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Phytochemical Compounds and Antioxidant Activity Modified by Germination and Hydrolysis in Mexican Amaranth

Eslim Sugey Sandoval-Sicairos[1] · Maribel Domínguez-Rodríguez[2] · Alvaro Montoya-Rodríguez[1] · Ada Keila Milán-Noris[1] · Cuauhtémoc Reyes-Moreno[1,2] · Jorge Milán-Carrillo[1,2]

Abstract

Amaranth (*Amaranthus* spp.) grains have become essential for human health and nutrition; due to the presence of bioactive compounds that have shown some biological activities. This study aimed to evaluate the effect of germination, enzymatic hydrolysis, and its combination on the phytochemical compounds and antioxidant activity in Mexican amaranth. Germinated amaranth flours (GAF) exhibited increases in the concentrations of soluble protein (SP), total phenolic content (TPC), total flavonoid content (TFC), total anthocyanin content (TAC) and antioxidant activity (AOX) by 35.7, 17.2, 163.0, 1472.2, and 54.3%, respectively, compared with ungerminated amaranth flours (UAF). In SDS-PAGE, both hydrolysates of UAF and GAF exhibited low molecular weight bands (< 10 kDa). The hydrolysates of UAFH and GAFH had the highest degree of hydrolysis at 205 min of sequential hydrolysis (pepsin with pancreatin) time with 73.4 and 60.3%, respectively. Both hydrolysates obtained from GAF and UAF released significantly SP, TPC, TFC after sequential enzymatic hydrolysis (up 205 min), which led to a remarkable improvement of AOX when compared to nonhydrolyzed amaranth samples. The UAFH and GAFH had the best AOX at 270 min of enzymatic hydrolysis with 983.1 and 1304.9 μmol TE/mg SP, respectively. Hence, the combination of germination and enzymatic hydrolysis could be used to produce functional ingredients for food product development.

Keywords Amaranth · Phytochemicals · Germination · Enzymatic hydrolysis · Antioxidant capacity

Abbreviations

GAF	Germinated amaranth flour
UAF	Non-germinated amaranth flour
GAFH	Germinated amaranth flour hydrolysate
UAFH	Non-germinated amaranth flour hydrolysate
DH	Degree of hydrolysis
AOX	Antioxidant activity

✉ Jorge Milán-Carrillo
jmilanc@uas.edu.mx

[1] Programa Regional de Posgrado en Biotecnología, Facultad de Ciencias Químico Biológicas, Universidad Autónoma de Sinaloa, Blv. de las Américas y Josefa Ortiz de Domínguez, S/N, Culiacán, Sinaloa, Mexico

[2] Posgrado en Ciencia y Tecnología de Alimentos, Facultad de Ciencias Químico Biológicas, Universidad Autónoma de Sinaloa, Privada Riace 2977, Col. Stanza Toscana, Culiacán, Sinaloa 80050, México

Introduction

Amaranth (*Amaranthus* spp.) grains have become essential for human health and nutrition due to the presence of bioactive compounds that have shown some biological activities, such as prevention of some types of cancer, hypertension, and lipid disorders [1, 2]. These purported health benefits are owing to the existence of biologically active compounds in amaranth, including phenolic acids, flavonoids, bioactive peptides, and proteins [3, 4].

Different food processing like extrusion, popping, roasting, germination, and enzymatic hydrolysis have been used in amaranth grains to develop products with excellent nutritional, sensorial, and nutraceutical characteristics [5]. In this sense, germination has been identified as an inexpensive and effective technology, which can cause significant changes in the biochemical characteristics of seeds. During this process, storage proteins can be degraded by proteases. It can also lead to modification of bioactive compounds and antioxidant activity; as a consequence, it can improve the nutraceutical value of cereals and legumes [6]. Enzymatic hydrolysis of plant

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proteins has been the preferred method for obtaining hydrolysates with specific bioactivities. Recently, amaranth hydrolysates with antioxidant activity have been prepared throughout digestive enzymes such as pepsin, pancreatin, and alcalase [7, 8]. However, there is no previous study showing the effect of germination and enzymatic hydrolysis on bioactive compounds and antioxidant activity present in amaranth. The current study determined the effect of germination, pepsin/pancreatin hydrolysis, and its combination of phytochemical compounds, and antioxidant activity in Mexican amaranth.

