weight, *FRAP* ferric reducing antioxidant power, *LPO* inhibition of lipid peroxidation, *ORAC* oxygen radical absorbance capacity, *TEAC* trolox equivalent antioxidant capacity, *TPC* total phenolic content

Black tea

Chinese steamed bread (CSB) was fortified with black tea with various tea to water (v/v) ratios [6]. Increasing the black tea concentration decreased the lightness (L), while increasing the redness (a) and yellowness (b) of CSB, which was attributed to the presence of black tea polyphenols. The addition of black tea greatly increased phenolic content and antioxidant activity along with the antimicrobial properties of CSB, with the best results seen at a 5:1 ratio of tea to water. There were fewer changes in the water activity, water content, texture and volumetric properties of fortified CSB when a lower ratio of tea was employed. The addition of black tea had little effect on sensory scores for brightness, crumb structure, elasticity and stickiness but resulted in decreased whiteness and smoothness.

Rice bran

Stabilised rice bran (RB) was added at different percentages to wheat breads [7]. All substituted breads showed higher protein, ash, fat and total dietary fibre contents than control bread. Inclusion of RB negatively affected crumb colour (L*), bread volume and sensory parameters such as colour, texture and overall acceptability, but improved hardness, aroma, taste, a* and b*. The highest and lowest values were seen in 30% RB substituted bread. Vitamin E content as indicated by tocopherol and tocotrienol content increased in proportion to the amount of RB. The addition of RB also increased the free and bound total phenolic content (TPC), total flavonoid content (TFC), ferulic acid, *p*-coumaric acid, sinapic acid, and ferric reducing antioxidant power (FRAP) and 2,2-diphenyl-1-picrylhydrazyl (DPPH) radical scavenging values compared to control bread, with the highest values seen in 30% RB substituted bread.

Green tea extract and microencapsulated green tea extract

Spray dried (SDMD) and freeze dried (FDMD) green tea polyphenols microencapsulated using maltodextrin were incorporated into bread to enhance its antioxidant properties [8]. Fortified bread had a significantly higher moisture content, a*, b* and ΔE as well as lower L* values than control bread. The reduced volume and increased crumb firmness of incorporated breads were statistically significant. These breads had lower overall sensory, taste, texture, crumb and crust colour scores than the control. Very similar amounts of polyphenols were present in the green tea extract (GTE) and in the encapsulates incorporated in the dough. However, after baking, the amount of polyphenols in incorporated breads was slightly reduced due to the degradation of polyphenols, but was still higher than that in control bread. The highest polyphenol content was seen in FDMD bread after baking and in non-encapsulated bread before baking. It was concluded that fortification of bread with green tea polyphenols can retain the functionality and quality characteristics of bread.

Stevia rebaudiana extracts (var. Morita)

Functional bread was prepared by partial replacement of sucrose with different amounts of *Stevia rebaudiana* extracts (var. Morita) [9]. Incorporated breads were rich in protein and crude fat. The substitution of up to 50% of sugar with stevia extracts did not adversely affect the softness of bread. The breads showed significantly higher antimicrobial activity with the highest value seen at 75% sugar substitution due to reduced sugar content. Multiple comparison tests revealed that sweetness decreased as the level of stevia extract increased. Bread with 50% replacement of sugar was well accepted. These breads had higher total dietary, insoluble and soluble fibre content than control bread, with a significant reduction in the caloric value. Bread containing stevia extract had good scavenging properties and efficiency in reducing polysaccharide digestion, which resulted in lower glucose absorption.

Quinoa leaves

Bread was fortified with different amounts of quinoa leaves (QL) as a source of antioxidants [10].
An increase in the percentage of QL resulted in a bread with decreased loaf volume and starch digestibility, and higher bread crumb hardness, cohesiveness and chewiness/gumminess, with an insignificant effect on elasticity. Bread fortification with QL showed a linear increase in antioxidant properties and TPC and had a positive influence

on FRAP, inhibition of linoleic acid peroxidation and chelating power. The maximum antiradical potential of bread was obtained with 5% QL supplementation.

Nejayote solids

Nejayote, a maize-processing wastewater, in the form of solids was used to enhance antioxidant activity in bread [11]. The addition of different amounts of nejayote solids (NS) to dough significantly enhanced water absorption, bread weight and apparent density with a slight decrease in bread volume, while the optimum mixing time, proof heights and bread heights were not significantly affected. Bread supplemented with NS showed higher dietary fibre, crude fibre and bioavailable calcium: two slices (64 g) of bread supplemented with 9% NS provided 29% of the recommended daily calcium intake. Ferulic acid increased proportionally with increased NS levels and significantly improved antioxidant activity. Flavour and odour received low sensory scores, while overall acceptability, texture and colour were not affected by 9% NS substitution as compared to control.

