

Effect of Popping on the Chemical Composition and Anti-Nutritional Factors of *Amaranthus caudatus* Grain

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ABSTRACT

Background and Objective: *Amaranthus caudatus* is a nutritious pseudo cereal with an optimal protein level similar to that of true cereals. However, it is known to have relatively high levels of antinutritional factors, making it essential to improve the nutritional value of *Amaranthus*-based feeds through processing. This study was designed to evaluate the effects of popping on the chemical composition and antinutritional factors of *Amaranthus*. **Materials and Methods:** *Amaranthus caudatus* grains were obtained from five farmers in Southwest Ethiopia, then cleaned, sun-dried and stored at 4°C. The grains were subsequently popped and milled for analysis. Proximate and mineral content analysis were performed at Jimma University and Horticoop Ethiopia, respectively, employing standardized methods, with each analysis conducted in triplicate to ensure accurate nutritional assessment. Phytate, tannin and oxalate contents were analyzed using established methods, followed by ANOVA for statistical differences between raw and popped samples. **Results:** The results indicated that the crude protein (CP (%)), ether extract (EE (%)) and metabolizable energy (ME) contents significantly decreased ($p < 0.05$) in popped grains compared with those in raw grains. Popping also significantly reduced ($p < 0.05$) the calcium and iron contents by 28.1 and 5.61%, respectively. Significant differences ($p < 0.05$) in antinutritional factors, particularly condensed tannins, phytates, alkaloids and saponins, were detected between the two groups. **Conclusion:** It provides significant amounts of protein, fat, gross energy and various macro and micro minerals, making it a valuable complement to cereals and legumes. Additionally, popping resulted in a notable decrease in crude protein, fat and energy content further investigation into alternative processing methods is crucial to fully unlock the nutritional potential of the crop *Amaranthus caudatus*.

KEYWORDS

Amaranthus caudatus, anti-nutritional factors, popping, proximate composition, phytate, oxalate contents

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INTRODUCTION

Amaranth (*Amaranthus* spp.) is a pseudo-cereal known for its high nutritional value, significant agronomic potential and diverse applications^{1,2}. Venskutonis and Kraujalis² highlighted the crop's growing interest due to its remarkable adaptability to various environmental conditions, including poor soil quality and limited water availability. Although there are many species of amaranth, only a few are used for human



consumption. It has been demonstrated that amaranth possesses an impressive nutritional profile and offers health benefits, such as antioxidant, anti-tumor and anti-hypercholesterolemic properties³. Additionally, amaranth is widely used in several countries as a grain, forage or silage crop for livestock, including cattle, chickens, pigs and rabbits⁴. Amaranth is renowned for its impressive nutritional profile, offering greater levels of protein, minerals and fats compared to cereals like barley, wheat, maize and sorghum^{5,6}. It also has an exceptional amino acid composition, being particularly rich in lysine and sulfur-containing amino acids, which are often lacking in cereals and legumes⁷. Additionally, amaranth oil is known for its high unsaturated fatty acid content and contains a notable amount of squalene, which may aid in preventing skin cancer and reducing serum cholesterol levels⁸. However, the nutritional advantages of amaranth can be hindered by the presence of mineral absorption inhibitors, especially phytates, which can vary significantly among different species, ranging from 0.52 to 2.24 g/100 g^{6,9}. Factors such as growing conditions, season, soil profile, harvesting techniques, maturation stage, species and genotype have been identified as key contributors to these variations in phytate content^{10,11}. Other potential mineral absorption inhibitors, such as tannins, have also been documented in various amaranth species across different regions^{5,9}. To reduce the anti-nutritional factor, seeds can undergo various processing methods such as popping, toasting, cooking, roasting, flaking, extruding or grinding¹².

In Ethiopia, amaranth can thrive in nearly all regions; however, it remains underutilized in many areas. In the Southwestern region, particularly in the Bench Majji Zone, the Me'enit ethnic group cultivates the crop and utilizes it in various forms¹². Despite its wide range of food applications, there is a lack of information on the nutritional value and mineral absorption inhibitors of amaranth grown in Ethiopia's agroecological conditions. Despite its nutritional benefits, amaranth contains significant levels of anti-nutritional factors that can hinder its utilization in food and feed applications. To address this challenge, processing methods such as popping have been explored to enhance the nutritional profile of amaranth grains. This study aims to investigate the effects of popping on the chemical composition and antinutritional factors of *Amaranthus caudatus*, providing insights into how thermal processing can improve its nutritional value and potential applications.

MATERIALS AND METHODS

Study area: The research was carried out at Jimma University College of Agriculture and Veterinary Medicine (JUCAVM), Jimma, Ethiopia from December 03-20, 2023.