Materials and Methods

Materials

Amaranth (*Amaranthus hypochondriacus*) grains were grown and harvested in 2017 in Temoac, Morelos, Mexico (18°50′ 23”N 90°10′32”W, at an average height of 1583 m above sea level). The grains were cleaned and stored in containers under refrigeration (4 °C) until analysis. Also, amaranth grains (500 g lots) were milled (UD Cyclone Sample Mill, UD Corp. Boulder, CO, USA) to pass through an 80-US mesh (0.180 mm) screen and packed in plastic bags. Nongerminated amaranth flours (UAF) were stored in containers under refrigeration (4 °C) until use.

Germination Process

The procedure described by Perales-Sánchez et al. [6] was used. Amaranth seeds were germinated during an equivalent time in the presence of light and dark for a total of 78 h at 30 °C and 80% of relative humidity. Samples were frozen, freeze-dried, and milled to obtain germinated amaranth flour (GAF) and were packed in plastic bags and stored at 4 °C until analysis.

Preparation of Amaranth Flour Hydrolysates

The *in vitro* simulated protein digestion was carried out according to Montoya-Rodríguez et al. [7]. Briefly, UAF and GAF were dissolved in water to obtain a 10% solution (*w*/*v*). The sequential hydrolysis with pepsin (enzyme/substrate ratio, 1:20) was conducted at 37 °C and pH 2, and pancreatin (enzyme/substrate ratio, 1:20) was performed at 37 °C and pH 7.5. The aliquots (50 mL) were collected at different time intervals during sequential hydrolysis until 360 min, and the aliquots were heated at 75 °C for 20 min to inactivate enzymes. The solutions were centrifuged at 20,000×*g* for 15 min at 4 °C, and once the hydrolysates were collected filtered and freeze-dried to obtain ungerminated amaranth flour hydrolysate (UAFH) and germinated amaranth flour hydrolysate (GAFH), then stored at -20°C until analysis.

Soluble Protein

It was determined using DC Protein Assay (Biorad Laboratories, Hercules, CA). In a 96-well plate, 5 μL of diluted samples (1:50) was mixed with 25 μL of reagent A and 200 μL of reagent B, agitated, and incubated for 15 min at room temperature. The absorbance was read at 630 nm in an Ultra Microplate Reader (Biotek Instruments, Winooski, VT). The protein concentration (mg SP/mL) was calculated using bovine serum albumin (BSA) standard curve [7].

Electrophoresis

SDS-PAGE electrophoresis of amaranth samples was analyzed using a Mini-Protean Tetra Cell (Bio-Rad Laboratories Inc., Hercules, CA, USA). Gels consisted of a 15% polyacrylamide resolving gel (pH 8.8) and a 5% stacking gel (pH 6.8). Samples were diluted with Laemmli Sample Buffer and loaded onto gels [9]. After running, the gels were fixed and stained with Coomassie Blue G-250. Gels images were analyzed using the Gel Doc™ XR+ Gel Documentation System of Bio-Rad.

Degree of Hydrolysis (DH)

The DH was determined by measuring the reaction of free amino groups using the O-phthaldialdehyde method (OPA, Sigma, St. Louis, MO, USA) described by Nielsen et al. [10].

Phenolic Compounds Determination

Total phenolic content (TPC) was determined using the colorimetric method described by Singleton et al. [11]. The results were expressed as mg gallic acid equivalents (GAE) *per* 100 g of dry weight basis (dw). Total flavonoid content (TFC) was determinate, as reported by Heimler et al. [12]. The results were expressed as mg catechin equivalents (CE) *per* 100 g of dry weight basis (dw). Total anthocyanin content (TAC) was determinate, as reported by Abdel-Aal and Hucl [13]. The results were expressed as mg cyanidin 3-glucoside equivalents (CGE) *per* 100 g dry weight basis (dw). Condensed tannins content (CTC) was determined according to reported by Broadhurst and Jones [14]. The results were expressed as mg catechin equivalents (CE) *per* 100 g of dry weight basis (dw).

Antioxidant Activity

Oxygen radical absorbance capacity (ORAC) was measured as previously described by Ou et al. [15]. The ORAC value was expressed as micromoles of Trolox™ equivalents *per* mg of soluble protein (μmol TE)/ SP mg).

Statistical Analysis

All experiments were conducted at least in triplicate unless stated otherwise. Data were analyzed using the analysis of variance (ANOVA) with the group mean comparisons with the least significant differences (LSD) Fisher test ($p < 0.05$) and were performed using the Statistic 5.0 program. Correlation coefficients were obtained by multivariate analysis ($p < 0.05$) and were performed with the JMP 14 software from SAS Institute (Cary, NC, USA).

