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Barley and Malt as Base Ingredients for the Production of New Bio-Functional Foods

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Abstract

This work aimed to evaluate the chemical composition, bioactive compounds (phenolics and γ-aminobutyric acid, GABA), and antioxidant properties of different barley varieties (Overture, Charles, Sinfonía, Montoya, and Andreia) and their malts to weigh up them as potential ingredients for producing new bio-functional foods. For this, five barleys and five malts obtained from them were studied. Regarding chemical composition, total starch was the main component (≈62%) of barleys followed by total dietary fiber (≈22.6%) and proteins (≈9.5%). Potassium and phosphorus were the most abundant elements, with mean values being 3746.1 and 3679.1 g $100g^{-1}$d.w., respectively. Regarding the free amino acid profile, the proportion of hydrophobic free amino acids was higher than that of branched-chain amino acids or sulfur-containing amino acids and the mean value of GABA was 8.8 mg $100g^{-1}$. Ferulic acid was the most abundant free phenolic acid detected in the different barleys, followed by coumaric acid. All barley extracts showed ABTS and DPPH inhibitory activities and ferric-reducing antioxidant power (FRAP). As expected, total starch, total dietary fiber, and crude fat contents of malts were lower than those found for barley. However, the malting process increased GABA, ferulic acid, hydrophobic free amino acids, branched-chain

amino acids, and sulfur amino acid contents. Additionally, the antioxidant properties of malts were higher than those obtained for barleys. Barley flour could be successfully used as a bio-functional ingredient in many foods. Furthermore, given the high content of soluble solids (mainly carbohydrates, antioxidant compounds such as free phenolic acids and free amino acids, and GABA), malted flours would be novel ingredients for preparing beverages with bio-functional properties.

Keywords
Barley; bio-functional malt; GABA; phenolics acids; ABTS and DPPH inhibition; FRAP

1. Introduction

Barley (*Hordeum vulgare*, vulgare L.) is a grass belonging to the family Poaceae, the tribe Triticeae and the genus Hordeum. Globally, barley ranks fourth after wheat, rice, and corn in cereal crops, with a production of approximately 148 million tons under the area of 49 million ha in 2021 [1]. The top largest barley producers in 2021 were the Russian Federation, Australia, and France, which accounted for 30% of global production [2].

Barley is used primarily as animal feed (70%), and around 30% of the world's barley production is utilized for malting purposes. However, this cereal is also employed to a lesser extent as flour for human consumption [3]. In this regard, many traditional dishes in Russia, Poland, Tibet, Japan, and India are prepared with barley flour. In Western countries, pearled barley, whole, flaked, or flour are used in breakfast cereals, stews, soups, porridge, bakery flour blends, and baby foods [4]. Recently, there has been a growing interest in using barley for food production due to its various health effects, such as lowering blood cholesterol, regulating glycemic index, and antioxidant activity [5]. Mainly, hull-less barley as source of soluble and insoluble fiber receives considerable attention for developing functional foods with hypoglycemic and hypocholesterolemic properties [6]. Regarding this, FDA and EFSA have approved f health claims for β-glucans of barley and oats for reducing cholesterol and controlling the glycemic response [7]. Moreover, barley's phytochemicals (phenolic acids, flavonoids, lignans, phytosterols, and vitamins like B9 and E) are also involved in the health benefits [8].

Barley malting is the most widely known controlled germination process used to produce malt for brewing purposes and food applications [9]. Germination is a complex procedure triggering the grains' physical, chemical and structural changes. It has been identified as an inexpensive and effective technology for improving cereal quality [6]. In addition to improving the flour's nutritional properties, germination increases the bioactivity of malted flour, which is very important due to the increasing interest in natural products with bioactivity [10]. The studies in barley have shown an increment of γ-Aminobutyric acid (GABA) in soaked and germinated grains [11, 12]. GABA is a four-carbon non-protein amino acid occurring in plants and animals [13], which play an important role as neurotransmitter in mammal's brain cells [14]. On the other hand, most barley phenolic compounds have also been identified in malt, which implies that natural antioxidants present in barley make a large contribution to the antioxidant activity of malt [15].

Moreover, malt antioxidants play an essential role in preserving the oxidative stability of beer or malt products but are also crucial to the consumer's health [16].

Despite the functional properties of barley and its derivative products, human consumption of food products containing this cereal and malt has been negligible compared to other grains [4]. Therefore, the development of new products has been neglected. This work aimed to evaluate the chemical composition, the bioactive compounds and the antioxidant properties of different barleys and their malts to revalue them as base ingredients for the production of new bio-functional foods.

2. Materials and Methods

2.1 Raw Materials

The barley and malts were provided by Boortmalt Argentina S.a.U (Punta Alvear 2121, Rosario, Argentina). Five varieties were analyzed: Overture (741), Charles (745), Sinfonía (245), Montoya (753), and Andreia (758).

The barleys were soaked in the following sequence: 8 h steeping-15 h resting-2 h steeping-2 h resting. Malting conditions for germination and drying were shown in Table 1.

Table 1 Malting conditions.

Malt	Germination	Drying
741	20°C - 93 h	60°C - 8 h→68°C - 2 h→73°C - 1 h→80°C - 2 h→84°C - 1 h→90°C - 1 h→85°C - 1 h→85°C - 2 h
745	20°C - 95.5 h	58°C - 8 h→68°C - 2 h→73°C - 1 h→80°C - 2 h→84°C - 1 h→90°C - 1 h→85°C - 1 h→85°C - 2 h
245	20°C - 95.5 h	58°C - 8 h→68°C - 2 h→73°C - 1 h→80°C - 2 h→84°C - 1 h→90°C - 1 h→85°C - 1 h→85°C - 2 h
753	20°C - 78 h→ 15°C - 10 h	58°C - 8 h→68°C - 2 h→73°C - 1 h→80°C - 2 h→84°C - 1 h→90°C - 1 h→85°C - 1 h→85°C - 2 h
758	17°C - 109 h	58°C - 8 h→68°C - 2 h→73°C - 1 h→80°C - 2 h→84°C - 1 h→90°C - 1 h→85°C - 1 h→85°C - 2 h

Varieties: Overture (741), Charles (745), Sinfonía (245), Montoya (753), Andreia (758).