Broccoli sprout powder

Bread was enriched with various proportions of broccoli sprout (BS) powder for its antioxidant and anti-carcinogenic activity [12]. Partial replacement of flour with BS powder had a strong negative effect on aroma, texture, taste and overall acceptability and a slightly negative effect on colour. However, BS significantly enhanced the phenolic content and lipoxygenase enzyme (LOX) inhibition activity of the breads, with the highest values seen with 2% substitution, although a linear relationship between increased phenolic content and the percentage of added BS was not seen. Fortification resulted in an increase in the level of free amino groups but negatively influenced the antiradical potential, with highest values seen with 1% BS. Reducing power was decreased compared to control, whereas it substantially increased at higher levels compared to lower level of incorporation. The highest chelating activity was seen in bread with 3% BS, but was nevertheless still lower than in the control sample. Inhibition of lipid peroxidation was significantly lower in supplemented bread compared to control. Bread with 2% BS supplementation was determined to be best regarding consumer acceptability, antioxidant activity and ability to inhibit LOX and xanthan oxidase (pro-oxidative enzymes).

Onion skin powder

Bread supplemented with dry onion skin (OS) powder used as a source of antioxidants [13] showed an increase in quercetin concentration, scavenging activity, chelating power, lipid peroxidation and reducing power compared to control bread. As expected, the antioxidant properties (except for lipid peroxidation) of OS-fortified bread and overall acceptability were positively correlated with the addition of up to 2–3% OS. However, bread supplemented with 4–5% OS was less acceptable due to no further increase in antioxidant activity and the presence of excessive amounts of volatile compounds.

Chestnut flour

Different amounts of chestnut flour (CF) were incorporated into bread as a source of antioxidants and fatty acids [14]. Bread with up to 50% CF showed a significant linear increase in antioxidant activity and level of volatiles, including alcohols, followed by aldehydes, ketones and furans. The levels of compounds present in CF, such as furans including furfural, 5-methylfurfural and 2-furfuryl alcohol, increased rose strongly as amount of substitution increased. CF imparted nutty and smoky flavours due to the presence of guaiacol and 4-ethyl-guaiacol, demonstrating a positive effect on organoleptic properties. Bread made with 50% CF showed a homogeneous crumb structure, greater crumb hardness, more cohesiveness and a darker colour compared to control bread.

Banana pseudo-stem flour

A fixed amount of banana pseudo-stem flour (BPF) was used with xanthan gum (XG) or carboxy methyl cellulose (CMC) as a source of antioxidants in bread [15]. Proximate analyses showed that BPF breads had a high content of ash, crude fibre and soluble, insoluble and total dietary fibre, with a balanced insoluble dietary fibre/soluble dietary fibre ratio. The addition of BPF resulted in an increase in phenolic content and free radical scavenging activity compared to control. However, supplementation with XG or CMC in breads containing BPF resulted in a significant decrease in phenolic content and free radical scavenging activity, with 10% substitution having the strongest effect whreas physical analyses indicated that supplementation with 10% BPF with XG reduced loaf volume, weight loss, height and higher density. While all breads containing BPF had a darker crumb and lighter crust colour, mouth feel, aroma, gumminess and taste perceptions were not affected. All breads received better acceptability scores than control bread, with the highest overall acceptability score seen for bread with added CMC.

Fennel seed powder

Various amounts of fennel seed (FS) powder were incorporated into bread as a source of antioxidants [16]. Supplementation significantly affected physical properties, with a negative effect on texture and decreased loaf volume and specific loaf volume, but with an insignificant effect on loaf mass. With increasing addition of FS, the redness (a*) and yellowness (b*) of the bread significantly increased, while lightness (L*) significantly decreased. The appearance of the bread declined slightly, with the optimum taste and aroma scores obtained at 7–10% FS supplementation, while the best colour was achieved with 5% FS supplementation. A linear increase in TPC and FRAP values (but not DPPH radical scavenging activity which showed a reduction at 5% FS incorporation) was seen with increasing supplementation up to 7.0% FS, with further additions having little effect on antioxidant properties. Thus, supplementation with 5.0% and 7.0% FS powder was found to be optimum.