Results and Discussion

Changes of Soluble Protein, Phenolic Compounds, and Antioxidant Activity during Germination Process

The total soluble protein (SP) concentration of amaranth seeds significantly ($p < 0.05$) increased after germination bioprocess, SP value was up until 4.41 mg SP/mL, which was 35.7% higher than the initial concentration of ungerminated amaranth (Table 1). Previous studies also observed an increase of SP in germinated soybean and cowpea bean [15, 16]. Such changes in SP could be related to the effect of proteolysis and protein synthesis. During the initial stages of germination, some enzymes are activated through several biochemical mechanisms. Endopeptidase degraded storage proteins to provide free amino acids necessary to promote the synthesis of new proteins and tissues. Meanwhile, some other proteins can be hydrolyzed by proteases [17].

Phenolic compounds, such as total phenolic content (TPC), total flavonoid content (TFC), and total anthocyanins content (TAC) of germinated amaranth, are shown in Table 1. The phytochemical concentrations in ungerminated amaranth flour were 23.34 mg GAE/100 g dw for TPC, 4.60 mg CE/100 g dw for TFC, and TAC 0.58 mg CGE/100 g dw for TAC. In this study, the TPC, TFC, and TAC of germinated amaranth flour increased significantly ($p < 0.05$) by 17.2, 163.0, and 1, 472.2%, respectively, compared with counterparts unprocessed. The most significant increase of phenolic compounds during the germination of amaranth has been previously observed in edible amaranth grains [6, 18]. The increased in the phenolic compounds throughout the germination of amaranth could be due to *de novo* biosynthesis of the phytochemical through the increase of phenylalanine ammonialyase (PAL) enzyme during the initial stage of germination [19]. Otherwise, as a result of the germination process, the condensed tannin contents (CTC) of amaranth varied from 133.1 to 65.7 mg CE/100 g dw. This parameter had a decrease of 50.6% compared with its counterparts unprocessed (Table 1). Similar results were previously reported in germinated amaranth seeds [20]. Those CTC changes are due to the rise of enzymatic activity in the bioprocess, in which several components such as macronutrients and phenolic compounds are degraded and are associated with reduction of antinutritional compounds [21].

Antioxidant activity of the ungerminated and germinated amaranth are presented in Table 1. The highest ORAC value (641.6 μmol TE/mg SP) was found in germinated amaranth, which increased almost 54.3% compared with its counterpart unprocessed amaranth. Our results agree with studies showing an increase in antioxidant activity concentration during germination in amaranth and other seeds [6, 15, 19]. The AOX increments in the germination process have been related to the liberation of phenolic compounds from cell walls or protein-starch interaction [22] or phenolics biosynthesis by PAL activity [19]. Additionally, the presence of free amino acids or peptides in seeds flours, in particular, aromatic or with sulfur, may show antioxidant activity [16].

Effect of Hydrolysis Time on Processed Amaranth Hydrolysates

SDS–PAGE Protein Profile and Degree of Hydrolysis

The gastrointestinal digestion was simulated with the use of pepsin and pancreatin. This was made to get results close to reality when the food is ingested. The first enzyme used was pepsin at controlled conditions (37 °C and pH 2) for 180 min. During this time, the pH and temperature were controlled to make sure the highest enzyme activity. At the end of the hydrolysis with pepsin, the pH was increased to stop the pepsin activity and was raised and controlled at pH 7.5 for pancreatin activity. The temperature and pH conditions were controlled to assure the highest enzyme activity. Figure 1 presents the electrophoretic protein profile of unprocessed (A) and germinated (B) amaranth flours and their hydrolysates. As described in Fig. 1a, the UAF proteins presented the leading bands between 35 and 100 kDa, corresponding to components of the albumins, globulin, and glutelin fractions [8, 23]. The electrophoretic protein profile of unprocessed amaranth was similar, as previously reported by Silva-Sánchez et al. [24]. Also, SDS-PAGE showed a weak protein breaking as a result of the germination process. Some protein bands around 100, 75, and 50 kDa started to degrade during the process and reduced their band intensity, such as amaranth albumins (32 kDa), 7S fraction (28 kDa). Other authors, Aphalo et al. [25], showed in SDS-PAGE a high proportion of generation of peptides (<10 kDa) as an indication of the proteolysis that took place during the germination process.

