

The effect of amaranth, a pseudo-cereal, on the activity of *L. acidophilus* probiotic bacteria and its antioxidant activity in the gastrointestinal digestion process

Büşra KARKAR

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University of Bursa Uludağ, Faculty of Science and Arts, Department of Chemistry, Bursa/Türkiye

ORCID IDs of the authors:

B.K. 0000-0001-6547-5558

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ABSTRACT

Pseudo-cereals are an excellent source of nutrients, rich in carbohydrates, dietary fiber, protein, lipids, phytochemicals, and minerals such as magnesium, zinc, copper, sodium, potassium, and calcium. The positive effects of gluten-free pseudo-cereals on the digestive system are an alternative to natural cereals. Pseudo-cereals have prebiotic properties and strengthen digestion by positively affecting the development of probiotic bacteria, especially *Lactobacillus*. Therefore, the effect of amaranth, a pseudo-cereal, on the activity of *L. acidophilus* probiotic bacteria, which helps digestion, was determined. First, solvent, acidic, and basic hydrolysis extractions of amaranth in eight different solvent media were performed, and total phenolic content and antioxidant activity values were determined. The total phenolic content values in the gastrointestinal digestion process were investigated by applying three different consumption methods, milling, boiling, and drying, to amaranth grains. *L. acidophilus* probiotic bacteria were activated with milled, dried, and boiled amaranth, and the increase in viability was examined. While the viability of *L. acidophilus* activated with milled and dried amaranth increased by 9.47% and 7.46%, respectively, the viability of bacteria activated with boiled amaranth almost did not increase (0.60%).

Keywords: Pseudo-cereals, Amaranth, *L. acidophilus*, Probiotic bacteria, Antioxidant, Gastrointestinal

Correspondence:

Büşra KARKAR

E-mail: busrakarkar@uludag.edu.tr



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Introduction

Pseudo-cereals are the seed grains of dicotyledonous plants of different plant families, such as *Amaranthaceae* and *Chenopodiaceae*. This type of cereal is physically similar to natural cereal grains from monocotyledonous plants of the *Poaceae* or *Gramineae* family (Shewry, 2016). The most known pseudo-cereals are quinoa from *Chenopodiaceae*, chia from *Lamiaceae*, buckwheat from *Polygonaceae*, and amaranth from *Amaranthaceae* (Upasana & Yadav, 2022).

Pseudo-cereal grains are a good source of nutrients. It contains 50-70% of carbohydrates, 4-12% of dietary fibre, 7-16% of protein, 4-7% of lipid, and a high amount of micronutrients such as zinc, copper, manganese, potassium, sodium, calcium, and magnesium (Malleshi et al., 2020). Pseudo-cereal grains do not contain gluten, and they also contain low starch. Due to its high lipid and protein content, it is high in calories like natural cereals (Bekkering & Tian, 2019). Pseudo-cereals have antioxidant, antimicrobial, anti-inflammatory, and antihypertensive properties due to their rich phytochemical content. In addition, pseudo-cereals show protective activities for bone and gastrointestinal system health (Upasana & Yadav, 2022). The positive activities of cereals on the health of the gastrointestinal tract have been known for many years. However, in recent years, many studies have supported the idea that pseudo-cereals can be an alternative to natural cereals. The main emphasis of these studies is that pseudo-cereals contribute to the viability of probiotic bacteria, one of the most essential elements of the gastrointestinal system. Especially pseudo-cereals are the substrate of *Lactobacillus* species from probiotic bacteria and have prebiotic activity (Ugural & Akyol, 2022).

Amaranth is a plant of the *Amaranthaceae* family, cultivated in Asia, Africa, and Central and South America (House et al., 2020; Olawoye & Gbadamosi, 2020). Amaranth has been consumed as a vegetable in the Americas for thousands of years. Nowadays, various parts of the plant are used for different purposes; for example, the flowers are used to produce red dye, the seeds are used as grain for flour production and animal nutrition, and the leaves are used in food (López-Mejía et al., 2014). Various parts of the plant are rich in carbohydrates (61.4%), dietary fibre (20.6%), protein (16.5%), vitamins, and minerals, which are highly beneficial for human health (Petrova & Petrov, 2020; Shewry, 2016). These parts also contain components with antioxidant activity, such as flavonoids, phenolic acids, carotenoids, and tannins (Silva et al., 2021). Amaranth is approved as a superfood due to its rich content of beneficial components for human nutrition

and health. The literature has revealed that amaranth can effectively overcome nutrition-related health problems (Ruth et al., 2021).