Cinnamon powder

Different amounts of cinnamon powder (CP), which is rich in fibre, fats and ash, and has a high phenol content and antioxidant activity, were added to bread [17]. Baking absorption and loaf weight increased linearly with amount of CP added. Loaf volume and height increased up to 2% CP supplementation and decreased thereafter, while specific volume remained either equal to or less than that of control bread. Appearance and colour scores were high at 2% and 3% CP addition, with flavour, texture and overall acceptability scores highest at 2% CP incorporation. The hardness, gumminess, cohesiveness and chewiness of the bread significantly increased above 2% CP incorporation. In addition, an adequate microbial inhibitory effect was observed due to the presence of cinnamaldehyde and eugenol at 2% CP incorporation. Fibre, ash, protein and fat content, phenolic content and antioxidant activity showed a significant linear increase as the level of supplementation increased with highest values obtained at 4% CP incorporation.

Citrus peel

Unfermented (UF) and fermented (F) citrus peel (CP) was processed under different dry hot-air temperatures to make four CP powders which were incorporated into toasting bread to enrich its antioxidant properties [18]. The water adsorption capacity of the dough increased with greater content of CP powder, resulting in faster toasting time and improved strength and elasticity. The crude fat and crude protein content of the unfermented citrus powder (UFCP) was higher than that of the fermented citrus powder (FCP), with crude fibre inversely proportional to moisture in CP. The UFCP extract showed better antioxidant activity (DPPH and H_2O_2 test) and higher total polyphenols, with the best values seen in UFCP dried at 150°C, indicating enhancement of phenolic compounds and antioxidant activity at higher drying temperatures. Sensory evaluation indicated greater acceptability of UFCP-supplemented bread compared to FCP-supplemented bread. Bread containing UFCP dried at 150°C achieved the highest score for flavour, while bread containing UFCP dried at 100°C had the best scores for hardness and surface texture. Both these breads together with bread containing FCP dried at 100°C achieved high overall acceptability scores, with bread containing FCP dried at 150°C scoring the lowest.

Intermediated pearled wheat fraction

Bread was enriched with different amounts of pearled fractions from intermediate layers of wheat kernel as a source of antioxidants [19]. The L*, a* and hue values (h) of the crust of supplemented bread significantly increased with no difference in chroma (C*) values compared to control. Crust crunchiness, total bread energy and acoustic emissions were highest in the bread containing 0% and 5% pearled fractions with lowest values seen at 20–25% incorporation. A decrease in bread volume with a proportional increase in gumminess, chewiness and hardness accompanied the increasing percentage of pearled wheat fractions, while cohesiveness and resilience remained unaffected. The protein, dietary fibre, β-glucan and ash content along with the total antioxidant activity (measured as DPPH scavenging capacity) and TPC of bread increased linearly and significantly as supplementation increased, with maximum values seen at 25% substitution. However, the alkylresorcinol content was lower than expected, while the deoxynivalenol content increased linearly, indicating 10% substitution was the best level of supplementation.

Maca flour

Maca flour (MF) as a powerful antioxidant was incorporated in different amounts into bread to increase its functional properties [20]. Bread containing MF had considerably increased antioxidant activity, with highest values seen at 20% MF incorporation. Antioxidant properties were determined according to TPC, scavenging effect on DPPH, ABTS and hydroxyl radicals as well as percentage of fluorescence decay inhibition and anti-inflammatory activity. The study also demonstrated that MF could reduce sugar intake as shown by an enzymatic assay where α-amylase and α-glucosidase were progressively inhibited as MF content increased, with maximum values found at 20% MF addition. Results showed that the biological properties of maca were retained after the bread-making process.

Micronized by-products from debranned durum wheat

Different amounts of coarse and fine micronized bran fractions were incorporated both into sourdough and baker's yeast dough to make antioxidant-rich bread [21]. The intermediate and internal layers of durum wheat contain high amounts of protein, fat, starch and free amino acids and have a high peroxide value, while the external fraction has a high total dietary fibre content. Supplementation with up to 5% coarse fraction or bran fraction only changed water absorption capacity, development time and stability very slightly compared to control. However, the concentration of total free amino acids gradually increased with the addition of bran fraction and fermentation of dough. Supplementation and dough fermentation increased total phenol and scavenging activity, with wheat flour bread supplemented with 5% fine fraction having the highest values. The hardness, resilience and fracturability of breads baked at 220°C and enriched with bran fractions were lower than those of control. Bran fractions intensified crust colour, especially with sourdough fermentation, with increased sweetness, dryness and overall perception of taste, while elasticity decreased. Sourdough bread showed significantly higher acid flavour, acid taste and salty attributes for better overall consumer acceptability.