The sample size of barley was the following: from 550 ton batch, a 20 kg sample was taken by compensated sampling, and from this, a 1 kg sample was taken per cone and quarter method. After the mixing, a piece of 200 g was taken per cone and quarter method and milled. Regarding malt, from 480-ton batch, a 20 kg sample was taken by compensated sampling, and from this, 1 kg sample was taken per cone and quarter method. After the mixing, a sample of 200 g was taken per cone and quarter method and milled. Both barley and malt grains were milled using a cyclone sample mill (Belt Drive UD3010 UDY Corporation, Colorado, USA) with a sieve of 1 mm.

2.2 Reagents

Amino acid standard solution, α-amino butyric acid, γ-amino butyric acid, diethyl ethoxymethylenemalonate, 2,2-Azino-bis(3-ethylbenzothiazoline-6-sulfonic acid) diammonium

salt (ABTS), 2,2-Diphenyl-1-picrylhydrazyl (DPPH), 2,4,6-Tris(2-pyridyl)-s-triazine(TPTZ),(±)-6-Hydroxy-2,5,7,8-tetramethylchromane-2-carboxylic acid (TROLOX), caffeic acid, ferulic acid, p-coumaric acid and sinapic acid were obtained from Sigma Chemical Co. (St Louis, MO, USA). Other reagents were of analytical grade and obtained from Cicarelli Laboratorios (San Lorenzo, Santa Fe, Argentina).

2.3 Chemical Composition, γ-aminobutyric Acid, Free Amino Acid and Free Phenolic Acid Content

2.3.1 Chemical Composition

Protein, moisture, and ash content were determined according to AACC methods [17]. Total starch and soluble carbohydrates of malts and amylose content were measured according to Albarracín and Drago [18] and Belhadi et al. [19], respectively. Crude fat was determined by the AOAC method [20]. Total dietary fiber (TDF) was assessed using the Megazyme kit®. The contents of iron, zinc, calcium, and magnesium were determined by atomic absorption (Analyst 300-Perkin Elmer IL 551, Instrumentation Laboratory, Norwood, Massachusetts, USA). Sodium and potassium contents were determined by flame photometry, and phosphorus content by the AOAC method [20]. The determinations were made in triplicate.

2.3.2 γ-aminobutyric Acid and Free Amino Acid Content

To determine γ-amino butyric acid (GABA) and free amino acids, samples (0.2 g) were extracted with 8 g 100 mL^{-1}trichloroacetic acid, shaken for 60 min, and centrifuged at 3000 *xg* for 10 min using an Eppendorf centrifugue (Cabour 1675-D, Argentina). The supernatant (0.5 mL) was added with 1.5 mL of borate buffer (1 $molL^{-1}$, pH 9.0). The contents of GABA and free amino acids were determined according to Alaiz et al. [21] after derivatization with diethyl ethoxymethylenemalonate using D, α-L-amino butyric acid as the internal standard. The HPLC system consisted of a LC-20AT Prominance Liquid Chromatograph (Shimadzu Co., Kyoto, Japan) equipped with a 300 × 3.9 mm i.d. reverse-phase column Novapack C18 4 m (Waters®, Milford, Massachusetts, USA). The Shimadzu LC solution software was used for processing data. GABA was expressed as mg 100 g^{-1}d.w., using a concentration-response curve of 0-325 nmol GABA mL^{-1}. Free amino acid content was expressed as mg 100 g^{-1}d.w. Moreover, total branched chain amino acids, sulfur amino acids and hydrophobic amino acids were also calculated and expressed as mg 100 g^{-1}d.w. The analysis was made in triplicate.

2.3.3 Free Phenolic Acid Profile

To determine free phenolic acids, samples were extracted with distilled water at 4 g 100 mL^{-1}, shaken for 60 min at room temperature and centrifuged at 3000 *xg* for 10 min. The content of cinnamic acid derivatives of supernatant was determined according to Van de Velde et al. [22] using a Shimadzu Series LC-20AT pump with a Shimadzu SPD-M20A diode array detector (Shimadzu Co., Kyoto, Japan), equipped with a 250 × 4.6 mm i.d. reversed-phase column (Gemini 110A C-18 Phenomenex column). Experimental results were analyzed using Shimadzu LC solution software. Phenolic acid content was expressed as µg 100 g^{-1}d.w. All determinations were made in triplicate.

2.4 Antioxidant Properties

To determine the antioxidant properties, samples were extracted with distilled water at 4 g 100 mL^{-1}, shacked for 60 min at room temperature, and centrifuged at 3000 *xg* for 10 min using an Eppendorf centrifugue (Cabour 1675-D, Argentina). The ABTS and DPPH inhibition of samples was measured according to Cian et al. [23]. Ferric reducing antioxidant power (FRAP) of samples was determined according to Benzie and Strain [24]. ABTS and DPPH inhibition were expressed as percentages, while FRAP was expressed as µmol Trolox mL^{-1}. All determinations were made in triplicate.

2.5 Hydration Properties of Barleys and Malts

The swelling power, solubility at 95°C, and water absorption of samples were determined according to Albarracín and Drago [18]. All determinations were made in triplicate.

2.6 Statistical Analysis

All results were expressed as mean ± standard deviation. The data were analyzed by one-way analysis of variance using the software STATGRAPHICS Centurion XV 15.2.06 (Statpoint Technologies, Inc., Warrenton, VA, USA). The statistical differences between samples were determined using the least significant difference test (LSD test). The significance was established at $P < 0.05$. Pearson correlation tests were applied to correlate the antioxidant properties with total cinnamic acid derivatives content. Total branched-chain amino acids, sulfur amino acids, and hydrophobic amino acids of barley and their respective malts were compared by a *t*-test analysis.