The main aim of this study is to examine the effect of amaranth, which is a pseudo-cereal, on the activity of probiotic bacteria that help digestion and to determine its antioxidant activity. For this purpose, various extracts of amaranth were prepared, and total phenolic content and antioxidant capacity values were determined. In addition, amaranth was used as a prebiotic to increase the viability of *Lactobacillus acidophilus* probiotic bacteria, and its effect on bacterial viability was investigated. Also, with the preparation of consumable forms of amaranth, their digestion in the *in-vitro* gastrointestinal tract was investigated.

Materials and Methods

Chemical and Reagent

Acetic acid, acetone, *n*-butanol, ethanol, ethyl acetate, hexane, hydrochloric acid, methanol, petroleum ether, and potassium persulfate were purchased from Merck (Darmstadt, Germany). 2,2-azino-bis(3-ethylbenzothiazoline-6-sulfonic acid) diammonium salt, 2,4,6-Tris(2-pyridyl)-s-triazine, copper(II) sulfate pentahydrate, Folin-Ciocalteu reagent, gallic acid (HPLC grade), pancreatin, pepsin, sodium acetate trihydrate, sodium carbonate, sodium hydroxide, potassium sodium tartrate, and Trolox (HPLC grade), were supported from Sigma-Aldrich (St. Louis, MO., USA). Bile salt, potassium dihydrogen phosphate, and sodium chloride were purchased from Edukim, Isolab, and Tekkim, respectively.

Preparation of Amaranth (*Amaranthus*)

The amaranth used in the research was purchased commercially. The amaranth, which will be used for spectroscopic analysis, was milled, and its dimensions were determined in the 14-30 mesh range. For *in-vitro* gastrointestinal digestion analysis, amaranth was prepared in three ways: grinding into flour, drying in an oven at 100°C for one h, and boiling at 100°C for 30 min.

Extraction of Amaranth

In order to determine the total phenolic content (TPC) and antioxidant activity (AA) of amaranth, ultrasonic extractions (160 W, 50 Hz) were performed in eight different solvents (water, methanol, ethanol, acetone, ethyl acetate, *n*-butanol, petroleum ether, and hexane) environments in three different

ways: solvent extraction, acidic hydrolysis, and basic hydrolysis. For solvent extraction, 1 g of amaranth was extracted with 10 mL of solvent for 4 h at 45°C. For acidic hydrolysis extraction, amaranth (1 g) was extracted with solvent (10 mL) and 1 M HCl (0.1 mL) at 45°C for 4 h. At the same time, 1M NaOH was used instead of 1 M HCl for basic hydrolysis extraction. All the extracts were filtered through filter paper, and the extracts other than water, methanol, and ethanol were evaporated in an evaporator. The remaining residues were dissolved in methanol, and all extracts were kept at 4°C until analysis (Karkar & Şahin, 2022).

Spectroscopic Analysis

Total phenolic content (TPC) analysis

The Folin-Ciocalteu method was used to determine the TPC of amaranth extracts (Akkan & Aybastier, 2023; Güçlü et al., 2006; Karkar & Şahin, 2022; Singleton et al., 1999). According to the method, 0.25 mL of amaranth extract was mixed with Lowry C (2.5 mL) (prepared by mixing Lowry A (0.4% NaOH and 2.0% Na₂CO₃) and Lowry B (1.0% NaKC₄H₄O₆ and 0.5% CuSO₄) in a ratio of 50:1) solution and 0.67 N Folin reagent (0.25 mL). The total volume was made up of 4.75 mL of distilled water. The samples were kept in a dark environment for 30 min, and absorbance measurements were performed at 750 nm using a UV-VIS spectrometer. The TPC of amaranth was determined as mg gallic acid equivalent (GAE)/ 100 g amaranth.

The antioxidant activity (AA) analysis

The ABTS (Karkar & Şahin, 2022; Re *et al.*, 1999) and FRAP (Benzie & Strain, 1996; Karkar & Şahin, 2022) methods determined the AA of amaranth. ABTS* radical solution used in the AA analysis by ABTS method was prepared by mixing 2.45 mM K₂S₂O₈ with 7 mM ABTS solution. For the analysis, amaranth extract (0.1 mL), ethanol (3.9 mL), and ABTS* radical solution (1 mL - 1:10 diluted with distilled water) were mixed, and the samples were kept for 6 min. The absorptions of the samples were measured with a UV-VIS Spectrophotometer at 734 nm, and the AA values of Amaranth were determined as mg Trolox equivalent (TE)/100 g Amaranth.