Sorghum flour

Varying amounts of wholegrain white sorghum flour (WSF) and red sorghum flour (RSF), which both provide total dietary fibre, were incorporated into flat bread as a source of antioxidants [22]. The total phenolic content and antioxidant capacity of red sorghum flat bread was significantly higher than that of white sorghum flat bread, which in turn was significantly higher than that of control bread. RSF bread had a higher content of bound phenolics and free phenolics than WSF bread or control bread, resulting in increased TPC. RSF flat breads were darker in colour than controls, possibly due to the presence of higher levels of coloured polyphenolic compounds such as anthocyanins. Breads supplemented with 30% WSF and 40% RSF had the highest scores for sensory parameters like flavour and texture and the best overall acceptability scores.

Barley hull and flaxseed hull

Barley hull extracts, flaxseed hull extracts and both combined were used as sources of antioxidants in Chinese steamed bread (CSB) [23]. The phytochemical profile of the bread improved significantly with the addition of barley hull extracts, flaxseed hull extracts and both combined compared to control bread. Barley hull extract increased the content of ferulic acid and *p*-coumaric acid, and flaxseed hull extract introduced secoisolariciresinol diglucoside (SDG), ferulic acid glucoside (FeAG) and coumaric acid glucoside (CouAG) into CSB, while the combined extracts contributed their major phenolic compounds. The combined extracts significantly increased TPC, DPPH radical scavenging and oxygen radical absorbance capacity (ORAC) values compared to control.

Barley flour and barley protein isolate

Varying amounts of barley flour (BF) and barley protein isolate (BPI) were added as sources of antioxidants to pita bread [24]. The water holding capacity, solubility, emulsion and foaming properties of wheat flour fortified with BPI were superior compared to flour fortified with BF. Similarly, the content of essential and non-essential amino acids increased gradually as the amount of added BF and BPI increased rose from 0 to 15%. Bread made from 15% BPI had the highest levels of cystine, tyrosine, valine and essential amino acids. Protein, fat, ash, dry matter and fibre content increased with increasing supplementation, as did TPC, antioxidant activity, angiotensin converting enzyme (ACE) inhibitor activity and amylase inhibitory activity, with highest values seen at 15% BPI supplementation.

Coriander leaf powder

Varying amounts of coriander leaf powder (CLP) were used for antioxidant enrichment of bread [25]. Incorporation of dried CLP enhanced moisture retention capacity and baking characteristics, showing a higher crumb moisture up to 5% CLP supplementation and a slower staling rate. Physical parameters like crumb firmness and loaf weight increased, while specific volume and loaf volume values decreased linearly with increased supplementation. Similarly, as supplementation increased, the L*, a* and b* values of the crust decreased, while crumb L* values decreased and a* values increased, indicating a darker colour. TPC, DPPH radical scavenging activity and FRAP assay results showed a sharp and almost linear increase with level of supplementation. The aroma acceptance scores gradually increased, while colour and taste were less acceptable with high CLP supplementation. Consequently, the overall acceptability of 3% and 5% CLP-substituted bread samples was much better than those with higher levels of supplementation.

Ginger powder

Different amounts of ginger powder (GP) were used to obtain antioxidant-rich bread [26]. The addition of GP enhanced the elasticity of dough. Hardness and gumminess were significantly higher with 6% GP supplementation, although changes in dough characteristics and bread rheological properties were insignificant up to 4.5% GP supplementation. Breads had a fine and uniform crumb. A higher percentage of ginger lowered the L* and h values and elevated the C* value of crumb. The amount of phenolics and radical scavenging activity increased in proportion with increased GP content of both crumb and crust, but to a great extent in crust. Addition of the lowest percentage of GP (3%) resulted in bread with the highest overall acceptability as it had the least amount of shogaol.

Turmeric powder

Varying amounts of turmeric powder (TP) were used as a source of antioxidants in wheat bread [27]. Despite a loss of 32–54% of TPC after baking, the breads had a significantly higher phenolic content due to the presence of curcumin. Scavenging activity, thermal stability and antioxidant properties as measured by β-carotene-linoleate bleaching assays improved as supplementation was increased to 2–8% TP compared with control breads. The taste and overall acceptability of breads with turmeric powder was best at 0–4% TP supplementation, while aroma and texture did not vary. The best crumb colour was seen at 2% TP supplementation.