3. Results

3.1 Chemical Composition

The proximate composition of the different barleys and malts is shown in Table 2. The barley protein content varied from 8.9 to 10.2 g 100 g^{-1} d.w., being similar to that of the malts (9.3 to 11.2 g 100 g^{-1} d.w.). Moreover, there were no significant differences in protein content among samples ($p > 0.05$). Similarly, there were no significant differences in ash content among samples ($p > 0.05$).

Table 2 Proximate compositions of the different barleys and their malts.

Samples		Moisture (g 100g^{-1})	Protein (g 100 g^{-1}d.w.)	Crude fat (g 100 g^{-1}d.w.)	Ash (g 100 g^{-1}d.w.)	Total dietary fiber (g 100 g^{-1}d.w.)
Barley	**741**	12.3 ± 0.1^{b}	8.9 ± 0.4	1.7 ± 0.0^{b}	2.1 ± 0.0	22.3 ± 1.7^{b}
	745	11.2 ± 0.0^{b}	9.8 ± 0.1	1.8 ± 0.0^{b}	2.0 ± 0.1	23.8 ± 0.3^{b}
	245	10.9 ± 0.0^{b}	9.5 ± 0.2	1.9 ± 0.0^{b}	2.1 ± 0.0	23.4 ± 1.0^{b}
	753	11.2 ± 0.0^{b}	9.1 ± 0.0	1.8 ± 0.0^{b}	2.1 ± 0.2	21.4 ± 1.3^{b}
	758	11.7 ± 0.1^{b}	10.2 ± 0.2	1.8 ± 0.0^{b}	2.2 ± 0.1	22.1 ± 0.6^{b}
Malt	**741**	4.9 ± 0.1^{a}	9.3 ± 0.2	1.5 ± 0.0^{a}	2.2 ± 0.0	15.0 ± 1.0^{a}
	745	4.9 ± 0.0^{a}	9.7 ± 0.0	1.5 ± 0.0^{a}	2.1 ± 0.0	16.5 ± 0.6^{a}

245	4.8 ± 0.0[a]	9.7 ± 0.1	1.5 ± 0.0[a]	2.1 ± 0.0	16.8 ± 0.5[a]
753	5.3 ± 0.1[a]	9.8 ± 0.0	1.6 ± 0.0[a]	2.1 ± 0.1	16.5 ± 0.6[a]
758	5.0 ± 0.1[a]	11.2 ± 0.2	1.5 ± 0.0[a]	2.1 ± 0.0	16.0 ± 0.2[a]

Media ± standard deviation (SD). Different superscript letter in a column means significant differences among samples ($p < 0.05$). d.w.: dry weight. Varieties: Overture (741), Charles (745), Sinfonía (245), Montoya (753), Andreia (758).

The chemical analysis confirmed that the crude fat content of barleys was higher than that obtained for malts ($p < 0.05$). Similarly, the barleys showed the highest TDF content ($p < 0.05$), which ranged from 21.4 to 23.8 g 100 g^{-1}d.w. and 15.0-16.8 g 100 g^{-1}d.w. for barleys and malts, respectively.

On the other hand, the most abundant component in barleys and malts was starch (Table 3). Moreover, the total starch content of barleys was higher than that found for malts ($p < 0.05$). However, there were no significant differences in amylose content among samples ($p > 0.05$). The soluble carbohydrates of malts ranged from 7.2 to 12.3 g 100 g^{-1}d.w.

Table 3 Total starch and amylose content of the different barleys and malts.

Samples		Total starch (g 100 g^{-1}d.w.)	Amylose (g 100 g^{-1}d.w.)	Amylose/starch (%)
	741	65.2 ± 1.6[b]	15.9 ± 0.1	24.5
	745	62.0 ± 1.8[b]	15.8 ± 0.2	25.5
Barley	**245**	60.5 ± 2.3[b]	13.5 ± 0.6	22.3
	753	59.6 ± 2.9[b]	15.2 ± 0.6	25.5
	758	61.1 ± 2.2[b]	16.5 ± 0.4	27.0
	741	48.7 ± 1.2[a]	12.3 ± 0.3	25.3
	745	50.0 ± 1.3[a]	16.1 ± 1.3	32.2
Malt	**245**	47.4 ± 2.2[a]	13.7 ± 0.4	28.9
	753	51.5 ± 0.7[a]	13.1 ± 1.1	25.4
	758	43.0 ± 1.0[a]	12.8 ± 0.6	29.8

Media ± standard deviation (SD). Different superscript letter in a column means significant differences among samples ($p < 0.05$). d.w.: dry weight. Varieties: Overture (741), Charles (745), Sinfonía (245), Montoya (753), Andreia (758).

Iron, zinc, calcium, magnesium, phosphorus, potassium, and sodium content of the different barleys and malts are shown in Table 4. Except for the phosphorus and potassium content, there were no significant differences in mineral content among samples ($p > 0.05$). The phosphorus content of malts (3849.1-4121.6 mg 100 g^{-1}d.w.) was higher than that found for barleys (3447.5-3955.0 mg 100 g^{-1}d.w.). In contrast, barleys showed higher potassium content than malts (3473.0-4077.3 mg 100 g^{-1}d.w. and 3145.8-3267.8 mg 100 g^{-1}d.w. for barleys and malts, respectively).

Table 4 Mineral content of the different barleys and malts.