Sample collection and preparation: As 15 kg of *Amaranthus caudatus* grain was purchased from five farmers in Dankila Kebele, Guraferda Wereda, Bench Majji Zone and Southwest Ethiopia (Fig. 1). Composite samples from each farmer were prepared by cleaning, sorting and washing the grains to remove foreign materials and immature seeds. The cleaned seeds were then sun-dried and stored at 4°C. Following the method described by Amare *et al.*¹², the sun-dried amaranth grains were popped by placing them in a hot clay pan for 10-15 sec (Fig. 2). The raw and grains popped (Fig. 2a-c) were milled to pass through a 0.425 mm sieve and stored in polyethylene bags at 4°C for analysis.

Chemical analysis: The proximate analysis was done at the Animal Nutrition Laboratory of Jimma University College of Agriculture and Veterinary Medicine (JUCAVM). The grains were analyzed for dry matter, crude protein, ether extract, crude fiber and total ash content according to Baur and Ensminger¹³. The metabolizable energy content of all the feed ingredients and the treatments was estimated following a method by Wiseman¹⁴:

$$\text{Metabolizable energy (kcal/kg DM)} = 3951 + 54.4 \text{ EE} - 88.7 \text{ CF} - 40.8 \text{ Ash}$$

Where:

EE = Ether extract

CF = Crude fiber



Fig. 1: *Amaranthus caudatus* crop



Fig. 2(a-c): *Amaranth* seed grain popping process

Mineral content analysis: A 0.5 g homogenized sample of amaranth was placed in a microwave digestion vessel (Milestone, Sorisole, Bergamo, Italy). To establish the ideal conditions, 9 mL of 10 M HNO₃ and 3 mL of 10 M HCl were added in a 9:3 volume ratio according to Lee *et al.*¹⁵. The digestion was conducted for 45 min at a temperature of 180°C.

The vessels were securely capped and placed in the microwave digestion system until a clear solution was achieved. The resulting clear, colorless solution was then filtered through Whatman No. 42 filter paper into a 50 mL volumetric flask. The volume was adjusted with 2% HNO₃. Subsequently, the target minerals (Ca, Mg, K, Na, Fe, Mn, Cu, Zn and P) in the amaranth samples were analyzed using an ICP-OES Spectro-Arcos (Model: ARCOS FHS12, USA) at the Horticoop Ethiopia P.L.C. Chemistry Laboratory, Bishoftu, Ethiopia.

Anti-nutritional analysis: The phytate content was assessed using a method outlined by Haug and Lantzsch¹⁶ and Gebeyehu *et al.*¹⁷. A dried sample weighing 0.6 g was combined with 10 mL of 0.2 N HCl in methanol and allowed to sit at room temperature for 1 hr. Following this, the mixture was centrifuged at 3000 rpm for 30 min (FISHER SCIENTIFIC Model: ACCUSPIN 400 MPN: USA) to separate the clear supernatant, which was then used for phytate analysis. To this supernatant, 2 mL of Wade reagent (0.03% FeCl₃ in water) was added to 3 mL of the sample solution and the mixture was centrifuged once more. The absorbance at 500 nm was measured using a spectrophotometer (LBDI-UV5800 Kalaunta, India). The phytate concentration was calculated by subtracting the absorbance of a blank solution (comprising 3 mL of 0.2 N HCl and 2 mL of Wade reagent) from that of the sample, utilizing an acid standard curve. For tannin content, a modified Burns method was employed as described by Maxson and Rooney¹⁸. As 0.2 g sample of anchote flour was placed in a screw-cap test tube and extracted with 10 mL of 1% HCl in methanol over 24 hrs at room temperature while being mechanically shaken. After centrifugation at

3000 rpm for 5 min, 1 mL of the supernatant was combined with 5 mL of vanillin HCl reagent (made by mixing equal volumes of 8% HCl in methanol and 4% vanillin in methanol). The absorbance was measured and the tannin concentration was determined using a standard curve based on D-catechin, expressed in milligrams of D-catechin per 100 g of sample¹⁷.

Total oxalates were extracted following the method described by Savage *et al.*¹⁹. As 1.0 g freeze-dried powdered sample was thoroughly mixed with 40 mL of 2.0 M HCl and shaken at 21°C for 15 min. The oxalic acid content was analyzed using High-Performance Liquid Chromatography (HPLC) according to the specified method of Castellari *et al.*²⁰. The extract was first thawed, then homogenized and filtered through a 0.45 µm nylon syringe filter prior to examination with a Perkin-Elmer Series 200 HPLC system. The absorbance was measured at 215 nm and the data were integrated using Perkin-Elmer Total Chrom software (Version 6.2.1). The sample concentration was estimated using a calibration curve (0-900 ppm, $R^2 = 0.999$) based on the elution profiles of the oxalic acid standard¹⁷.