Wholegrain flour and fibres

Wheat, rye, barley and oat wholegrain flours and cellulose (insoluble fibre) and xanthan gum (soluble fibre) in different proportions were added to wheat bread [28]. Bread containing oats had the highest levels of free phenolics, while rye bread had the highest level of bound phenolics and the best scavenging capacity. The antioxidant properties of breads containing cellulose and xanthan gum were very similar to those of control bread, indicating that inclusion of fibre does not affect the antioxidant properties of high fibre bread. Incorporation of wholegrain and fibre increased total dietary fibre, while the addition whole wheat flour increased protein content. In general, there was a marked reduction in average specific and loaf volume. The overall quality of wholegrain-rich breads was acceptable except for that with added cellulose.

Anka (fermented rice)

Monascus-fermented rice (anka, red koji) flour was incorporated into bread to enhance its antioxidant properties [29]. Bread enriched with anka flour had a slightly lower specific volume and fewer carbohydrates but higher amounts of reducing sugars, fat and fibre than rice and wheat breads. Anka-enriched bread had an attractive red colour with lower L* and whiteness index (WI) values and higher a* and b* values than rice and wheat breads. White bread and anka-enriched bread showed comparable antioxidant activity but were slightly less effective than rice-enriched bread regarding reducing power and scavenging ability as measured by the elimination of DPPH radicals and EC_{50} values. Substantial amounts of the functional components monacolin K and GABA were found in anka-enriched bread, which slightly decreased during baking. The colour and mouth feel of anka-enriched bread were rated higher, but there were no differences in its appearance, flavour and overall sensory attributes compared to white bread.

Grape by-product

Grape by-products (GP) containing very large amounts of ash and total dietary fibre were incorporated in different amounts to enhance the antioxidant properties of rye bread [30]. The levels of some phenolic compounds together with free radical scavenging activity (DPPH) and total antioxidant activity (FRAP) values increased gradually and significantly with increasing amounts of GP. The improved antioxidant properties of GP-enriched breads were attributable to the incorporation of phenolic compounds, mainly catechins and procyanidins. Considerable amounts of quercetin and quercetin-3-β-D-glucoside were also found. The hardness and gumminess of breads increased significantly with increasing amounts of GP, while insignificant changes were seen in cohesiveness, resilience, chewiness and springiness up to 8% GP supplementation. Sensory evaluation showed that as GP level increased, bread volume, porosity and overall acceptance decreased. Differences in aroma attributes were due to dominant fruity, alcoholic and sharp notes. However, different types of volatile esters and carbonyl compounds increased gradually.

Pseudocereal flour

Varying amounts of different pseudocereal (buckwheat, amaranth and quinoa) flours were used as a source of antioxidants for bread [31]. The incorporation of pseudocereal flour improved the antioxidant and scavenging properties of bread compared to control wheat bread. The addition of buckwheat flour improved antioxidant activity more effectively than amaranth or quinoa flour. Bread supplemented with 30% buckwheat flour had the highest phenolic content, TFC and Trolox equivalent antioxidant capacity (TEAC) values as evaluated by FRAP and DPPH, while bread supplemented with 15% buckwheat flour showed the highest flavonoid content. Organoleptic evaluation of pseudocereal-substituted breads indicated they were moderately acceptable. Buckwheat bread had a better sensory profile than amaranth and quinoa breads with higher scores for colour and odour.

Grape seed extract

Grape seed extract (GSE) added to bread linearly enhanced antioxidant activity with increased GSE [32]. However, baking degraded GSE proanthocyanidins, thus lowering GSE antioxidant capacity. No significant differences in hardness were observed between bread samples, but with increased addition of GSE, L* and the E index decreased while a* and b* values increased, indicating that GSE affected bread colour. Addition of GSE did not affect quality attributes such as sweetness, porosity, astringency and stickiness but did improve bread colour compared to the control. The catechins and proanthocyanidins in GSE greatly enhanced the antioxidant activity of bread. The abundant phenolics in GSE may help reduce the health risks associated with Ne-(carboxymethyl) lysine (CML).