Regarding both hydrolysates UAFH and GAFH, the expression of protein bands around 15 to 100 kDa was completely hydrolyzed after enzymatic hydrolysis (pepsin/pancreatin at 270 min) showing protein bands around 10 kDa, which were the most intense for both hydrolysates. The presence of low molecular weight bands in both hydrolysates (UAFH and

Table 1 Soluble protein content, phenolic compounds and antioxidant activity in amaranth flours and hydrolysates obtained by germination and enzymatic hydrolysis time

Digestion	Hydrolysis time (min)	SP (mg SP/mL)		TPC (mg GAE/100 g dw)		TFC (mg CE/100 g dw)		TAC (mg CGE/100 g dw)		CTC (mg CE/100 g dw)		AOX (μmol TE/mg SP)	
	Flours	UAF	GAF	UAF	GAF	UAF	GAF	UAF	GAF	UAF	GAF	UAF	GAF
	0	$3.3 \pm 0.3_{B}$	$4.4 \pm 0.4_{A}$	$23.3 \pm 1.2_{B}$	$27.3 \pm 1.8_{A}$	$4.6 \pm 0.1_{B}$	$12.1 \pm 0.0_{A}$	$0.5 \pm 0.0_{B}$	$8.8 \pm 0.3_{A}$	$133.1 \pm 3.7_{A}$	$65.7 \pm 5.3_{B}$	$415.9 \pm 34.7_{B}$	$641.6 \pm 60.7_{A}$
with pepsin	Hydrolysates	UAFH	GAFH	UAFH	GAFH	UAFH	GAFH	UAFH	GAFH	UAFH	GAFH	UAFH	GAFH
	10	$3.2 \pm 0.2^{f}_{B}$	$4.5 \pm 0.1^{f}_{A}$	$193.3 \pm 4.8^{f}_{B}$	$349.2 \pm 6.1^{cd}_{A}$	$8.4 \pm 0.0^{g}_{B}$	$54.4 \pm 2.0^{fg}_{A}$	ND	ND	ND	ND	$463.3 \pm 35.7^{f}_{B}$	$720.5 \pm 70.9^{a}_{A}$
	25	$3.4 \pm 0.2^{f}_{B}$	$4.7 \pm 0.1^{e}_{A}$	$213.4 \pm 3.3^{e}_{B}$	$368.8 \pm 6.7^{c}_{A}$	$8.0 \pm 0.6^{gh}_{B}$	$57.1 \pm 0.6^{ef}_{A}$	ND	ND	ND	ND	$590.5 \pm 42.1^{de}_{B}$	$742.2 \pm 75.7^{e}_{A}$
	60	$3.8 \pm 0.1^{e}_{B}$	$5.2 \pm 0.1^{d}_{A}$	$266.9 \pm 3.6^{d}_{B}$	$398.1 \pm 9.2^{b}_{A}$	$12.3 \pm 0.6^{f}_{B}$	$59.5 \pm 0.6^{de}_{A}$	ND	ND	ND	ND	$633.6 \pm 25.4^{d}_{B}$	$737.9 \pm 66.0^{e}_{A}$
	90	$4.3 \pm 0.1^{d}_{B}$	$5.3 \pm 0.2^{d}_{A}$	$298.1 \pm 3.2^{c}_{B}$	$409.9 \pm 8.2^{ab}_{A}$	$9.2 \pm 0.6^{g}_{B}$	$56.3 \pm 0.6^{ef}_{A}$	ND	ND	ND	ND	$616.2 \pm 30.8^{d}_{B}$	$824.5 \pm 58.2^{d}_{A}$