Statistical analysis: Data was analyzed using One-way Analysis of Variance (ANOVA) to determine significant differences between the raw and popped methods. All data were analyzed following statistical procedures of SAS version 9.3 and subjected to Analysis of Variance (ANOVA) using a General Linear Model (GLM) whenever treatment effects were significant, the means were separated using the Tukey's Multiple Range Test at 5% level of significance.

RESULTS AND DISCUSSION

Proximate composition of raw amaranth grains: Table 1 presented the proximate composition of *Amaranthus caudatus* grain. The protein content of raw amaranth was found to be 16.2%, which is higher than the 14.0-15.5 g/100 g DM reported by Amare *et al.*¹². Bender and Schönlechner²¹ reported that the protein content ranges between 12.5 and 17.6%. Consistent with this study, Cai *et al.*²² reported protein content of 16.6% and other studies have found values ranging from 12.5 to 17.6% in selected light-seed varieties². Previous studies have also shown that amaranth grains are excellent sources of high-quality proteins, surpassing the protein content of common cereal grains (8-12%)²³.

The crude fat content of the amaranth grain was 6.6%, which is lower than the 7.2% reported by Cai *et al.*²². Depending on the species, crude fat content in amaranth grains has been reported to range from 2 to 10%²⁴⁻²⁶. The crude fat content in amaranth grain is higher compared to most conventional cereals such as wheat and maize²⁷. The crude fiber content was 4.6%, which is similar to the values of 4.1²² and 5.8%¹². The crude fiber content in amaranth is higher than that in common cereal grains such as rice, maize, sorghum and wheat²⁸.

The ash content was 2.6%. Similar ash contents were reported by Emire and Arega⁷, Caselato-Sousa and Amaya-Farfán²⁵ (2.9 and 2.4%, respectively), while Cai *et al.*²² and Escudero *et al.*²⁹ reported higher ash contents of 3.4 and 3.3%, respectively, compared to the current study. The ash content of amaranth grains is comparable to the ash content (2.7-3.0%) reported in grain teff²⁸.

Compared to the current value of 3795 kcal/kg, amaranth grain has previously been found to have metabolizable energy (ME) values of 2859 kcal/kg. Nüket *et al.*³⁰ and Ravindran *et al.*³¹ reported ME values of untreated amaranth species (*A. edulis*) as 3145 kcal/kg. Amaranth grain is known for its high nitrogen-free extract (NFE) content, which represents the carbohydrates in the grain, excluding fiber. In the current study, 70% NFE was obtained, which is higher than the mean content of nitrogen-free extracts ranging from 52.10 to 57.2 g/100 g of dry matter³².

Table 1: Proximate and metabolizable energy contents of raw and popped amaranth grains

Treatments	DM (%)	CP (%)	EE (%)	CF (%)	Ash (%)	NFE (%)	ME (kcal/kg)
Raw	92.1	16.2*	6.6*	4.6	2.6	70.0	3795.9
Popped	93.3	15.7*	5.1*	4.8	2.9	71.5	3684.4
SEM	0.06	0.12	0.11	0.15	0.11	0.16	13.35
p-value	0.09	0.002	0.0001	0.08	0.06	0.08	0.063

Values are presented as mean of triplicates±SEM, DM: Dry matter, CP: Crude protein, EE: Ether extract, CF: Crude fibre, NFE: Nitrogen-free extract, ME: Metabolizable energy (kcal/kg) and *Significant difference

Table 2: Mineral content of raw and popped amaranth seed

Treatments	Mineral concentrations (mg/100 g DM)								
	Ca	P	K	Mg	Na	Cu	Fe	MN	Zn
Raw	186.45*	322.45	480.20	282.30	18.73	1.23	17.58*	3.32	3.726
Popped	158.35*	326.34	490.40	289.10	19.78	1.07	11.97*	4.54	3.317
SEM	10.02	36.30	20.02	10.02	0.08	0.43	0.95	0.61	0.95
p-value	0.03	0.06	0.53	0.83	0.09	0.40	0.0001	0.063	0.081

Values are presented as mean of triplicates±SEM, Ca: Calcium, P: Phosphorus, K: Potassium, Mg: Magnesium, Na: Sodium, Cu: Copper, Fe: Iron, MN: Manganese, Zn: Zinc and *Significant difference

Effect of popping on proximate composition: The proximate composition results for popped amaranth grains were presented in Table 1. There were no significant differences ($p > 0.05$) in dry matter (DM (%)), crude fiber (CF (%)), nitrogen-free extract (NFE (%)) and total ash (ASH (%)) between the popped and raw grains. However, the crude protein (CP (%)), ether extract (EE (%)) and metabolizable energy (ME) content were significantly ($p < 0.05$) reduced in the popped amaranth grains compared to the raw ones.