According to the AA of the FRAP method, 0.1 mL of amaranth extract and 2.9 mL of FRAP reagent (prepared by mixing pH 3.6 acetate buffer, 20 mM FeCl₃ solution, and 10 mM TPTZ solution (by 40 mM HCl) in a ratio 10:1:1) were mixed. The samples were kept in a dark environment for 30 min, and absorbance measurements were performed at 593 nm using a UV-VIS spectrometer. The AA of amaranth was determined as mg trolox equivalent (TE)/ 100 g amaranth.

Statistical Analysis

The analysis data were statistically analysed using the MINITAB 17.0 (Minitab Inc., Stage College, PA) statistical program with Fit General Linear Model ANOVA (solvent, acidic, and basic hydrolysis extracts separately for each method ($p < 0.01$), and TPC, ABTS and FRAP methods separately for each extract ($p < 0.01$)). Analyses were performed in two repetitions.

In-vitro Gastrointestinal Digestion Analysis

An analysis examined how the human digestive system breaks down amaranth. Two different simulated digestive fluids were created for gastrointestinal digestion. The simulated gastric fluid (SGF; pH 2.00) was prepared using sodium chloride (0.2%; w/v) and porcine pepsin (1600 U/mL of final volume), and the pH was adjusted to 2.0 ± 0.2 (0.2 N HCl). The simulated intestinal fluid (SIF; pH 7.00) was prepared using potassium dihydrogen phosphate (0.68%; w/v), bile salt (0.3%; w/v), and porcine pancreatin (800 U/mL of final volume), and pH was adjusted to 7.0 ± 0.2 (0.2 N NaOH) (Tipigil, 2015).

The SGF medium (10 mL) was added to the milled, dried, and boiled amaranth samples (1 g), and gastric digestion was carried out for 2 h in a shaker incubator at 37°C and 100 rpm. After gastric digestion, the pH of the medium was adjusted to 7.0 with 0.2 N NaOH, and the SIF medium (10 mL) was added. For intestinal digestion, the medium was incubated for 2 h in a shaker incubator at 37°C and 100 rpm. During gastric and intestinal digestion, a 1 mL of sample was taken every 30 min from the digestive fluid, kept in an ice bath for 15 min, and centrifuged at 6000 rpm for 15 min. Then, the TPC of the samples (1 mL) taken from the media was determined, and the amounts of phenolic compounds released from the amaranth samples into the environment during gastrointestinal digestion were determined.

The Effect of Amaranth on Probiotic Bacterial Viability

The effect of amaranth on the viability of probiotic bacteria was investigated using the prebiotic effect of amaranth. The analysis used three forms of amaranth - milled, dried, and boiled - while *Lactobacillus acidophilus* species from the *Lactobacillaceae* family was used as probiotic bacteria. *L. acidophilus* (DSM 20079) bacterial strain was activated by incubation at 37°C for 24 h under anaerobic conditions in 5 mL of De Man, Rogosa, and Sharpe (MRS) medium (sterilised at 121°C for 15 minutes). After incubation, the bacterial culture was sequentially activated in MRS-Broth medium twice un-

der the same anaerobic conditions, and a stock bacterial culture was prepared. *L. acidophilus* stock bacterial culture was activated by incubating at 37°C for 24 h under anaerobic conditions in MRS-Broth containing 1% (w/v) milled, dried, and boiled amaranth. Only *L. acidophilus* was activated under the same incubation conditions as the control group. The viability of the *L. acidophilus* strain was determined using the pour plate technique. Serial dilutions of activated bacterial strains in physiological saline were prepared and planted on sterile plates, and their growth in MRS-Agar medium was examined. Plates were placed in anaerogenic jars with AnaeroGen Gas Packs and left for 72 h under anaerobic incubation at 37°C. Colony forming units (cfu) per mL of probiotic bacteria (log cfu/mL) were determined by counting the colonies formed after incubation. The effect of amaranth on the increase in bacterial viability was determined by comparing the viability of ground, roasted, and boiled amaranth-containing bacteria with the control group. The results (two repetitions)

were statistically analysed using the MINITAB 17.0 (Minitab Inc., Stage College, PA) statistical program with One-Way ANOVA (*L. acidophilus* bacterial viability for each amaranth sample $p < 0.01$)).

Results and Discussion

Total Phenolic Content and Antioxidant Activity of Amaranth

The TPC and AA values of different amaranth extracts are given in Table 1. The effect of solvent medium, extraction method, and solvent medium x extraction method interaction on the TPC and AA results of amaranth extracts was statistically significant at a 99% confidence level according to ANOVA analysis (Table 2). The TPC and AA values of amaranth were seen to vary at different solvent mediums and extraction methods.