Sugar beet molasses-based ingredients

Fruit and vegetable powders obtained with osmotic dehydration (OD) in beet molasses and pure beet molasses were incorporated into white bread to increase its antioxidant properties [33]. The OD vegetable powders contained significantly more protein, crude fibre, reducing sugars, saccharose, K, Na, Mg and Ca than the OD fruit powders. Bread supplemented with 10% OD red cabbage powder had the highest total moisture, ash, protein, crude fibre and mineral content, while bread supplemented with 10% OD plum powder had the highest crumb moisture, and bread supplemented with 10% OD apple powder had the highest reducing sugar content. The addition of molasses and molasses-based ingredients significantly increased the antioxidant activity of bread, with the highest values observed in bread formulated with 10% OD plum powder. Specific volume greatly decreased and colour darkened with increased supplementation. The crumb and crust of bread supplemented with 10% OD powders and molasses showed a decrease in yellowness (b*) compared to bread made with 5% OD powders. Increased supplementation resulted in a gradual but significant increase in crumb pore roughness and a decrease in crumb elasticity. The taste attributes of bread supplemented with 5% OD were acceptable.

Barley flour

Varying amounts of barley flour (BF) from different varieties (Tyra, Cindy and STS 2-11) were added to wheat flour to make breads rich in antioxidants [34]. There were significantly more free phenolics consisting of flavonols and tocopherol than bound phenolics, including phenolic acids, in supplemented bread. The bread supplemented with the Tyra variety had significantly higher levels of free phenolics and total antioxidant activity than control bread. The total amount of phenolics and total antioxidant activity were highest in breads supplemented with the Tyra and Cindy varieties. During baking, free phenolics decreased, while bound phenolics greatly increased and total antioxidant activity only changed slightly, with highest values seen in the Cindy and Tyra supplemented breads. The antioxidant properties of breads depended on the barley variety and the flour extraction rate. Other factors such as storage and baking procedure were less significant but did affect sensory attributes. Tyra-supplemented bread was the least acceptable with the highest scores for bitterness, off-odour and off-flavour, followed by the Cindy variety.

Garlic and basil

Garlic and sweet basil were added in different amounts to bread [35]. Total antioxidant activity (TAC) and polyphenol content were higher in the garlic and basil-flavoured breads than in white bread, and increased with increased levels of supplementation. Basil-flavoured bread showed higher TAC and polyphenol content than garlic-flavoured bread, with the maximum antioxidant activity seen in bread supplemented with 1.5% basil. Bread made with garlic and basil could be a source of antioxidants in the diet.

Auricularia auricula polysaccharide flour

Various amounts of *Auricularia auricula* (commonly called black woody ear or tree ear fungus) polysaccharide flour (AAPF) were added to wheat bread [36]. AAPF has a high content of crude fibre, carbohydrates and minerals such as Fe, Ca, P and Mg compared to wheat flour. AAPF-supplemented bread showed a marked increase in concentration-dependent scavenging activity, which increased as supplementation increased. Up to 9% AAPF could be added without affecting the sensory qualities of the bread. Loaf weight, loaf volume and loaf height were slightly affected by 9% supplementation, but significantly negatively affected by 12% supplementation. Sensory characteristics like aroma, texture, taste and mouth feel were not affected at supplementation levels of 3–12%, except for colour which was affected by 12% supplementation. Bread made with 9% AAPF was considered the most suitable.

Yam flour

Different amounts of yam flour (YF) were added to wheat bread. YF contains a lot of ash, crude fibre and nitrogen free extract (NFE), the latter mainly as starch [37]. Loaf weight, loaf volume and loaf height showed no significant changes at 5% supplementation, while maximum changes were seen at 25% substitution. Free radical scavenging activity and total antioxidant activity showed a marked increase as the proportion of YF in the breads increased. The sensory score for colour increased as substitution increased. No statistically significant difference was observed in other sensory characteristics for bread made with up to 20% YF compared to wheat bread.

Factors affecting the antioxidant properties of bread

This literature review shows that supplementation of wheat flour with sources of natural antioxidant can produce a bread suitable for the functional food market. The quantity and quality of the final product are determined by different factors, as shown in Table 1. Enhancement of antioxidant properties mainly depends on the phenolic content of the natural sources and the amount of supplementation. Total phenolic and flavonoid content shows a positive correlation with antioxidant activity as seen in the case of *Lens culinaris* Medikus seeds [38], and so is used to determine product quality. A similar trend was observed when fennel seed powder, black tea, chestnut flour, banana pseudo-stem flour and yam flour were added to bread. However, antioxidant activity did not increase with higher levels of supplementation when broccoli sprout powder was used.