Samples		Fe (mg kg^{-1}d.w.)	Zn (mg kg^{-1}d.w.)	Ca (mg kg^{-1}d.w.)	Mg (mg kg^{-1}d.w.)	P (mg kg^{-1}d.w.)	K (mg kg^{-1}d.w.)	Na (mg kg^{-1}d.w.)
	741	31.7 ± 0.3	19.9 ± 0.3	329.7 ± 5.07	1043.3 ± 88.4	3447.5 ± 222.3^{a}	3655.4 ± 41.0^{b}	140.3 ± 5.3
	745	35.6± 4.0	17.4 ± 2.1	264.2 ± 29.1	953.2 ± 66.2	3518.1 ± 185.4^{a}	3473.0 ± 19.6^{b}	149.5 ± 1.4
Barley	**245**	32.7 ± 0.3	19.7 ± 0.7	279.2 ± 1.1	1456.4 ± 88.6	3904.6 ± 30.6^{a}	4077.3 ± 49.8^{b}	68.8 ± 1.1
	753	31.7 ± 1.5	18.4 ± 1.1	284.0 ± 19.6	1293.8 ± 41.8	3570.5 ± 346.4^{a}	3543.1 ± 391.6^{b}	133.3 ± 1.3
	758	40.9 ± 2.5	21.4 ± 0.4	289.1 ± 22.8	1184.2 ± 76.0	3955.0 ± 375.8^{a}	3711.9 ± 26.0^{b}	87.5 ± 0.4
	741	28.7 ± 1.0	23.1 ± 1.2	424.4 ± 44.8	1547.2 ± 68.9	3924.8 ± 201.9^{b}	3267.8 ± 154.5^{a}	85.2 ± 2.4
	745	36.2 ± 0.6	20.8 ± 0.8	308.7 ± 27.6	1505.4 ± 79.3	3999.2 ± 27.1^{b}	3233.56 ± 86.3^{a}	88.4 ± 0.4
Malt	**245**	30.7 ± 0.1	20.9 ± 1.0	301.6 ± 5.6	1252.5 ± 78.9	3849.1 ± 73.2^{b}	3241.8 ± 49.8^{a}	144.7 ± 0.5
	753	32.2 ± 0.2	20.4 ± 0.4	303.1 ± 17.4	1144.2 ± 85.18	3975.0 ± 39.1^{b}	3145.8 ± 200.4^{a}	151.7 ± 0.2
	758	35.1 ± 1.1	19.8 ± 0.4	282.3 ± 7.3	1574.0 ± 62.9	4121.6 ± 248.6^{b}	3238.8 ± 40.7^{a}	112.7 ± 1.60

Media ± standard deviation (SD). Different superscript letter in a column means significant differences among samples ($p < 0.05$). d.w.: dry weight. Varieties: Overture (741), Charles (745), Sinfonía (245), Montoya (753), Andreia (758).

3.2 Gama-Amino Butyric Acid, Free Amino Acids Content and Free Phenolic acids

Figure 1 shows the γ-amino butyric acid content of different barleys and malts. Except for malt 754(C), malts showed higher GABA content than those found for barleys ($p < 0.05$). Thus, the malting process increased the content of GABA. Moreover, the highest GABA content in malts corresponded to 741, 754(S) and 758.

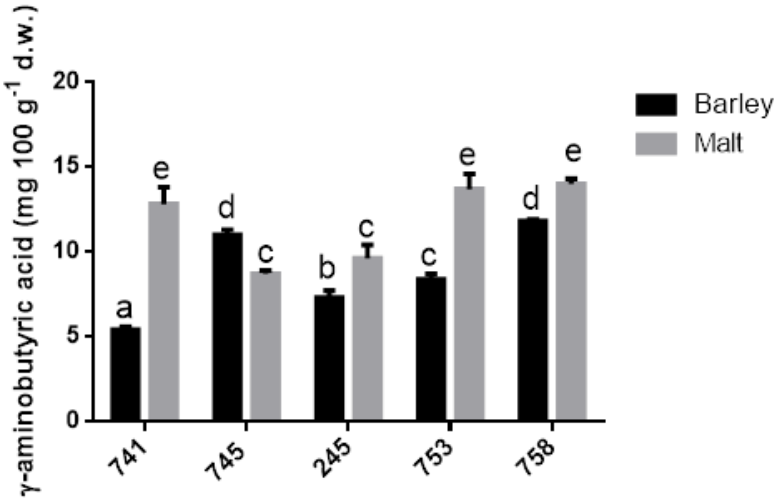

Figure 1 Gamma amino butyric acid content of different barleys and malts. Different superscript letters in a column mean significant differences among samples ($p < 0.05$). d.w.: dry weight. Varieties: Overture (741), Charles (745), Sinfonía (245), Montoya (753), Andreia (758).

Table 5 shows the free amino acid content of barleys and malts. Serine, glutamic acid, and alanine were the most abundant amino acids in barley. Moreover, malts showed a high proportion of serine, glutamic acid, and phenylalanine. The highest content of branched-chain, sulfur, and hydrophobic amino acids in barleys correspond to 753 and 758 samples. However, these values were lower than that found for malts ($p < 0.05$). Thus, the malting process increased the content of branched-chain, sulfur, and hydrophobic amino acids. Moreover, malt 758 presented the highest values of these groups of amino acids.

Table 5 Free amino acid content of the different barleys and malts.