	120	$4.5 \pm 0.1^{c}_{B}$	$5.7 \pm 0.1^{c}_{A}$	$325.3 \pm 6.0^{b}_{B}$	$420.2 \pm 7.6^{a}_{A}$	$6.9 \pm 0.6^{h}_{B}$	$54.7 \pm 0.6^{f}_{A}$	ND	ND	ND	ND	$630.8 \pm 20.0^{d}_{B}$	$813.9 \pm 84.2^{d}_{A}$
	180	$5.2 \pm 0.2^{b}_{B}$	$6.0 \pm 0.2^{b}_{A}$	$346.1 \pm 1.7^{a}_{B}$	$426.3 \pm 9.7^{a}_{A}$	$11.6 \pm 0.6^{f}_{B}$	$68.1 \pm 2.9^{c}_{A}$	$0.4 \pm 0.0^{a}_{B}$	$2.8 \pm 0.4^{a}_{A}$	$244.4 \pm 3.8^{a}_{A}$	$249.4 \pm 7.2^{a}_{A}$	$627.5 \pm 35.5^{d}_{B}$	$731.9 \pm 19.9^{e}_{A}$
with pepsin +	190	$5.3 \pm 0.1^{b}_{B}$	$6.1 \pm 0.1^{b}_{A}$	$172.7 \pm 8.9^{g}_{B}$	$335.5 \pm 5.9^{d}_{A}$	$36.3 \pm 0.6^{a}_{B}$	$66.5 \pm 2.9^{c}_{A}$	ND	ND	ND	ND	$551.9 \pm 39.9^{e}_{B}$	$910.8 \pm 53.8^{c}_{A}$
pancreatin	205	$5.5 \pm 0.1^{a}_{B}$	$6.3 \pm 0.1^{a}_{A}$	$178.7 \pm 5.5^{g}_{B}$	$337.5 \pm 9.3^{d}_{A}$	$30.8 \pm 1.1^{b}_{B}$	$75.5 \pm 2.0^{b}_{A}$	ND	ND	ND	ND	$795.6 \pm 50.2^{c}_{B}$	$916.3 \pm 73.9^{c}_{A}$
	240	$5.4 \pm 0.3^{a}_{B}$	$6.2 \pm 0.1^{ab}_{A}$	$171.8 \pm 2.7^{g}_{B}$	$279.1 \pm 3.6^{e}_{A}$	$26.9 \pm 0.6^{c}_{B}$	$82.2 \pm 3.3^{a}_{A}$	ND	ND	ND	ND	$878.9 \pm 88.5^{b}_{B}$	$1156.9 \pm 93.7^{b}_{A}$
	270	$5.4 \pm 0.2^{ab}_{B}$	$6.1 \pm 0.3^{ab}_{A}$	$155.7 \pm 7.4^{h}_{B}$	$267.5 \pm 2.2^{e}_{A}$	$25.9 \pm 1.3^{d}_{B}$	$72.0 \pm 1.1^{b}_{A}$	ND	ND	ND	ND	$983.1 \pm 38.1^{a}_{B}$	$1304.9 \pm 86.9^{a}_{A}$
	300	$5.3 \pm 0.1^{ab}_{B}$	$6.0 \pm 0.3^{ab}_{A}$	$153.2 \pm 3.6^{h}_{B}$	$262.7 \pm 7.1^{e}_{A}$	$24.6 \pm 0.6^{d}_{B}$	$62.2 \pm 4.4^{d}_{A}$	ND	ND	ND	ND	$937.0 \pm 65.3^{a}_{B}$	$1211.4 \pm 99.0^{ab}_{A}$
	360	$5.3 \pm 0.1^{ab}_{B}$	$5.9 \pm 0.3^{ab}_{A}$	$176.9 \pm 6.2^{g}_{B}$	$268.4 \pm 4.6^{e}_{A}$	$21.0 \pm 0.6^{e}_{B}$	$50.8 \pm 0.0^{g}_{A}$	$0.4 \pm 0.0^{a}_{B}$	$2.1 \pm 0.0^{b}_{A}$	$153.9 \pm 7.6^{b}_{A}$	$123.5 \pm 4.0^{b}_{B}$	$973.9 \pm 78.6^{a}_{B}$	$1246.4 \pm 45.9^{ab}_{A}$

