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Proceeding Paper

Effects of Germination Periods on Proximate, Mineral, and Antinutrient Profiles of Pearl Millet (*Pennisetum glacum*) and Grain Amaranth (*Amaranth cruentus*) Flours †

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Abstract: Protein and minerals are central to human diets. This study investigated the effects of germination periods (24 h, 48 h, and 72 h) as a pretreatment process on the nutritional and antinutritional components of pearl millet and amaranth grain flours (28 ± 0.1 °C). The results showed significant differences ($p < 0.05$) in the proximate, mineral, and antinutrient factors of the samples. The highest protein content, 8.61% in the millet and 17.37% in the amaranth, was observed after 24 h of germination. Specifically, the protein content in the millet after 24 h of germination, 8.61%, was significantly higher than after 72 h, which was 8.07%, and also higher than in the ungerminated millet flour, which was 7.71%. Furthermore, the concentration of iron (Fe) in the millet after 24 h, 48 h, and 72 h of germination was 4.77 mg/100 g, 4.90 mg/100 g, and 4.96 mg/100 g, while in the amaranth; they were 4.10 mg/100 g, 5.86 mg/100 g, and 5.89 mg/100 g. The iron concentration in the ungerminated millet flour, 3.31 mg/100 g, was significantly lower than what was observed in the germinated millet across the periods. A similar trend was observed in the amaranth. In terms of antinutrients, a notable reduction in their concentration was observed as the germination periods increased. The concentration of phytates in the millet ranged from 0.173 g to 0.836/100 g. However, the phytate concentration significantly decreased from 0.836 g/100 g in the ungerminated millet flour to 0.326 g/100 g, 0.230 g/100 g, and 0.173 g/100 g after 24 h, 48 h, and 72 h of germination, respectively. This study shows the potential influence of germination periods on the quality of grains such as millet and amaranth.

Keywords: germination; cereals; nutrients; antinutrients; bioavailability

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1. Introduction

Cereals are a staple food in many diets, providing a significant amount of energy and nutrients. They are a good source of carbohydrates, protein, vitamins, minerals, and fiber. Whole grains are particularly important because they are high in nutrients and can help improve diet quality. Cereal-based foods are usually consumed as dietary staples by adults, while nursing mothers use them in the making of complementary food, especially by poor ones who cannot afford commercial baby foods [1,2]. The major cereal-based foods in most developing regions are derived from millets, sorghum, maize, and rice [3]. Infant cereal is one of the foods most commonly introduced when an infant has shown signs of readiness for solids. Because infant cereal is an excellent source of iron and zinc, it is an ideal choice to support the developmental needs of the growing infant [4].

Millet is an important drought-resistant grain that is widely cultivated in arid and semi-arid regions of Asia and Africa. It serves as a vital component of traditional diets and a primary source of carbohydrates, proteins, and essential phytonutrients for rural populations and those in impoverished urban areas [5]. The millet variety includes kodo, finger, foxtail, proso, barnyard, and pearl millet. However, pearl millet is widely cultivated in Africa and Asia [5].

Recently, there has been a growing interest in grain amaranth, which is regarded as a pseudo-cereal. Amaranth is a versatile and resilient crop that is tolerant to a wide range of weather conditions [6]. It has a well-balanced amino acid profile, being especially rich in lysine, which is lacking in many other grains. Amaranth is also a good source of iron, zinc, magnesium, phosphorus, and manganese. It is regarded to possess superior protein content compared to other staple cereal crops [7]. Because of its high protein content, amaranth can be utilized singly or as a food fortifier in cereal-based mixes. Amaranth seeds are popped, ground to make flour, eaten as porridge, or incorporated into breakfast cereals. Amaranth flour is used in beverages, weaning foods, and baked products like bread and cookies [6]. The development of improved complementary infant foods sourced from locally available, underutilized food materials has been suggested as a probable approach that could be adopted to reduce the problem of protein energy malnutrition [8].

Although cereals are rich in macronutrients, micronutrients, and phytochemicals that are essential in human nutrition, they contain antinutritional factors that form complexes with essential nutrients within food matrixes, thereby reducing their bioavailability [9]. However, germination, which is an inexpensive processing technique that could be adopted at the household level, has been suggested as being capable of reducing these antinutritional factors, which include phytates, oxalate, tannins, trypsin inhibitors. The germination of seeds activates enzymes like phytase, which degrades phytates and leads to decreased phytic acid concentration in the food material [10]. Germination not only deactivates antinutritional factors to improve the concentration of nutrients such as protein and minerals, including iron, zinc, and calcium, it also helps in protein digestibility. Germination improves protein digestibility by breaking down antinutritional factors and activating enzymes that break down complex molecules into simpler components [11]. It is expedient, therefore, to determine the appropriate germination period suitable for a visible improvement in protein and mineral concentration in millet and amaranth grains. This could guide rural women who might want to use germination processes as a pretreatment in making weaning foods from millet and amaranth gains. This study aimed to evaluate the effects of different germination periods as a pretreatment for nutrient improvement in millet and amaranth grain flours.