Amino acid (mg 100 g^{-1}d.w.)	Barley 741	Malt 741	Barley 745	Malt 745	Barley 245	Malt 245	Barley 753	Malt 753	Barley 758	Malt 758
Asp	23.1 ± 1.3	18.9 ± 0.8	26.5 ± 0.8	24.4± 0.1	19.1 ± 0.7	19.0 ± 0.4	25.4 ± 0.0	19.7 ± 0.3	25.3 ± 0.2	25.8 ± 0.4
Glu	36.8 ± 3.8	56.4 ± 2.2	66.3 ± 0.5	56.7 ± 2.1	47.3 ± 1.7	51.1 ± 2.0	64.4 ± 2.3	63.7 ± 2.5	47.8 ± 0.2	66.0 ± 5.5
Ser	86.8 ± 0.7	68.9 ± 6.2	114.3 ± 10.0	99.9 ± 4.1	100.4 ± 2.9	28.0 ± 2.5	92.6 ± 5.5	114.1 ± 1.2	109.3 ± 5.1	92.8 ± 4.3
His	3.2 ± 0.4	20.1 ± 0.5	6.6 ± 0.5	22.6 ± 0.2	9.4 ± 0.2	29.2 ± 1.9	5.8 ± 0.3	25.0 ± 0.7	4.1 ± 0.2	39.3 ± 2.6
Gly	2.9 ± 0.2	7.2 ± 0.3	5.2 ± 0.2	6.6 ± 0.3	3.9 ± 0.1	8.9 ± 1.0	4.7 ± 0.3	8.2 ± 0.6	4.1 ± 0.2	13.9 ± 1.3
Thr	3.4 ± 0.1	14.8 ± 0.5	8.2 ± 0.9	15.6 ± 1.7	3.7 ± 0.2	15.8 ± 0.8	7.1 ± 0.5	14.2 ± 0.8	6.6 ± 0.1	27.6 ± 0.9
Arg	7.8 ± 0.2	37.6 ± 2.1	15.8 ± 0.6	46.2 ± 1.3	10.3 ± 0.2	52.2 ± 2.3	12.2 ± 0.3	45.0 ± 0.2	13.6 ± 1.0	79.5 ± 4.0
Ala	10.4 ± 0.5	25.3 ± 0.6	23.6 ± 0.7	24.5 ± 2.5	15.6 ± 0.4	27.3 ± 2.6	18.8 ± 0.7	28.5 ± 0.6	20.9 ± 0.4	36.3 ± 1.4
Pro	3.1 ± 0.3	7.6 ± 0.7	4.1 ± 0.4	16.5 ± 1.2	2.6 ± 0.3	1.3 ± 0.0	3.5 ± 0.2	11.4 ± 1.3	4.4 ± 0.1	46.4 ± 3.4
Tyr	3.3 ± 0.0	24.3 ± 2.4	6.1 ± 0.6	22.8 ± 1.9	4.7 ± 0.1	30.6 ± 2.6	5.5 ± 0.0	27.1 ± 2.1	3.9 ± 0.2	43.6 ± 0.8
Val	4.2 ± 0.1	24.6 ± 1.1	8.3 ± 0.1	24.5 ± 1.8	6.9 ± 0.1	25.9 ± 1.5	8.6 ± 0.3	27.5 ± 0.2	6.6 ± 0.5	40.0 ± 1.5
Met	0.7 ± 0.1	4.5 ± 0.4	2.1 ± 0.1	5.6 ± 0.1	1.5 ± 0.0	6.1 ± 0.1	1.9 ± 0.2	7.8 ± 0.4	1.4 ± 0.0	11.4 ± 1.1
Cys	1.5 ± 0.1	5.2 ± 0.6	3.0 ± 0.2	3.3 ± 0.4	2.3 ± 0.1	6.5 ± 0.5	4.0 ± 0.3	4.5 ± 0.2	1.7 ± 0.1	6.2 ± 0.0
Ile	2.8 ± 0.2	15.0 ± 1.6	6.3 ± 0.2	9.1 ± 1.0	3.8 ± 0.3	17.7 ± 1.0	5.5 ± 0.3	11.1 ± 0.0	4.5 ± 0.6	29.7 ± 2.0
Leu	5.2 ± 0.1	31.4 ± 0.9	6.8 ± 0.3	24.5 ± 2.6	4.9 ± 0.1	12.3 ± 0.6	7.0 ± 0.4	30.8 ± 0.3	3.9 ± 0.3	16.0 ± 0.6
Phe	5.3 ± 0.2	33.3 ± 3.6	9.1 ± 0.1	32.8 ± 1.1	7.2 ± 0.6	37.3 ± 3.7	9.2 ± 0.4	45.3 ± 0.3	6.8 ± 0.1	59.7 ± 0.4
Lys	5.5 ± 0.4	15.9 ± 1.3	11.7 ± 1.0	19.2 ± 1.8	7.3 ± 0.7	19.9 ± 1.1	9.6 ± 0.2	20.5 ± 0.8	7.5 ± 0.3	29.1 ± 0.9
BCA*	96.8 ± 0.8^{a}	563.6 ± 28.5^{b*}	124.4 ± 0.2^{c}	449.7 ± 15.1^{a*}	170.8 ± 4.6^{b}	465.2 ± 42.5^{a*}	168.4 ± 3.4^{c}	553.3 ± 3.9^{b*}	120.4 ± 2.2^{b}	689.3 ± 2.7^{c*}
SCA*	11.0 ± 1.0^{a}	51.9 ± 0.1^{a*}	19.6 ± 0.8^{bc}	68.0 ± 1.2^{a*}	26.8 ± 1.5^{ab}	51.1 ± 0.7^{b*}	29.5 ± 2.5^{c}	70.9 ± 3.7^{b*}	101.9 ± 7.3^{d}	101.9 ± 7.3^{c*}
HA*	315.7 ± 13.6^{a}	1241.1 ± 70.2^{a*}	426.7 ± 0.8^{e}	1152.3 ± 53.8^{a*}	610.4 ± 0.6^{b}	1207.1 ± 84.4^{a*}	539.9 ± 8.2^{d}	1408.3 ± 28.1^{b*}	498.4 ± 8.8^{c}	2123.2 ± 39.1^{c*}

Media ± standard deviation (SD). Different superscript letter in a line means significant differences among samples ($p < 0.05$). d.w.: dry weight. Varieties: Overture (741), Charles (745), Sinfonía (245), Montoya (753), Andreia (758). * Expressed as µEq 100 g-1 d.w. BCA, SCA and HA: branched chain amino acids, sulfur amino acids and hydrophobic amino acids, respectively.

Table 6 shows the cinnamic acid derivatives content in the different barleys and malts. Caffeic acid was only detected in malts. Thus, the malting process released these cinnamic acid derivatives. Moreover, there were no significant differences in this phenolic acid among malt samples ($p > 0.05$). Except for the 758 malts, no sinapic acid was detected. On the other hand, the content of coumaric and ferulic acid in malts was higher than that found for barleys ($p < 0.05$). Thus, the malting process increased these free cinnamic acid derivatives. In line with these results, the total content of cinnamic acid derivatives in malts was 3.6 times higher than that obtained for barleys.

Table 6 Cinnamic acid derivatives content of the different barleys and their malts.