UAF: Unprocessed amaranth flour; GAF: Germinated amaranth flour; UAFH: Unprocessed amaranth flour hydrolysate; GAFH: Germinated amaranth flour hydrolysate; SP: soluble protein; TPC: total phenolic content; TFC: total flavonoid content; TAC: Total anthocyanin content; CTC: Condensed tannin content; AOX: Antioxidant activity. ND: No determined; dw: Dry weight. Values are means ± SD. Uppercase letters within a row for each parameter indicate significant differences by germination ($p < 0.05$). Lowercase letters within a column for each parameter indicate significant differences by enzymatic hydrolysis ($p < 0.05$)

GAFH) suggests that hydrolysis was extensive and, subsequently, that peptides with biological activity may be present. Our study revealed that the enzymatic hydrolysis (pepsin/pancreatin at 270 min) increased in the percentages of peptides of molecular mass < 10 kDa when compared to nonhydrolyzed amaranth flour extract.

The degree of hydrolysis (DH) method is the most widely used for measuring the hydrolysis of protein, which could impact the molecular size and amino acid composition of the peptides, therefore the biological activity of protein hydrolysate [26]. In the current study, hydrolysis of the UAF and GAF were carried out by treatment with pepsin for 180 min, followed by treatment with pancreatin for 180 min, as previously reported (Fig. 2) [7]. The UAFH and GAFH had the highest DH values at 205 min of sequential hydrolysis time with 73.4 and 60.3%, respectively (Fig. 1b). Based on the results, after 205 min of hydrolysis, the DH of both hydrolysates (UAFH and GAFH) reached the plateau, confirming that the hydrolysis had been completed, indicating that the number of peptides was small. The percentage of DH of both hydrolysates showed the same tendency to increase with increasing hydrolysis time, showing no significant differences ($p > 0.05$) between 205 to 360 min (Fig. 2).

Soluble Protein

The SP of both hydrolysates UAFH and GAFH are displayed in Table 1. The SP values of both hydrolysates were significantly ($p < 0.05$) increased continuously during sequential enzymatic hydrolysis (pepsin + pancreatin). Montoya-Rodríguez et al. [7] reported that the enzymatic hydrolysis of unprocessed *A. hypochondriacus* increased SP 75% after 360 min of pepsin/pancreatin hydrolysis. The increase in the concentration of SP after enzymatic hydrolysis could be due to the generation of peptides with small molecular masses [7, 24], as it could be supported in Fig. 1. The UAFH and GAFH had the highest SP value at 205 min of sequential hydrolysis with 5.53 and 6.37 mg SP/mL. Respectively, these values were 70.2 and 41.3% higher when compared with nonhydrolyzed amaranth samples. After that point, SP values in the continuous hydrolysis (205–360 min) were not significantly different ($p > 0.05$). It could be because the protein