Table 1. The TPC (mg GAE/100 g amaranth) and AA (mg TE/100 g amaranth) of amaranth

Solvent		TPC	ABTS	FRAP
W	SE	581.80 ± 18.40 ^c	148.01 ± 1.37 ^b	32.28 ± 0.33 ^{ij}
	AH	606.70 ± 18.98 ^b	97.92 ± 1.36 ^c	15.84 ± 0.12 ^j
	BH	722.11 ± 18.68 ^a	176.53 ± 0.89 ^a	60.70 ± 2.34 ^{g,h,i}
M	SE	70.17 ± 4.29 ^{h,i}	47.30 ± 1.27 ^e	37.08 ± 2.17 ^j
	AH	134.90 ± 5.37 ^e	46.60 ± 1.24 ^e	40.43 ± 1.14 ^{h,i,j}
	BH	88.25 ± 2.88 ^g	79.73 ± 0.79 ^d	138.89 ± 3.59 ^{d,e}
E	SE	113.25 ± 5.04 ^f	11.89 ± 0.87 ^m	231.97 ± 12.85 ^a
	AH	364.11 ± 0.70 ^d	7.45 ± 0.14 ⁿ	207.65 ± 4.10 ^{a,b}
	BH	66.62 ± 0.92 ^{h,i,j}	34.52 ± 0.79 ^g	159.08 ± 17.76 ^{c,d}
A	SE	41.65 ± 0.43 ^{l,m,n}	19.00 ± 1.11 ^j	152.88 ± 0.40 ^{c,d}
	AH	59.10 ± 3.66 ^{j,k}	25.36 ± 0.60 ^h	131.22 ± 27.56 ^{d,e}
	BH	40.31 ± 0.42 ^{l,m,n}	16.81 ± 1.30 ^{k,l}	130.38 ± 42.72 ^{d,e}
EA	SE	34.70 ± 0.40 ⁿ	14.51 ± 0.90 ^{l,m}	159.86 ± 5.61 ^{c,d}
	AH	52.54 ± 1.27 ^{k,l}	20.14 ± 1.10 ^j	196.72 ± 6.31 ^b
	BH	47.49 ± 2.88 ^{k,l,m,n}	22.86 ± 0.42 ^{h,i}	180.25 ± 5.02 ^{b,c}
B	SE	53.66 ± 0.66 ^{j,k,l}	24.58 ± 1.57 ^h	112.06 ± 0.84 ^{c,f}
	AH	77.79 ± 1.67 ^{g,h}	39.47 ± 2.83 ^f	35.98 ± 0.86 ^{ij}
	BH	60.01 ± 2.63 ^{j,k}	35.48 ± 3.71 ^g	88.95 ± 1.43 ^{f,g}
PE	SE	36.71 ± 3.30 ^{m,n}	15.34 ± 0.71 ^{k,l,m}	160.68 ± 8.65 ^{c,d}
	AH	45.75 ± 1.36 ^{k,l,m,n}	15.15 ± 0.91 ^{k,l,m}	72.19 ± 3.90 ^{g,h}
	BH	42.38 ± 2.33 ^{l,m,n}	18.54 ± 1.58 ^{j,k}	151.59 ± 17.19 ^{c,d}
H	SE	48.81 ± 1.21 ^{k,l,m}	14.23 ± 0.22 ^{l,m}	149.83 ± 9.47 ^{c,d}
	AH	66.86 ± 4.93 ^{h,i,j}	17.92 ± 0.11 ^{j,k,l}	84.79 ± 3.86 ^{f,g}
	BH	47.03 ± 4.82 ^{k,l,m,n}	16.80 ± 0.46 ^{k,l}	148.91 ± 7.56 ^{c,d}

*mean ± standard deviation (two replicates)

W: water, M: methanol, E: ethanol, A: acetone, EA: ethyl acetate, B: butanol, PE: petroleum ether, H: hexane, SE: solvent extraction, AH: acidic hydrolysis, BH: basic hydrolysis, TPC: total phenolic content, AA: antioxidant activity; a–n: Lowercase superscripts indicate significant differences in TPC and AA values of amaranth under different extraction conditions for each method ($p < 0.01$).