Samples		Caffeic acid (µg 100g^{-1})	Coumaric acid (µg 100 g^{-1}d.w.)	Sinapic acid (µg 100 g^{-1}d.w.)	Ferulic acid (µg 100 g^{-1}d.w.)	Total content (µg 100 g^{-1}d.w.)
Barley	**741**	N.D.	1.0 ± 0.0^{b}	N.D.	13.4 ± 1.1^{b}	14.4
	745		1.4 ± 0.1^{b}		16.9 ± 2.1^{b}	18.2
	245		0.7 ± 0.0^{a}		12.2 ± 0.3^{a}	12.8
	753		0.5 ± 0.1^{a}		14.1 ± 0.9^{b}	14.7
	758		1.3 ± 0.1^{b}		13.4 ± 1.0^{b}	14.6
Malt	**741**	2.8 ± 0.1	7.6 ± 0.4^{d}	N.D.	39.5 ± 3.4^{d}	49.9
	745	1.5 ± 0.2	8.3 ± 0.2^{d}		52.4 ± 0.9^{e}	62.2
	245	2.3 ± 0.2	6.4 ± 0.1^{d}		51.3 ± 0.6^{e}	60.0
	753	2.1 ± 0.0	5.1 ± 0.1^{c}		45.1 ± 0.6^{d}	52.2
	758	2.6 ± 0.0	7.2 ± 0.3^{d}	2.3 ± 0.2	30.4 ± 0.6^{c}	42.4

Media ± standard deviation (SD); d.w.: dry weight. Varieties: Overture (741), Charles (745), Sinfonía (245), Montoya (753), Andreia (758). N.D.: Not detected.

3.3 Antioxidant Properties of Barleys and Malts

Figure 2 shows the antioxidant properties of different barleys and malts. Malts showed higher ABTS and DPPH inhibition and ferric-reducing antioxidant power (FRAP) than malts ($p < 0.05$). Thus, the malting process increased the antioxidant properties. Moreover, the highest ABTS and DPPH inhibition activity correspond to 754(S) malt ($p < 0.05$). However, there were no significant differences in FRAP among malt samples ($p > 0.05$). Additionally, a linear correlation was obtained between the total content of cinnamic acid derivatives and ABTS inhibition for malts ($r = 0.9771$).

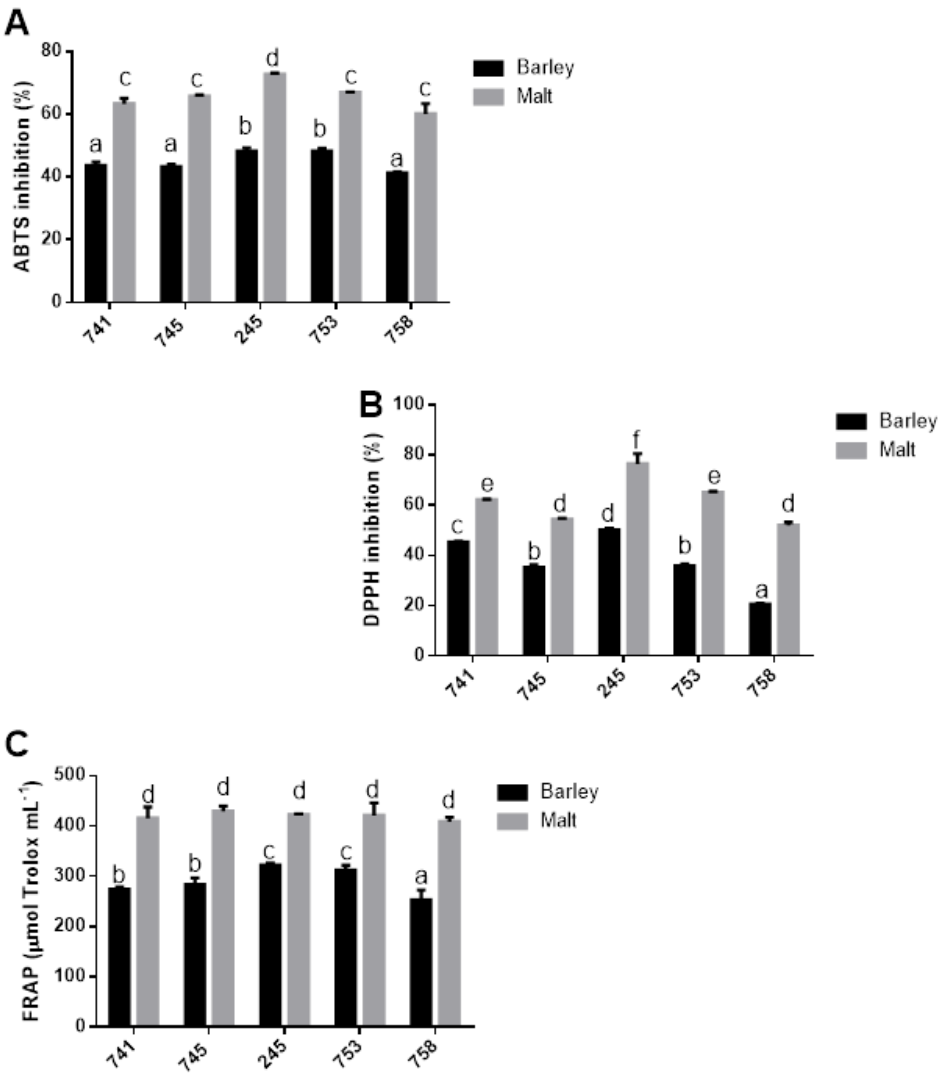

Figure 2 ABTS inhibition (**A**), DPPH inhibition (**B**), and reducing antioxidant power (**C**) of different barleys and malts. Different superscript letter in a column means significant differences among samples ($p < 0.05$). Varieties: Overture (741), Charles (745), Sinfonía (245), Montoya (753), Andreia (758).

3.4 Hydration Properties

The hydration properties of the different barleys and malts are shown in Table 7. The malts showed lower swelling power and higher solubility than those found for barleys ($p < 0.05$). However, there was no defined pattern for water absorption. Except for 741 and 745(S) barleys, there were no significant differences in water absorption among samples ($p > 0.05$).