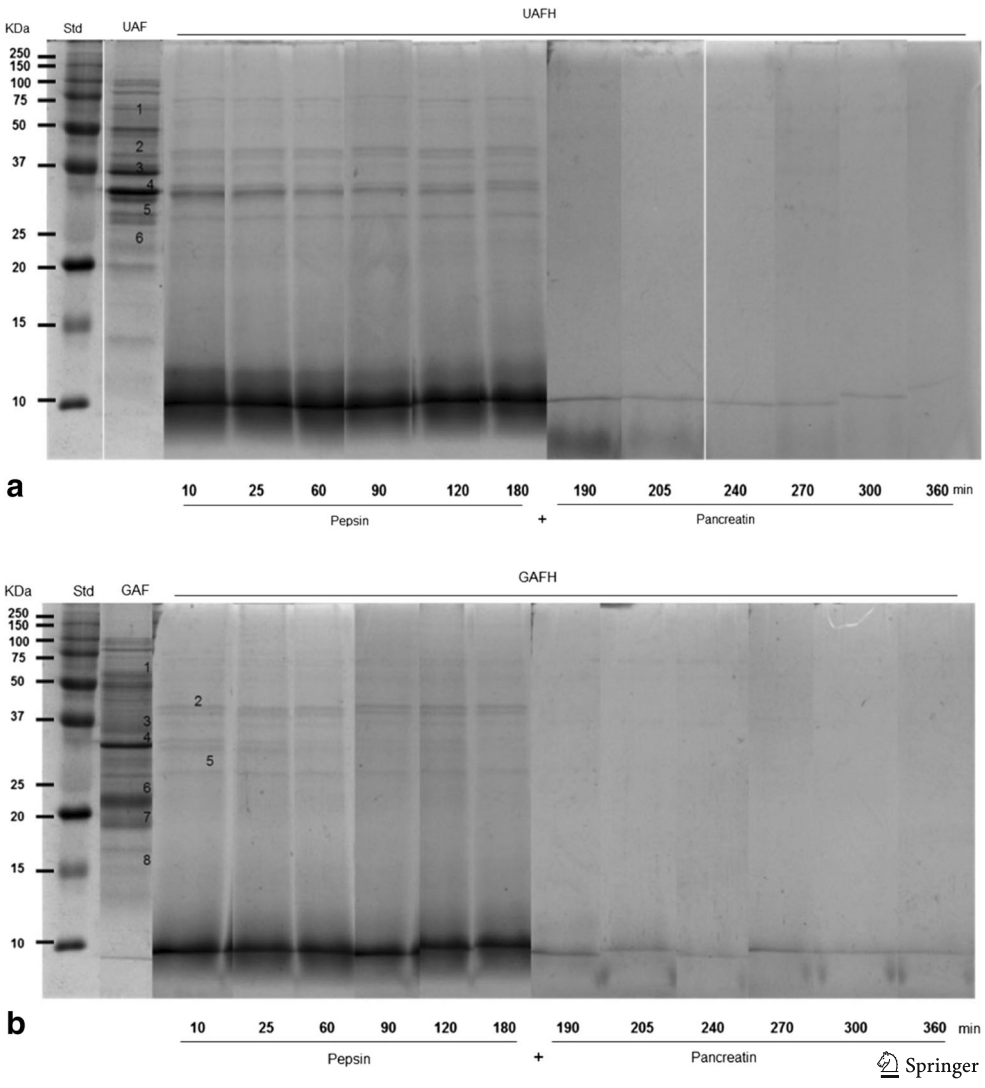

Fig. 1 SDS-PAGE electrophoresis protein profile of unprocessed (**a**) and germinated (**b**) amaranth flour and hydrolysates of the sequential hydrolysis with pepsin + pancreatin until 360 min. 1: Globulin 11S; 2: Glutelin; 3: Amaranth albumin 1; 4: Globulin 7S; 5: Albumin; 6: Prosystemin; 7: RING Zinc finger protein; 8: Superoxide dismutase [Cu-Zn]. The protein molecular weight standard was included. UAF: Unprocessed amaranth flour; GAF: Germinated amaranth flour; UAFH: Unprocessed amaranth flour hydrolysate; GAFH: Germinated amaranth flour hydrolysate

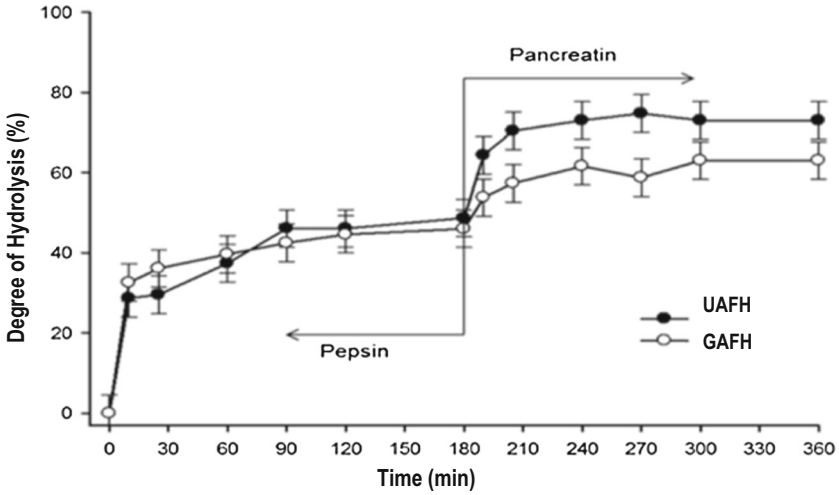

Fig. 2 Degree of hydrolysis of ungerminated (UAFH) and germinated (GAFH) amaranth hydrolysates. The hydrolysis was carried out with pepsin at 10 to 180 min, followed by hydrolysis with pancreatin at 190 to 360 min. The vertical bars indicate LSD = 4.67% ($p < 0.05$)

hydrolysis has been completed at 205 min according to the DH values of both hydrolysates (Fig. 2).

Total Phenolics Content

Table 1 shows the total phenolics content in UAFH and GAFH during enzymatic hydrolysis. The TPC values of both hydrolysates were significantly ($p < 0.05$) increased continuously through pepsin hydrolysis (0–180 min), followed by a significant ($p < 0.05$) decrease during stage (190–360 min) continuous hydrolysis with pancreatin. The highest TPC values for UAFH and GAFH were up to 346.1 and 426.3 mg GAE/100 g dw, respectively. These values were 14.9 and 15.5-fold higher compared with nonhydrolyzed samples. Pazinatto et al. [27] reported that the TPC in *Amaranthus cruentus* had significantly increased after enzymatic digestion. The release of phenolic compounds during enzymatic hydrolysis is associated with the type of phenolic compound in the samples. In cereals, most phenolic compounds are found insoluble and linked to other molecules, such as proteins, starch, and fiber [22].