2. Material and Method

2.1. Raw Material Collection

The raw materials were obtained as follows: the pearl millet (*Pennisetum glaucum*) was sourced from the Bodija market in Ibadan, Nigeria, while the amaranth grain seeds (*Amaranthus cruentus*) were obtained from the fields at the National Horticultural Research Institute (NIHORT) in Ibadan, Nigeria.

2.2. Preparation of Germinated Millet and Amaranth Grain Flours

The germinated millet and amaranth grain flours were prepared according to the methodology outlined by [12,13]. The grains underwent sorting, thorough washing, and immersion in water at a grain-to-water ratio of 1:3 (*w*/*v*) for 24 h at a constant room temperature of 28 ± 0.1 °C. Subsequently, the grains were enveloped in a clean white cloth and subjected to varying germination periods of 24, 48, and 72 h. Following germination, the grains were subjected to drying at temperatures ranging from 45 to 50 °C and were subsequently milled using a Marlex Excella electric grinder, model 750. The resultant flour was finely powdered through a 0.25 mm-diameter sieve and then stored in an airtight container.

2.3. Proximate Determination

The samples were analyzed for moisture, crude protein, ash, fat, and crude fiber using the AOAC method [14]. The carbohydrate content was calculated as % carbohydrate = 100 − % (moisture + crude protein + fat + ash + crude fiber). The energy content was determined by multiplying the percentage values of the protein, fat, and carbohydrates by the water.

2.4. Mineral Analysis

The mineral content was analyzed as prescribed by [15]. Approximately 2 g of the samples were weighed and then heated at 550 °C in a muffle furnace (Model: Kejian Kinsgeo KJ-3020). Then, the ash was dissolved in 100 mL of 1 M HCl. The dissolved ash was analyzed for zinc, iron, calcium, potassium, sodium, and magnesium contents using an atomic absorption spectrophotometer (Model: Buck Scientific ACCUSYS 211).

2.5. Antinutritional Factor Determination

The phytate content was determined by following the method described by [16]. The oxalate content was determined as described by [17]. The tannin content was determined according to the method described by [18], and the trypsin inhibitor unit was determined using the method described by [19].

3. Result and Discussions

3.1. Proximate Compositions of Millet and Amaranth Flours as Influenced by Germination Periods

The protein content in the germinated millet flour was found to be 8.61% after 24 h, exhibiting a significant increase ($p < 0.05$) compared to the values recorded at 48 h (8.54%) and 72 h (8.07%) and in the ungerminated millet (7.71%). Furthermore, the highest protein content of 17.37% was observed in germinated amaranth grain after 24 h of germination, representing a significant increase compared to other time periods and ungerminated amaranth (Table 1).

Table 1. Effects of germination period on proximate composition of millet and amaranth grain flour.