Free radical scavenging properties are generally evaluated using the DPPH (lipophilic stable free radicals), ABTS (hydrophilic radicals) and hydroxyl radical methods, as well as other experimental methods such as inhibition of lipid peroxidation and metal chelating activity evaluation. Increased FRAP, inhibition of lipid peroxidation and improvement of antiradical potential were shown when flour was supplemented with 4% and 5% quinoa leaves. Chelating power was also improved but only with 2% supplementation. This type of non-linear relationship may be partially explained by interactions between food components (especially phenolics, protein and starch) and gastrointestinal fluid [10]. A positive correlation was observed between increased levels of broccoli sprout powder and onion skin powder and protein–phenolic interactions. In a continuation of the chemical reaction, free amino acids were reduced and resistant starches increased, with a negative effect on protein and starch digestibility, highlighting the importance of adding the correct amount of functional ingredients. This finding may be due to the fact that flour samples with a low phenolic content are used at up to 20–40% supplementation levels, while spices, herbs and other extracts such as green tea and grape seed with a high phenolic content, are added at much lower supplementation levels of 2–10%.

Further *in vivo* studies on the production and marketing of naturally fortified antioxidant-rich breads are required. In previous research, bread enriched with a 0.5% nutraceutical compound (capsaicin extracted from red peppers, magnesium from barley germ and minerals) and eaten with salad when included in the diet for 3 months showed a significant increase in hydrosoluble antioxidants and decrease in oxidative stress in subjects compared to control [39]. This research also demonstrated the benefits of multicomponent functional food enriched with antioxidant compounds obtained from plant extracts.

Individual antioxidant compounds have been analysed in previous studies. Like vitamin E, ferulic acid, *p*-coumaric acid, sinapic acid from rice bran, curcumin from turmeric, and quercetin from onion skin improve the functional properties of bread. Antioxidant profiles can be studied using a method combining spectrophotometry and HPLC [40]. Antioxidant activity is determined as the total effect of free and bound phenolic compounds. In wholegrain products like bread, baking resulted in an increase in free phenolic acids but a decrease in bound phenolic acids with ferulic acid as the principal phenolic [41]. In bread supplemented with rice bran, both free and bound phenolics increased, but barley free phenolics decreased due to the interaction of phenolic compounds with proanthocyanidins and minerals such as iron. In contrast, fermentation and baking showed a positive effect by liberating bound ferulic acid in bread supplemented with nejayote. The loss of antioxidant properties during cooking can be avoided by the use of the microencapsulation technique as shown when supplementation with green tea extract is compared to supplementation with microencapsulated green tea extract. Thus, although baking reduces antioxidant activity, fortification results in increased antioxidant activity compared to unfortified breads.

The types of agents used in fermentation, an essential step in bread making, also influence antioxidant activity by altering the fermenting agent. In brewer's spent grain breads, sourdough and enzymes did not change the phenolic profile but did increase the in vitro antioxidant activity of breads [42]. The addition of legume (chickpea, lentil and bean) flours to wheat flour bread with a sourdough fermentation agent containing lactic acid bacteria showed a marked increase in DPPH radical quenching ability and phytase activity [43]. Similarly, when a micronized by-product of durum wheat was added to bread, better nutritional quality was observed in sourdough bread compared with yeast-fermented dough. In contrast, *Monascus* mould improved bread enriched with anka (fermented rice) by supplying monacolin K, GABA and dietary fibre with beneficial health effects. However, fermented citrus peel showed lower antioxidant activity than unfermented citrus peel when added to bread. Newer stress-resistant yeast strains producing antioxidant enzymes are being developed to enhance the fermentation ability of bread dough [44].

Finally, baking of bread in an oven or on a hearth significantly affects its phenolic profile and scavenging activity due to the formation of new products from sensitive compounds like polyphenols, reducing sugars and protein. The Maillard reaction and caramelization are the main chemical reactions during baking. The Maillard reaction is a chemical reaction between amino acids and reducing sugars that gives browned food its desirable flavour and in baking causes the formation of a brown crust. Maillard reaction products also contribute to antioxidant activity. In bread fortified with intermediated pearled wheat fraction, more TPC content was found than supplied by the raw materials due to formation of new intermediate phenolic products through the Maillard reaction. The antioxidant activity of rye bread, especially in the crust, was also affected by the Maillard reaction occurring during baking. Advanced Maillard reaction products resulted in good scavenging of peroxyl and ABTS radicals and increased TPC, enhancing the formation of antioxidants during rye bread baking [45]. Typical chestnut flour volatiles such as furans and pyrroles were formed due to same type of reaction, producing toasty and nutty notes which improved consumer acceptability. Also, natural antioxidant sources like grape seed extract showed inhibitory effects on the formation of the detrimental compound CML in crust during baking. The interaction between phenolics (flavonoids) and protein affects the antioxidant efficacy of flavonoids, as observed following onion skin fortification of bread [46].