Total Flavonoids

Both hydrolysates (UAFH and GAFH) had a progressive increase of TFC during enzymatic hydrolysis time (Table 1). The GAFH presented higher TFC values ($p < 0.05$) all enzymatic times than those UAFH. The highest values of TFC were observed at 190 min in UAFG (36.3 mg CE/100 g dw) and 240 min in GAFH (82.2 mg CE/100 g dw), which increase up to 7.9 and 6.8-fold by digestion, respectively, compared with nonhydrolyzed amaranth. Noteworthy, TFC showed a positive correlation with SP in unprocessed ($r = 0.7714$, $p = 0.0033$) and germinated ($r = 0.6517$, $p = 0.0217$) hydrolysates. Previous studies by Pellegrini et al. [28] also found a TFC increase in quinoa after enzymatic hydrolysis. The release of TFC may be attributed to the bioavailability of these compounds, and the interaction with macronutrients [28].

Total Anthocyanins

In this study, the effect of the enzymatic hydrolysis with pepsin (only 180 min) and pancreatin (360 min) on TAC are shown in Table 1. The TAC of UAFH decreased ($p < 0.05$) by 17.2% during pepsin hydrolysis, while the continuous action with pancreatin hydrolysis decreased TAC until 18.9% compared to the raw sample. Similarly, pepsin hydrolysis (180 min) also decreased TAC of GAFH had also decreased ($p < 0.05$) from 8.80 to 2.85 mg CGE/100 g dw and later after 360 min with pepsin/pancreatin hydrolysis reduced to 2.11 mg CGE/100 g dw. Soriano Sancho et al. [29] showed that simulated digestion of beans reduced anthocyanin content because they are broadly sensitive to the alkaline conditions of the intestinal digestion, as well as the impact caused by digestive enzymes and bile salts.

Condensed Tannins

In this study, the effect of the enzymatic hydrolysis with pepsin (only 180 min) and pancreatin (360 min) on CTC are shown in Table 1. The *in vitro* simulated digestion had a similar effect in both hydrolysates (UAFH and GAFH). The CTC of UAFH had increased ($p < 0.05$) from 133.1 to 244.4 mg CE/100 g dw after the first 180 min of pepsin hydrolysis but afterward decreased to 153.9 mg CE/100 g dw at 360 min of pancreatin hydrolysis. Regarding the CTC of GAFH had increased ($p < 0.05$) from 65.7 to 249.4 mg CE/100 g dw for pepsin hydrolysis (at 180 min) but subsequently reduced to 123.5 mg CE/100 g dw during pancreatin hydrolysis (at 360 min). The degradation of tannins during simulated digestion was previously observed in beans [29], which

was attributed to the interaction between these compounds and pancreatic enzymes and to the intestinal pH.

Antioxidant Activity (AOX)

The AOX of both hydrolysates UAFH and GAFH are presented in Table 1. At the different digestion times, the hydrolysate of GAF had higher AOX than most of the hydrolysates generated from GAF. The AOX of both hydrolysates were significantly ($p < 0.05$) increased continuously during sequential enzymatic hydrolysis (pepsin + pancreatin). It took 270 min to maximum reach values of AOX, but afterward, until the final continuous hydrolysis (270–360 min), the AOX values were not significantly different ($p > 0.05$). The UAFH and GAFH had the best AOX at 270 min of hydrolysis with 983.1 and 1304.9 μmol TE/ SP mg, respectively, and were 136.4 and 103.4% higher compared with nonhydrolyzed amaranth samples. Researchers have demonstrated that the hydrolysates of food proteins, such as amaranth, bean, and soybean, possess significant antioxidant activities [6, 9, 16, 30]. These antioxidant properties could be due to the synergistic action of polyphenols and peptides released by enzymatic hydrolysis [26]. Even though, AOX showed a positive correlation with SP unprocessed ($r = 0.7032$, $p = 0.0107$) and germinated ($r = 0.6204$, $p = 0.0314$) and a negative correlation with TPC in germinated data ($r = -0.8985$, $p = <0.0001$). Our results showed that germination of Mexican amaranth in combination with enzymatic hydrolysis with pepsin and pancreatin could be used to produce functional ingredients that contain a very high concentration of antioxidant activity.

Conclusions

This study revealed that phytochemical compounds increased significantly in amaranth grains during germination followed by sequential enzymatic hydrolysis (TPC by 17.2%; TFC by 163.0%; and TAC by 1,472.2%), which led to a remarkable improvement of antioxidant activities (an increment of 54.3%). Consequently, amaranth germinated flour and hydrolysates provide a potential application as functional ingredients for food product development.

Compliance with Ethical Standards

Conflict of Interest There are no conflicts of interest to declare.

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