Table 2. *P-values* of the effect of solvent medium and extraction method on TPC and AA of amaranth

	TPC	<i>p</i> -value ABTS	FRAP
Solvent medium	< 0.01	< 0.01	< 0.01
Extraction method	< 0.01	< 0.01	< 0.01
Solvent medium x Extraction method	< 0.01	< 0.01	< 0.01

TPC: total phenolic content, AA: antioxidant activity

The effects of solvent media and extraction method separately on TPC and AA values of amaranth at a 99% confidence level were investigated by ANOVA analysis (Table 3). When the TPC values of amaranth extracts were examined, it was found that the TPC of water extracts was the highest, while the TPC of petroleum ether extract was the lowest. As the solvent polarity decreased, the TPC of amaranth decreased. Compared to extraction methods, amaranth's TPC is generally highest in acidic hydrolysis and lowest in solvent extraction (Table 3). While the TPC of the aqueous basic hydrolysis extract of amaranth was found to be 722.11 ± 18.68 mg GAE/100g amaranth in the highest amount, it was determined as 34.70 ± 0.40 mg GAE/100g amaranth in the lowest amount of ethyl acetate solvent extract (Table 1). According to Peiretti *et al.* (2017), the TPC of the methanolic extract of amaranth seeds was determined as 4.35 ± 0.19 mg/g. Sandoval-Sicairos *et al.* (2020) determined the TPC of unprocessed amaranth seed as 23.3 ± 1.2 mg/100g DW and the TPC of germinated amaranth seed as 27.3 ± 1.8 mg/100g DW. In another study, the total phenolic content of amaranth flour after fermentation with water was 2.55 ± 0.20 mg GAE/g (Yeşil & Levent, 2022). According to Sarker, Oba, & Daramy (2020), in a study conducted with different genotypes of amaranth, the TPC range was between 78.22 ± 0.35 and 228.66 ± 0.42 µg/g DW.

When the AA results of amaranth are compared according to solvent mediums, the highest AA was observed in water solvent mediums in the ABTS method, while the lowest was in petroleum ether solvent mediums (Table 3). It was observed that the AA value increased with the ABTS method as the solvent polarity increased. In the FRAP method, while the highest AA was detected in the ethanol solvent medium, the lowest was observed in the methanol solvent medium (Table 3). It was observed that the AA value was higher in the medium polarity solvent with the FRAP method. When the antioxidant activities of amaranth were compared according to the extraction methods, it was determined that the basic hydrolysis results were the highest and the acidic hydrolysis results were the lowest in both analysis methods (Table 3). The

AA of amaranth was found in the highest basic hydrolysis extract of water (176.53 ± 0.89 mg TE/100g amaranth) with the ABTS method and the lowest in the acidic hydrolysis extract of ethanol (7.45 ± 0.14 mg TE/100g amaranth), while the highest in the ethanol extract (231.97 ± 12.85 mg TE/100g amaranth) with the FRAP method and the lowest in the acidic hydrolysis extract of water (15.84 ± 0.12 mg TE/100g amaranth). According to Sarker *et al.* (2020), in a study conducted with different genotypes of amaranth, the AA range was found to be between 16.71 ± 0.06 and 49.64 ± 0.04 µg/g DW by the ABTS method.

The most commonly used solvents in the extraction of phenolic compounds are water, ethanol, methanol, acetone, and their acidic/non-acidic water mixtures. Studies on this subject have reported that ethanol and methanol solvents are more effective than other solvents in extracting phenolic compounds. However, TPC and AA values of amaranth samples were generally determined to be higher in extracts obtained with water solvent than in methanol and ethanol solvents. Phenolic compounds do not show similar AA in hydrophilic and hydrophobic solvent environments. In the AA measurements, solvent type and polarity affect the hydrogen atom and electron transfer. Most of the phenolic compounds responsible for antioxidant properties are hydrophilic. Therefore, hydrogen atom bonding in polar solvents causes significant changes in the H-atom donor activities of phenolic compounds and affects the measured AA. Although there is a solvent effect in frequently used AA determination methods, the type and properties of the solvent affect each method differently. Therefore, each method gives different AA results even in the same solvent environment. When the effects were examined, the AA values of amaranth were found to be high in the water extract with the ABTS method, while they were increased in the ethanol extract with the FRAP method. ABTS and FRAP methods can be applied to hydrophilic and lipophilic phenolic compounds. However, while the ABTS method is in neutral conditions, the FRAP method is in acidic conditions (Boeing *et al.*, 2014; Çelik, 2011; Karaaslan *et al.*, 2018; Turkmen *et al.*, 2006).