Samples	Moisture (%)	Protein (%)	Fat (%)	Ash (%)	Fiber (%)	CHO (%)	Energy (Kcal/100 g)
Millet							
Ungerminated	9.44 ± 0.06 ^{d}	7.71 ± 0.02 ^{d}	7.67 ± 0.02 ^{a}	1.11 ± 0.01 ^{abc}	2.26 ± 0.01 ^{b}	71.49 ± 0.01 ^{d}	366.69 ± 0.01 ^{d}
24 h germination	8.30 ± 0.01 ^{c}	8.61 ± 0.01 ^{a}	5.72 ± 0.01 ^{bcd}	1.17 ± 0.01 ^{ab}	3.07 ± 0.01 ^{a}	73.79 ± 0.01 ^{b}	385.02 ± 0.01 ^{a}
48 h germination	8.81 ± 0.01 ^{b}	8.54 ± 0.15 ^{ab}	5.76 ± 0.15 ^{b}	1. 19 ± 0.10 ^{a}	3.03 ± 0.0 ^{c}	74.38 ± 0.02 ^{a}	382.42 ± 0.10 ^{b}
72 h germination	8.10 ± 0.01 ^{a}	8.07 ± 0.01 ^{c}	5.73 ± 0.01 ^{bc}	1.19 ± 0.01 ^{a}	3.08 ± 0.01 ^{a}	73.40 ± 0.01 ^{bc}	379.33 ± 0.10 ^{c}
Amaranth							
Ungerminated	8.41 ± 0.01 ^{a}	16.08 ± 0.12 ^{c}	5.75 ± 0.01 ^{d}	2.19 ± 0.01 ^{d}	4.37 ± 0.01 ^{d}	63.28 ± 0.10 ^{b}	371.16 ± 0.01 ^{d}
24 h germination	7.20 ± 0.01 ^{d}	17.37 ± 0.01 ^{a}	6.75 ± 0.05 ^{c}	2.65 ± 0.01 ^{ab}	4.62 ± 0.01 ^{c}	62.46 ± 0.01 ^{d}	379.61 ± 0.01 ^{a}
48 h germination	7.51 ± 0.01 ^{c}	16.73 ± 0.01 ^{b}	7.64 ± 0.01 ^{b}	2.47 ± 0.01 ^{c}	4.68 ± 0.02 ^{b}	63.18 ± 0.11 ^{c}	375.03 ± 0.01 ^{b}
72 h germination	7.70 ± 0.46 ^{b}	16.05 ± 0.56 ^{cd}	8.13 ± 0.20 ^{a}	2.69 ± 0.02 ^{a}	4.97 ± 0.01 ^{a}	63.61 ± 0.11 ^{a}	374.69 ± 0.01 ^{bc}

Note: Values are means ± standard deviation of duplicate determinations. Values with the same superscript letters in the same column are not significantly different at $p < 0.05$.

Some documented studies reported an increase in crude protein levels in germinated grains, including oats, waxy wheat, and barley. The authors of [20,21] observed a higher protein concentration (7.40%) after 24 h of germination compared to 48 h (6.83%) during the germination of bean seeds. The authors of [22], in a study on the physicochemical and nutritional changes in two amaranth species (Amaranthus quitensis and Amaranthus caudatus), noted that a short germination period of 24 h appeared to be sufficient for inducing significant alterations in the physicochemical and nutritional properties of amaranth, as the protein concentration substantially increased after 24 h compared to ungerminated seeds.

3.2. Minerals of Millet and Amaranth Grain Flours as Influenced by Germination Periods

The mineral compositions of germinated millet and amaranth grain flours as influenced by germination periods is presented in Table 2. During the 24-h germination period, the iron (Fe) concentration in the millet flour was 4.77 mg/100 g, which was lower than the concentrations observed at 48 h (4.90 mg/100 g) and 72 h (4.94 mg/100 g) of germination. However, it was significantly higher than the concentration in the ungerminated millet flour, which was 3.31 mg/100 g. These results demonstrate that the longer the germination period is, the higher the concentration of minerals will be. A similar improvement in mineral concentration was also observed in the germinated amaranth flour compared to the ungerminated.

Table 2. Effects of germination period on mineral composition of millet and amaranth grain flours.

Samples	Fe (mg/100 g)	Ca (mg/100 g)	Zn (mg/100 g)	Mg (mg/100 g)	K (mg/100 g)	Na (mg/100 g)
Millet						
Ungerminated	3.31 ± 1.10 ^{d}	7.32 ± 0.02 ^{c}	3.32 ± 0.02 ^{c}	9.41 ± 0.01 ^{d}	251.73 ± 0.63 ^{d}	30.01 ± 0.01 ^{d}
24 h germination	4.77 ± 0.01 ^{c}	9.40 ± 0.03 ^{ab}	4.92 ± 0.02 ^{b}	12.07 ± 0.01 ^{a}	257.50 ± 0.51 ^{c}	33.01 ± 0.02 ^{c}
48 h germination	4.90 ± 0.02 ^{ab}	9.49 ± 0.01 ^{b}	4.94 ± 0.01 ^{b}	11.29 ± 0.00 ^{c}	268.83 ± 0.55 ^{b}	33.66 ± 0.11 ^{b}
72 h germination	4.94 ± 0.01 ^{a}	10.82 ± 0.01 ^{a}	5.60 ± 0.08 ^{a}	12.03 ± 0.01 ^{b}	282.73 ± 0.55 ^{a}	43.54 ± 0.11 ^{a}
Amaranth						
Ungerminated	3.50 ± 0.12 ^{d}	33.14 ± 0.31 ^{d}	3.17 ± 0.01 ^{d}	90.01 ± 0.01 ^{d}	250.83 ± 0.60 ^{d}	25.01 ± 0.01 ^{d}
24 h germination	4.10 ± 0.01 ^{c}	38.74 ± 0.01 ^{b}	4.92 ± 0.02 ^{b}	94.16 ± 0.02 ^{b}	271.43 ± 0.60 ^{b}	30.20 ± 0.01 ^{c}
48 h germination	5.86 ± 0.01 ^{ab}	38.59 ± 0.01 ^{bc}	4.81 ± 0.01 ^{bc}	94.30 ± 0.01 ^{b}	264.30 ± 1.92 b^{c}	30.81 ± 0.02 ^{b}
72 h germination	5.89 ± 0.01 ^{a}	40.80 ± 0.01 ^{a}	5.75 ± 0.01 ^{a}	95.81 ± 0.01 ^{a}	291.36 ± 0.50 ^{a}	37.70 ± 0.01 ^{a}