Table 7 Swelling power, solubility at 95°C, and water absorption of the different barleys and malts.

Samples		Swelling power (g g^{-1}d.w.)	Solubility (95°C) (g 100 g^{-1}d.w.)	Water absorption (mL g^{-1}d.w.)
Barley	**741**	5.9 ± 0.0^{b}	7.0 ± 0.4^{a}	3.5 ± 0.2^{b}
	745	5.8 ±0.1^{b}	12.6 ± 0.5^{b}	2.4 ± 0.1^{a}

	245	6.2 ± 0.2[b]	12.7 ± 0.8[b]	3.7 ± 0.3[b]
	753	5.8 ± 0.0[b]	7.7 ± 0.5[a]	2.9 ± 0.1[a]
	758	5.9 ± 0.0[b]	7.7 ± 0.9[a]	3.1 ± 0.1[a]
	741	2.0 ± 0.1[a]	64.8 ± 0.1[c]	2.9 ± 0.2[a]
	745	2.1 ± 0.0[a]	63.7 ± 0.5[c]	2.8 ± 0.1[a]
Malt	**245**	2.1 ± 0.1[a]	64.0 ± 2.3[c]	2.6 ± 0.1[a]
	753	2.2 ± 0.0[a]	63.3 ± 0.3[c]	2.8 ± 0.1[a]
	758	2.1 ± 0.0[a]	65.0 ± 1.2[c]	2.5 ± 0.2[a]

Media ± standard deviation (SD). Different superscript letter in a column means significant differences among samples ($p < 0.05$). d.w.: dry weight. Varieties: Overture (741), Charles (745), Sinfonía (245), Montoya (753), Andreia (758).

4. Discussion

The chemical composition of barleys showed that total starch was the main component (≈62%), followed by TDF (≈22.6%) and proteins (≈9.5%). These values agree with those reported by Schlörmann et al. [25] for the chemical composition of different raw and roasted barley products. As it is known, barley starch accounts for around 70% of total dry weight [5]. Based on the concentration of barley amylose, starch can be divided into standard (≈20-30% amylose), waxy (<5% amylose), and elevated amylose (>35% amylose) [26]. In the present work, barley amylose content was around 15%. This amylose level can be related to normal starch barley. Note that the ranges of the amylose content for starch classification can vary because the amylose level is related to the granule size, and it is affected by barley growing conditions [4]. Several reports claimed that normal amylose barley genotypes have an essential role in manufacturing thermoplastics and films [5]. Moreover, barley flour with standard amylose starch gave extruded products with higher expansion and lower density than those obtained with waxy starch flour [4]

As mentioned above, total dietary fiber was the second most abundant barley component. In this regard, it was reported that TDF in this cereal can reach up to 28% [27]. Barley contains high levels of β-glucans as soluble fiber (up to 20%) [15]. These macromolecules are found mainly in the cell walls of the endosperm (75%), while the insoluble dietary fiber fraction, i.e. cellulose, arabinoxylans, and lignin, is found mainly in barley bran [26]. Health-promoting effects of barley β-glucans include reduction of blood cholesterol and glucose and weight loss by increased satiety, and therefore, control of heart disease and type-2 diabetes [27]. These effects have been demonstrated both by animal experiments and human clinical trials [4]. Regarding malts, there was a 28.5% reduction in TDF with respect to barley. This is related to the modification of the cell wall occurring during germination. Particularly the activation of several enzymes called –β-glucanases that degrade β -glucans. These enzymes include endo- β -1,3:1,4 glucanases, endo- β -1-3 glucanase, and exo- β -1-4-glucanase, which acting together are capable of hydrolyzing β-glucans to disaccharides (cellobiose and laminarobiose) [28]. Thus, even though these disaccharides could not be measured by the method used for the determination of TDF, mainly cellobiose could be fermented by the colonic microorganisms and have a prebiotic effect [29].

The crude fat content of barley was similar to that reported by Schlörmann et al. [25] for the chemical composition of different raw and roasted barley products. Moreover, ash content values agree with those found by Farooqui et al. [6] for barley flour. The mineral composition of barley,

showed that potassium and phosphorus were the most abundant elements, mean values being 3746.1 and 3679.1 g 100 g^{-1}d.w., respectively. In agreement with these results, Geng et al. [30] reported that these minerals in barley account for 0.3%-0.6% of dry matter. In this sense, potassium is an essential macronutrient for maintaining electrical potential, hydrostatic pressure, and biochemical activity for many enzymes in barley, while phosphorus contributes with the growth and development of plants [30]. Moreover, similar iron, zinc, calcium, magnesium, and sodium content were reported by Cieslik et al. [31] for different spring barley cultivars.

GABA is a four-carbon non-protein amino acid occurring in plants and animals, which plays a vital role as a neurotransmitter in mammal brain cells [32]. GABA provides beneficial effects for human health by decreasing blood pressure, preventing chronic alcohol-relating diseases, and inhibiting cancer cell proliferation. Other physiological functions such as relaxation, sleeplessness, and depression have been treated with GABA [10]. In this work, the GABA content varied according to the different barley cultivars. The mean value of GABA for the different barleys was 8.8 mg 100 g^{-1}. This value agrees with that reported by Nogata et al. [32] for different barley cultivars. These authors found that the average GABA content in barley samples without Lys mutation was 8 mg 100 g^{-1}.

The mean free amino acid content value for the different barley cultivars was 276.5 mg 100 g^{-1}. Similar results were reported by Nogata et al. [32] for different breeding line barleys, such as Kankei n553 (252.3 mg 100 g^{-1}) and Kankei n554 (209.5 mg 100 g^{-1}). On the other hand, the proportion of hydrophobic amino acids (Gly, Ala, Val, Leu, Ile, Met, Phe, and Pro) in barley was higher than those found for branched-chain amino acids or sulfur amino acids. This result could be associated with extracting hordein fractions such as γ-hordein, the most hydrophobic subunit [33]. Glutelin is the second most abundant protein fraction in barley and contains high levels of hydrophobic amino acids such as proline and glycine [34]. Therefore, hydrophobic free amino acids could also be contributed from this reserve protein.