Apart from their antioxidant activity, natural sources of antioxidants affected the digestive system. Negative effects resulting from the binding of phenolic compounds to protein and starch have been mentioned above. Similarly, inhibition of enzymes (protease and amylase) also reduces protein and starch digestibility, resulting in increased levels of free amino groups and resistant starch [10]. The anti-inflammatory activity associated with maca-enriched bread, in vitro release of catalase activator and the anticancer activity of broccoli sprout-fortified bread demonstrate the dynamic role of natural antioxidant sources. Supplementation of wheat flour usually increased dietary fibre, which consequently affected glucose absorption. *S. rebaudiana* extracts added to wholegrain flours decreased glucose absorption by increasing dietary fibre, while the addition of maca flour had an inhibiting effect on α-amylase and α-glucosidase, thus also hindering glucose absorption. Different types of fibre have different impacts. When whole wheat flour, which has a high content of insoluble dietary fibre, was supplemented with cellulose, the amount of resistant starch increased while the amount of digestible starch decreased, thus delaying starch digestion.

In contrast, barley, oat and rye contain higher levels of soluble dietary fibre and so have less effect on lowering glucose absorption [28].

The type of wheat flour used was also a major factor affecting antioxidant activity. The effect of the coat colour of wheat was studied. Investigations showed that red and white wheat grain flour showed different phenolic acid profiles despite having comparable total phenolic acid content (TPAC) [47]. A similar effect was observed when red and white sorghum flours were added to wheat bread. Kisra, a traditional flat Sudanese bread, was prepared using two different sorghum cultivars. The two types of bread showed significantly different FRAP and DPPH values, which were both enhanced by baking, with increased fermentation time also resulting in higher values and hence increased antioxidant activity [48]. Therefore, a change in type of dough flour can lead to a remarkable change in antioxidant activity. Another investigation found that, despite loss of TPC and antioxidant activity following baking, breads containing pseudocereals showed significantly higher antioxidant capacity than bread made only with wheat flour [49].

The physical properties of a fortifying agent have a combined effect on overall acceptability. Gluten quality and quantity are important characteristics of bakery flour. Gluten quantity decreases when natural sources of antioxidants such as turmeric, ginger and coriander powder are added to wheat flour. High fibre and gluten-free flours such as rice bran, banana pseudo-stem, chestnut and sorghum, and spices such as fennel and cinnamon affect the gluten chain by diluting it with fibre and fibre-induced increased water absorption capacity. With a decreased bread volume, high moisture content also effects bread quality negatively. Consequently, a higher level of coriander supplementation resulted in improved bread due to the lower level of fibre. The addition of microionized by-products of debranned durum wheat to sourdough also increased loaf volume compared to the same supplementation of yeast dough.

Hydrocolloids like CMC when added to banana pseudo-stem-fortified bread improved physical properties by balancing soluble and insoluble dietary fibre content with decreased antioxidant activity. The hydrocolloid-like properties of naturally formed alkali-treated fibre in nejayote solids also provide another method of improving bread.

The darkness of crumb and crust is directly proportional to phenolic content and Maillard reaction. A higher level of reducing sugar in chestnut flour and higher levels of protein in rice bran increased the darkness of bread crust, while the crumb colour was affected by turmeric and coriander.

Another important parameter is taste which is perceived as flavour combined with aroma. The acceptability of the sweet aroma of fennel seed, the pungency of ginger and the woody notes of chestnut is related to the amount of fortification. Differences in processing like employing sourdough instead of yeast fermentation, masked the inferior flavour of grape by-product and improved the sensory profile of bread containing microionized by-products from debranned durum wheat. At increased level of *Stevia rebaudiana* the product becomes less sweet than control due to the presence of chlorophyll pigment and glycosides. In general, higher levels of fortification with natural sources of antioxidants negatively affect bread quality, so it is important to identify of optimum levels of supplementation.

In addition to the various processing parameters, storage conditions also affect antioxidants in bread. One study showed that the TPC and oxidative stability of bread samples decreased during storage depending on the manufacturing process. However, frozen dough bread lost less phenolics and had higher oxidative stability compared to frozen baked bread [50]. This indicates the fortification ingredient as well as various environmental, physical and chemical factors affect the antioxidant capacity of breads enriched with natural sources of antioxidants.

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Conflicts of Interest

The authors report no conflict of interest. The authors alone are responsible for the content and writing of the paper.

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