Note: Values are means ± standard deviation of duplicate determinations. Values with the same superscript letters in the same column are not significantly different at $p < 0.05$.

Iron and zinc are trace minerals that are essential for infants' normal growth and development, especially during their first six months of life. Iron deficiency can lead to anemia, which is common in early childhood and late infancy [23]. Zinc deficiency in malnourished children can result in stunted growth and increased susceptibility to infections, such as diarrhea and pneumonia [24].

3.3. Antinutritional Factors of Millet and Amaranth Grains as Influenced by Germination Periods

The antinutritional contents of germinated millet and amaranth grain flours are summarized in Table 3. Various degrees of reduction in the concentration of the antinutritional factors were observed in the germinated millet and amaranth flours compared to ungerminated. The concentrations of tannins and phytates in millet flour significantly ($p < 0.05$) reduced from 0.850 g/100 g and 0.836 g/100 g to 0.416 g/100 g and 0.326 g/100 g, which represents reductions of 48.9% and 61.0%, respectively, after a 24-h germination period. A significant reduction in antinutrient concentration was also observed in the amaranth flour as influenced by periods of germination.

Antinutritional factors are secondary metabolites that affect the nutritional value of foods [25]. However, their concentration can be reduced using traditional food processing techniques such as germination [26]. Germination lowers antinutritional factors by activating enzymes such as phytase, amylase, and protease and consequently increases the bioavailability of essential nutrients such as minerals and protein [27]. Thus, germination processes could be traditionally engaged in the treatment of cereals to reduce their antinutrients, as observed in this study.

Table 3. Effects of germination period on antinutrients in millet and amaranth grain flour.

Samples	Tannin (g/100 g)	Oxalate (g/100 g)	Phytate (g/100 g)	Trypsin. I (TIU/g)
Millet				
Ungerminated	0.850 ± 0.00 [a]	0.620 ± 0.12 [a]	0.836 ± 0.01 a	0.570 ± 0.00 a
24 h germination	0.416 ± 0.05 [b]	0.253 ± 0.01 [b]	0.326 ± 0.05 b	0.363 ± 0.01 b
48 h germination	0.333 ± 0.11 [c]	0.251 ± 0.01 [bc]	0.230 ± 0.00 c	0.341 ± 0.00 c
72 h germination	0.323 ± 0.05 [cd]	0.213 ± 0.01 [d]	0.173 ± 0.11 d	0.340 ± 0.01 [cd]
Amaranth				
Ungerminated	0.633 ± 0.12 [a]	0.520 ± 0.52 [a]	0.613 ± 0.81 [a]	0.656 ± 0.85 [a]
24 h germination	0.273 ± 0.05 [b]	0.223 ± 0.21 [b]	0.286 ± 0.18 [b]	0.513 ± 0.46 [c]
48 h germination	0.163 ± 0.05 [c]	0.213 ± 0.22 [c]	0.226 ± 0.21 [c]	0.460 ± 0.59 [b]
72 h germination	0.154 ± 0.33 [cd]	0.196 ± 0.19 [d]	0.210 ± 0.23 [cd]	0.446 ± 0.44 [d]

Note: Values are means ± standard deviation of duplicate determinations. Values with the same superscript letters in the same column are not significantly different at $p < 0.05$.

4. Conclusions

The rising demand in global food consumption of cereals and protein-rich grains like amaranth is propelled by their provision of complementary proteins and an array of critical nutrients that benefit human health. Though they have immense nutritional values, their antinutritional factors, such as phytates, trypsin, tannins, oxalates, and saponins, may limit the bioavailability and bioaccessibility of the nutrients inherent in them. Thus, germination was identified as a promising, economical, and re-emerging process that improves their nutritional values for optimal utilization. In this study, notably, a 24-h germination period was identified as giving the highest protein content, a critical nutritional component in both adult and infant nutrition for growth and development.

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