Table 3. The effects of solvent media and extraction method on TPC and AA of amaranth

Solvent Medium	N	TPC (mg/100g)	ABTS (mg/100g)	FRAP (mg/100g)
W	6	646.46 ^a	140.57 ^a	36.28 ^e
M	6	96.76 ^c	57.88 ^b	72.13 ^d
E	6	181.33 ^b	17.95 ^{e,f}	199.57 ^a
A	6	47.02 ^{e,f}	20.39 ^d	138.16 ^c
EA	6	44.91 ^f	19.17 ^{d,e}	178.94 ^b
B	6	63.82 ^d	33.18 ^c	79.00 ^d
PE	6	41.76 ^f	16.21 ^f	128.15 ^c
H	6	54.23 ^e	16.23 ^f	127.84 ^c
Extraction Method	N	TPC (mg/100g)	ABTS (mg/100g)	FRAP (mg/100g)
SE	16	123.90 ^c	36.81 ^b	129.58 ^a
AH	16	176.44 ^a	33.66 ^c	98.11 ^b
BH	16	140.77 ^b	50.13 ^a	132.34 ^a

W: water, M: methanol, E: ethanol, A: acetone, EA: ethyl acetate, B: butanol, PE: petroleum ether, H: hexane, SE: solvent extraction, AH: acidic hydrolysis, BH: basic hydrolysis, TPC: total phenolic content, AA: antioxidant activity; a–f: Lowercase superscripts indicate significant differences in TPC and AA values of amaranth under different solvent mediums and extraction methods for each method ($p < 0.01$).

According to the results of the LSD (Least Significance Difference) test performed to determine the difference between extracts in terms of TPC, ABTS, and FRAP values, it is seen that all samples are in different groups ($p < 0.01$). The correlation of TPC, ABTS, and FRAP of amaranth obtained under different extraction conditions with various solvents was determined using the MINITAB 17.0 (Minitab Inc., Stage College, PA) statistical program with Basic statistical analysis. The inter-method correlation coefficient stated in the 99% confidence interval is given in Table 4. The structure of phenolic compounds and the application conditions of AA methods cause different antioxidant activities obtained from different solvent environments. The TPC method is carried out in basic conditions, the ABTS method in neutral conditions, and the FRAP method in acidic conditions. The different application conditions of the methods lead to differences in the antioxidant activity values of extracts prepared with solvents of different polarities. This situation also affects the correlation between methods. A positive correlation was observed between TPC and ABTS, while a negative correlation was observed between FRAP/ABTS and FRAP/TPC.

In-vitro Gastrointestinal Analysis

In *in-vitro* gastrointestinal analyses, digestion time and pH of the medium in simulated gastric fluid differ between researchers. Digestion in the simulated gastric medium is considered 90 min in some research, while 120 min in some research. It has been observed that the average pH range of gastric fluid

varies between 1.5 and 2.5. For the gastric medium, the digestion time and pH of SGF were chosen to be 120 min and 2.00 ± 0.20 , respectively. After gastric digestion, the samples were taken directly into the intestinal medium, and intestinal digestion was performed for 120 min. The TPC values of the samples taken from the gastric and intestinal mediums every 30 min were determined and examined against digestion time.

The TPC of milled, dried, and boiled amaranth after *in-vitro* gastrointestinal digestion is given in Figure 1. The initial TPC of milled amaranth was 4.52 ± 0.22 mg GAE/g sample, while the initial TPC of dried and boiled amaranth decreased (3.45 ± 0.13 and 2.38 ± 0.17 mg GAE/g sample, respectively). When amaranth was directly heat treated, a decrease in TPC value was observed compared to milled amaranth. Likewise, the TPC value of boiled amaranth in an aqueous medium decreased more than that of dried amaranth. Due to the complex structure of phenolic substances, some phenolic compounds are inactivated due to heat treatment, while others can become free. As a result, some phenolics in dried amaranth appear to be inactivated after heat treatment. In boiled amaranth, while some of the phenolics were inactivated after heat treatment, it was accepted that some were extracted by passing into the aqueous medium. Therefore, the initial TPC value of untreated amaranth (milled) was higher than that of treated (dried and boiled) amaranth samples.

After gastric digestion, the TPC of milled amaranth was increased by 46.03% to 6.59 ± 0.18 mg GAE/g sample, the TPC

of dried amaranth increased by 9.61% to 3.79 ± 0.20 mg GAE/g sample, and the TPC of boiled amaranth was increased by 13.93% to 2.71 ± 0.07 mg GAE/g sample. The amaranth samples were passed directly into the intestinal environment after gastric digestion, and an increase in TPC was observed in all amaranth samples. After intestinal digestion, the TPC of milled amaranth was increased by 114.62% to 9.69 ± 0.48 mg GAE/g sample, while the TPC of dried and boiled amaranth was increased by 21.02% (4.18 ± 0.35 mg GAE/g sample) and 27.96% (3.05 ± 0.20 mg GAE/g sample).