Popping led to a significant decrease ($p < 0.05$) in protein content from 16.2 to 15.7% g DM. This reduction in protein content might be due to the partial oxidation of heat-sensitive amino³³. This finding was consistent with reports by Amare *et al.*¹², who also noted a reduction in crude protein content after popping and roasting raw amaranths.

Popping resulted in a 1.5% reduction in crude fat content, likely due to the removal of free fatty acids during the heating process. This result aligned with the study by Pisarikova *et al.*³³ which also reported higher ether extract content in raw amaranth. The current results are in agreement with those reported by Bressani *et al.*²⁴, who found a 3.9% reduction in lipid content due to dry heating of *A. caudatus* seeds. However, Amare *et al.*¹² reported a significant increase in fat content from 7.6 to 9.2 g/100 g DM during popping, possibly due to the partial removal of the pericarp, which is low in fat. No differences were observed in the crude fiber content of raw and popped amaranth seeds. Laovoravit *et al.*³⁴ reported similar findings, noting that dry heated amaranth seed flour contained 2.48% of crude fiber, which is comparable to the 2.43% found in raw seed flour. The ME content was significantly decreased by heat treatments, with popping resulting in a 111.6 kcal/kg ME reduction. Reports in food and feed science suggest that heat treatment can reduce the negative effects on the energetic efficiency of grains³⁴. Previous estimates of ME (kcal/kg) in heat-treated amaranth grain ranged from 2859³⁴ to 3650³⁵. Differences are likely due to variations in amaranth variety, physicochemical properties and processing methods³⁶.

Mineral composition amaranth grains: Table 2 presented the macro and micro mineral levels in both raw and popped amaranth seeds. The calcium (Ca) content in this study is 186.45 mg/100 g, which is higher than the values reported by Caselato-Sousa and Amaya-Farfán²⁵ at 159 mg/100 g and Whitney and Rolfes³⁷ at 153 mg/100 g. In contrast, Amare *et al.*¹² reported a higher Ca content of 215 mg/100 g for brown grain amaranth. The potassium (K) levels were found to be lower than the 508 mg/100 g reported by Caselato-Sousa and Amaya-Farfán²⁵, yet higher than the values of 366.7 mg/100 g reported by Schakel *et al.*³⁸. Magnesium (Mg) content in the studied amaranth grains was measured at 282.3 mg/100 g, which is comparable to the values of 248.0 mg/100 g²⁵ and 266.7 mg/100 g³⁸.

Sodium (Na) content was recorded at 18.73 mg/100 g, indicating that amaranth grains have a moderate sodium level, yet this is still higher than typical cereal grains, as noted by Schakel *et al.*³⁸. Previous reports indicated sodium levels of 20 mg/100 g³⁸⁻⁴¹ and a range of 3.4 to 11.9 mg/100 g³⁹. Generally, the variation in mineral composition of the grains from the different studies might be due to the nature of the soil and the variety of the plant. Copper (Cu) content in the current study was found to be 1.23 mg/100 g, aligned with Temesgen and Bultosa²⁸ findings of 0.8 to 1.1 mg/100 g, though slightly higher than 0.8 mg/100 g. The iron (Fe) content was measured at 17.58 mg/100 g, which falls within the range of 17.5 to 32.5 mg/100 g reported by Temesgen and Bultosa²⁸. This finding is notably higher than the typical value of 7.6 mg/100 g cited by Caselato-Sousa and Amaya-Farfán²⁵ and Schakel *et al.*³⁸ while approaching the higher end of the range reported by Kachiguma *et al.*⁴⁰, which spans from 3.6 to 22.5 mg/100 g. Variations in iron content can be attributed to agronomic factors related to the conditions under which the amaranth grains were harvested, as noted by Bultosa⁴¹. The manganese (Mn) content was consistent with the findings of previous studies⁴²⁻⁴⁵, reporting at 3.3 mg/100 g. The zinc (Zn) levels in this study were consistent with the findings of Amare *et al.*¹², reporting at 3.4 mg/100 g, but lower than those reported by Mustafa *et al.*⁵ amaranth has a higher amount of Ca, P and Fe than rice and maize and a comparable amount of Fe in wheat⁴³.