Ferulic acid was the most abundant free phenolic acid detected in the different barleys studied, followed by coumaric acid. In agreement with this result, Carvalho et al. [15] reported that catechin and ferulic acid were the most abundant phenolic compounds identified in barley's free and bound fractions.

As expected, the total starch, TDF, and crude fat content of malts was lower than that found for barley. This can be due to the malting process. During malting, partial degradation of the cell walls and starchy endosperm occurs. The grain produces the hydrolytic enzymes responsible for this process during the first stages of germination [34]. Moreover, β-glucanases are the primary enzymes breaking down barley endosperm cell walls [35]. Thus, starch and soluble fiber are partially degraded. In this regard, the soluble carbohydrates of malts reached up to 12.3 g 100 g^{-1}d.w., which confirms a partial hydrolysis of starch.

On the contrary, the contents of GABA, free amino acids, and free phenolic acids in malts were higher than those obtained for barley. As is known, the level of GABA in plant tissues is low (0.3-20 mg 100 g^{-1} fresh weight). Still, it increases several folds in response to diverse stimuli, including heat shock, mechanical stimulation, hypoxia, and phyto-hormones [36]. The malting process includes soaking, germination, and drying, which produce an increment of GABA. In agreement, Garzón and Drago [10] reported that the sorghum malting increased the GABA, free amino acids, and total free phenolic compounds contents. Note that in this work, the free coumaric and ferulic acid contents of malts were, on average, seven and three times higher than those obtained for

barley, respectively. These changes were attributed to the enzymatic release of bound phenolic compounds from barley during germination [15]. Additionally, ferulic acid is better able to withstand the malting process and is, therefore, the most abundant phenolic compound in malt [37].

Regarding antioxidant properties, malts showed higher ABTS and DPPH inhibitory properties than barleys. Moreover, ferric-reducing antioxidant power (FRAP) increased after malting. It was reported that the antioxidant properties of malt is usually associated with phenolic compounds [37]. In fact, phenolic acids have been reported as solid antioxidants due to their ability to donate hydrogen and electrons and the formation of stable radical intermediates, which prevent oxidation of other compounds [38]. However, phenolic compounds in malt account for only a part of the overall antioxidant capacity [15]. It was reported that the antioxidant capacity of malt can increase during kilning and roasting, not only because of the modification or release of phenolic compounds but also due to the development of reductones through the Maillard reaction [15] and the release of free amino acids [10]. In the present work, malts showed higher content of branched-chain, sulfur, and hydrophobic amino acids than barleys. Thus, these amino acids could contribute to the increase of antioxidant properties of malts. In this regard, free amino acids such as tryptophan, histidine, and tyrosine have antioxidant activity [39]. Moreover, sulfur-containing amino acids such as methionine and cysteine exert stronger DPPH, ABTS, and O_2^-radical scavenging actions [40]. Garzón and Drago [10] related the antioxidant activity of malts measured by different mechanisms with the sulfur-containing amino acid, phenolic amino acid, and charged amino acid content. These authors found a positive correlation between the reducing power and sulfur-containing amino acids.

Swelling power is a measure of the ability of starch granules to bulk freely when heated in excess water. The swelling power decreased after the malting process, as was reported by Singh et al. [41], who evaluated the swelling power during the different stages of sorghum germination. The authors found that this parameter decreased with germination time and temperature. During malting, starch degradation occurs by the action of amylolytic enzymes. Thus, the crystallinity of the starch is altered, losing the ability to swell [42].

On the other hand, malts showed higher solubility than those found for barleys. This result can be due to the hydrolysis of different macromolecules during the malting process (starch, β-glucans, etc.), increasing the soluble species' content. Note that malts' free amino acid content was higher than that obtained for barleys. In addition, soluble carbohydrates were detected in the malt samples. In this regard, Elkhalifa and Bernhardt [43] reported that the germination time increased the nitrogen solubility index due to the proteolysis of the grain proteins, which produced the release of free amino acids and small peptides.

Finally, it is worth mentioning that all these physicochemical analyses were carried out on five barley varieties and their corresponding malts. Other barley varieties or cultivars and other malting processes could impact the results of chemical composition and bioactive properties of barley and malt flours.

5. Conclusions

Barleys had a high content of starch (around 60 g 100 g^{-1}) and total dietary fiber (22.6 g 100 g^{-1}). In addition, the presence of coumaric acid, ferulic acid, and GABA was observed. On the other hand, all barley varieties exhibited *in vitro* antioxidant properties, which could be related to the presence of free phenolic acids and free amino acids. Thus, the barley could be successfully used as a bio-functional ingredient in many foods, adding nutritional value. On the other hand, a reduction of macronutrients was observed after the malting process, mainly starch (8 to 18 g 100 g^{-1} lower content) and fiber (5 to 7 g 100 g^{-1} lower content). However, malts showed higher antioxidant properties than barleys, probably due to increased free phenolic compounds and amino acids. Moreover, malts showed the highest GABA content (18 to 124% higher than barleys). Finally, the malting process reduced swelling power and increased water absorption, principally by the action of hydrolytic enzymes on starch during germination. The increase in soluble solids, primarily carbohydrates, antioxidant compounds (free phenolic acids and free amino acids), and GABA would make malted flours a novel ingredient for preparing foods and beverages with bio-functional properties.

Further research, including more barley varieties and *in vivo* studies to validate the *in vitro* bio-functional properties, should be performed to deepen barley and malt bioactive properties.

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Author Contributions

Raúl Esteban Cian: Data curation; Formal analysis; Investigation; Methodology; Writing-original draft; Writing-review & editing. Antonela Garzón: Data curation; Formal analysis; Investigation; Methodology. Micaela Albarracín: Formal analysis; Investigation; Methodology. Silvina R. Drago: Conceptualization; Funding acquisition; Data curation; Methodology; Project administration; Supervision; Validation; Writing-original draft; Writing-review & editing.

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Competing Interests

The authors have declared that no competing interests exist.

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