Phenolic compounds in food materials are generally linked to chemical bonds by carbohydrates, proteins, and dietary fibres (de Araújo *et al.*, 2021; Jakobek, 2015). Phenolic compounds become more resistant to *in-vitro* gastrointestinal conditions as they are present in foods in glycosylated form (de Araújo *et al.*, 2021; Pavan *et al.*, 2014). At the same time, as a result of chemical reactions with pH and enzyme changes during *in-vitro* gastrointestinal digestion, the TPC value and antioxidant properties of the food material may change (Dantas *et al.*, 2019; de Araújo *et al.*, 2021). The increase in TPC values of various forms of amaranth (milled, dried, and boiled) after *in-vitro* gastrointestinal digestion indicates that phenolic compounds were released more into the medium during digestion. At the same time, this increase shows that phenolics are hydrolysed into different forms, such as glycosides, in acidic and basic environments.

The Effect of Amaranth on Probiotic Bacterial Viability

Amaranth (prebiotic) with high carbohydrate and dietary fibre content was used to increase the viability of *L. acidophilus* probiotic bacteria, which is naturally found in the human microbiota and widely used in the dairy industry. The viability results of *L. acidophilus* probiotic bacteria are given in Table 5. The bacterial viability of *L. acidophilus* without amaranth as a control was 7.18 ± 0.01 log cfu/mL. In order to determine the prebiotic effect of amaranth on *L. acidophilus* probiotic bacteria, amaranth was added while the bacteria were activated, milled, dried, and boiled. It was observed that the viability of probiotic bacteria activated with amaranth increased. Compared to the control, a log increase of 0.68 units was seen in the viability of *L. acidophilus* bacteria activated with milled amaranth. Likewise, an increase of log 0.54 and log 0.05 units was detected in the bacterial viability of *L. acidophilus* activated with dried and boiled amaranth, respectively. While milled amaranth increased bacterial viability by 9.47%, dried amaranth increased by 7.46%, and boiled amaranth increased by 0.60%. Based on the statistical analysis, it was observed that the viability of bacteria activated by milled and dried amaranth increased significantly compared to the control (initial) *L. acidophilus* probiotic bacteria. However, no significant increase in viability was observed in bacteria activated by boiled amaranth.