Effect of popping on mineral composition amaranth grains: Table 2 presented the mineral content in raw and popped amaranth seeds. There were no significant differences ($p > 0.05$) in the levels of phosphorus, potassium, magnesium, sodium, sulfur, copper, manganese and zinc between the raw and popped grains. However, popping significantly reduced ($p < 0.05$) the Ca and Fe content by 28.1 and 5.61%, respectively. This reduction in calcium and iron could be due to the loss of the pericarp during popping, as over 66% of the total minerals are located in the bran and germ fractions of amaranth⁴⁴. Amare *et al.*¹² also noted that the outer layer of amaranth grain contains high amounts of iron and calcium. Gamel *et al.*⁴⁴ reported that the levels of essential elements (magnesium, phosphorus, potassium, calcium, manganese, iron and copper) were not affected by popping at 180°C for 10 sec on a hot plate. Similarly, Pedersen *et al.*⁶ observed no significant changes in the levels of phosphorus, calcium, iron, copper and zinc after popping at 200°C for 15-20 sec.

Anti-nutritional content of raw and popped amaranth grains: Table 3 presented a summary of the anti-nutritional content of raw and popped amaranth grains. A significant difference ($p < 0.05$) was found between the two groups, particularly in the levels of condensed tannins, phytates, alkaloids and saponins.

Raw amaranth grains had a significantly higher ($p < 0.05$) condensed tannin content of 7.23 mg catechin/g. Popping reduced the tannin content by 50.7%, consistent with findings by Odunitan-Wayas *et al.*⁴⁵, who reported decreased tannin levels following heat treatment. While tannins are often considered anti-nutritional, their effects can vary based on their chemical structure, the amount consumed and the specific animal species involved.

The phytate content in raw amaranth grains was significantly higher ($p < 0.05$) at 88.85 µg/g. Popping resulted in a 30.4% reduction in phytate levels compared to raw amaranth, supporting the results of Odunitan-Wayas *et al.*⁴⁵.

Table 3: Anti-nutritional factor composition of raw and popped amaranth seed

Treatments	Condensed tannin (mg catechin/g)	Phytate (µg/g)	Oxalate (g)	Alkaloids (%)	Saponin (%)
Raw	7.23*	88.85*	0.0090	3.1*	2.28*
Popped	3.56*	61.82*	0.0088	1.7*	1.04*
SEM	0.07	2.55	0.01	0.07	0.04
P value	0.0001	0.001	0.321	0.0001	0.0001

Values are presented as mean of triplicates ± SEM, Values marked with an asterisk (*) indicates a significant difference

Alkaloid content decreased by 45.1% after popping, while oxalate levels remained similar between the two groups. Additionally, saponin content was significantly lower ($p < 0.001$) in popped amaranth grains, with a 54.4% reduction compared to raw grains, consistent with findings from Odunitan-Wayas *et al.*⁴⁵. In conclusion, popping effectively reduced the anti-nutritional factors present in raw amaranth grains, except for oxalate content. Anti-nutritional factors reduce the overall absorption of nutrients, particularly minerals, proteins and vitamins, thereby hampering optimal nutrient utilization and reducing nutritive values³⁷.

Further investigation is necessary to assess the *in vivo* and *in vitro* bioavailability of essential nutrients across different varieties of grain amaranth, as well as to examine their interactions with anti-nutritional factors such as phytates and oxalates. Notably, *Amaranthus caudatus* is characterized by elevated levels of mineral absorption inhibitors that considerably hinder nutrient availability. While popping was evaluated as a potential method to reduce these inhibitors, it proved insufficient, leaving ongoing challenges regarding mineral binding and bioavailability unaddressed.

CONCLUSION

Amaranth cultivated in southwestern Ethiopia provides significant amounts of protein, fat, gross energy and various macro and micro minerals, making it a valuable complement to cereals and legumes. Additionally, popping resulted in a notable decrease in crude protein, fat and energy content. Therefore, further exploration of alternative processing techniques is essential to fully harness the nutritional potential of this crop.

SIGNIFICANCE STATEMENT

This study addresses the nutritional challenges associated with *Amaranthus caudatus*, a highly nutritious pseudo cereal known for its protein content but also its antinutritional factors. By evaluating the effects of popping on its chemical composition, the research highlights how processing can alter nutrient availability. The findings reveal a decrease in essential nutrients and an alteration in antinutritional factors, emphasizing the need for alternative processing methods to enhance the crop's nutritional value. This work contributes to the broader understanding of how food processing can optimize the benefits of underutilized crops, ultimately supporting food security and nutritional improvement in diverse diets.

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