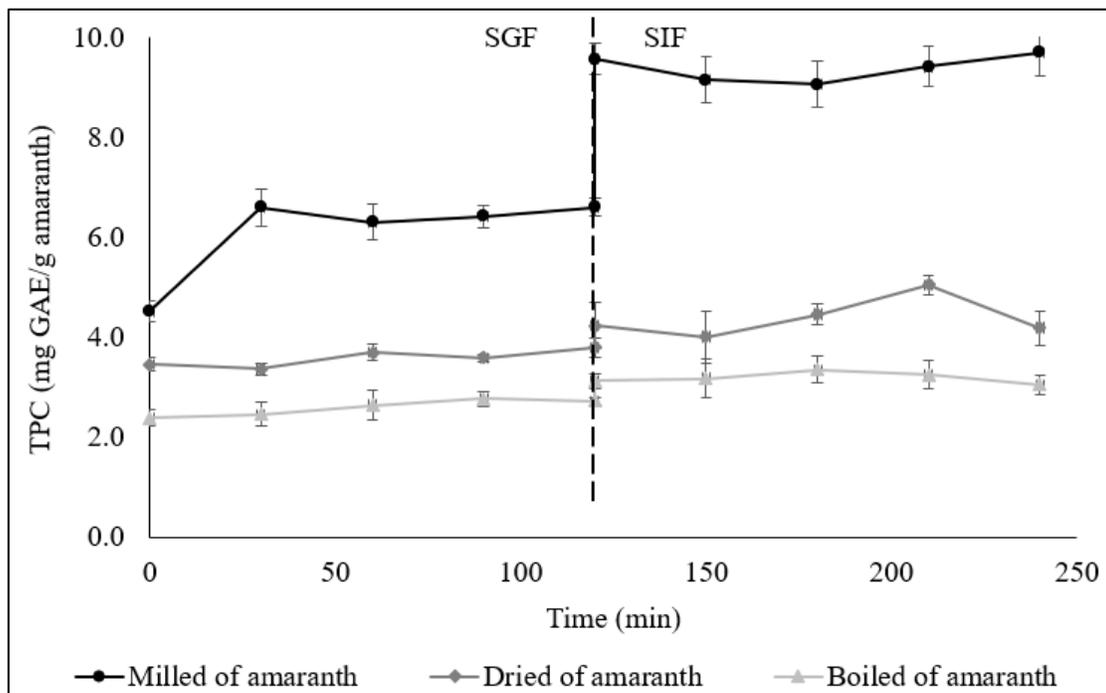


Figure 1. Gastrointestinal digestion of milled, dried, and boiled amaranth

Table 4. Correlation of TPC, ABTS, and FRAP of amaranth

		TPC	ABTS
ABTS	R ²	0.853	
	p-value	<0.01	
FRAP	R ²	-0.426	-0.578
	p-value	<0.01	<0.01

TPC: total phenolic content

Table 5. The effect of amaranth on the viability of *L. acidophilus* probiotic bacteria

	Viability of bacteria* (log cfu/mL)	Increase in vitality (%)
Control	7.18 ±0.01 ^c	
LA-MA	7.86 ±0.03 ^a	9.47
LA-DA	7.72 ±0.09 ^b	7.46
LA-BA	7.23 ±0.02 ^c	0.60

*mean ± standard deviation (two replicate)

Control: *L. acidophilus*, LA-MA: *L. acidophilus*-1% milled amaranth, LA-DA: *L. acidophilus*-1% dried Amaranth, LA-BA: *L. acidophilus*-1% boiled amaranth; a-c: Lowercase superscripts indicate differences in *L. acidophilus* bacterial viability for each amaranth sample.

Kockova *et al.* (2013) conducted a study to investigate the effect of various pseudocereals on the viability of *Lactobacillus rhamnosus* GG probiotic bacteria. The researchers incubated the bacteria with different grains and flours, including rye flour, rye grain, barley flour, whole barley flour, amaranth flour, amaranth grain, buckwheat flour, whole buckwheat flour, whole oat flour, and millet grain at a temperature of 37°C for 18 h. They then examined the increase in bacterial viability after fermentation with each of these substrates. The viability increased after fermentation with rye flour (log 2.37), rye grain (log 2.73), barley flour (log 1.99), whole barley flour (log 2.06), amaranth flour (log 3.57), amaranth grain (log 2.60), buckwheat flour (log 2.31), whole buckwheat flour (log 2.33), whole oat flour (log 1.95), and millet grain (log 2.61) (Kocková *et al.*, 2013).

Different processing and cooking methods applied to amaranth grains affect the nutritional composition of amaranth. Studies were shown that the nutritional content of amaranth, such as protein, fat, carbohydrate, dietary fiber, magnesium, iron, calcium, and phenolic compounds, decreases by boiling and cooking with various methods (Ugural & Akyol, 2022). When bacterial viability increases are examined, boiled amaranth's effect on bacterial viability is quite low. Accordingly, boiling amaranth significantly reduces its prebiotic properties. Considering the literature studies, boiling amaranth reduces its nutritional content (especially carbohydrates and dietary fibre) much more than drying cooking.

Conclusion

Pseudo-grains, which protect the health of *in-vitro* gastrointestinal digestion, positively affect the vitality of probiotic bacteria, are a good source of nutrients, and are an alternative to natural grains. This study determined the TPC and AA values of the extracts of amaranth, which is a pseudo-grain prepared in various solvent environments. In general, it was observed that TPC and AA values of amaranth increased with increasing solvent polarity. The change in TPC values of amaranth consumed today using different cooking techniques was investigated after *in-vitro* gastrointestinal digestion. Therefore, the increase in TPC values of milled, dried, and boiled amaranth after *in-vitro* gastrointestinal digestion was highest in milled amaranth and lowest in boiled amaranth. In addition, these forms of amaranth on the viability of *L. acidophilus* probiotic bacteria, which positively affects *in-vitro* gastrointestinal digestion, were investigated. As a result, it was observed that the prebiotic effect of boiled amaranth decreased due to the decrease in nutritional values, and it did not affect the increase in the number of live bacteria. However, the viability of *L. acidophilus* probiotic bacteria activated with milled amaranth increased by 9.47%. In this case, it is predicted that consuming amaranth, which has a very high nutritional value and a prebiotic effect, will be more beneficial for human health without applying heat treatment.

Compliance with Ethical Standards

Conflict of interests: The author(s) declares that for this article, they have no actual, potential, or perceived conflict of interest.

Ethics committee approval: Authors declare that this study includes no experiments with human or animal subjects. Ethics committee approval is not required for this study.

Data availability: Data will be made available on request.

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