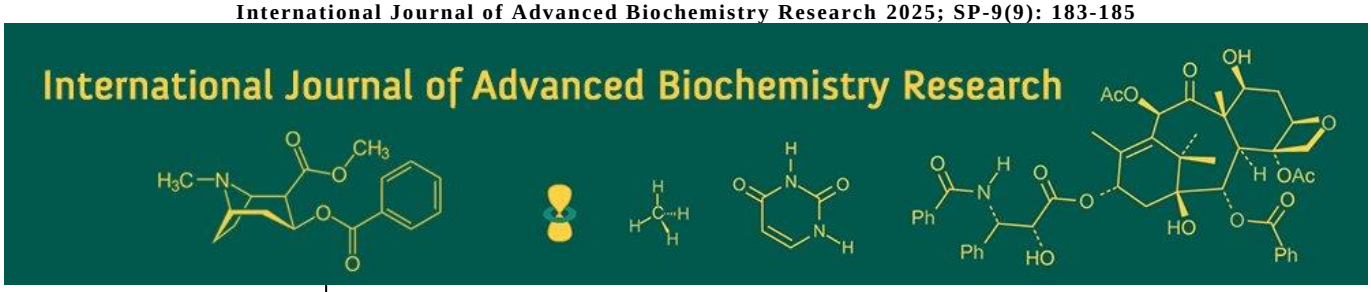

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Impact of soaking and germination on the nutritional and mineral composition of amaranth grain

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Abstract
Amaranth has attracted the attention of nutritionists and food technologists due to its high nutritional value. Also, it can be a useful diet for people with gluten sensitivities because of the gluten-free pseudocereal. Amaranth grain quality may be improved by processing procedures, which are thought to be an efficient method. The oldest and most efficient methods for increasing food grains' nutritional components are soaking and germination. There was a significant ($p \leq 0.05$) increase in amaranth protein, crude fibre and carbohydrate content from 15.07 to 17.02, 5.27 to 12.87 and 75.62 to 77.25 percent, while minerals like Fe, Mn, Zn, Cu, Ca and Mg increased from 6.67 to 12.53, 1.77 to 2.27, 3.02 to 3.72, 0.87 to 1.28, 130.3 to 162.2 and 215.9 to 276.6 mg/100 g, respectively after 18 h of soaking and 48 h of germination. Thus, soaking and germination turned out to be great ways to increase the nutritional and mineral content of these underutilised grains.

Keywords: Amaranth, pseudocereal, soaking, germination

1. Introduction
Pseudocereals are dicotyledonous plants that resemble real cereals in both appearance and starch substance. They are gluten-free. The three most widely consume pseudocereals are amaranth (*Amaranthus* L. spp.), buckwheat (*Fagopyrum esculentum* Moench and *Fagopyrum tataricum* (L.) Gaertn), and quinoa (*Chenopodium quinoa* Willd). They have gained more attention due to their nutritional and health-promoting properties, as well as their compatibility for those with gluten sensitivity and coeliac disease (Kaur, 2016) [1].
The Amaranthaceae family, commonly known as the Amaranth family, includes nutritionally superior pseudocereals. The name "Amaranthus" is derived from the Greek word "Anthos," meaning "flower," and signifies something "everlasting" or "unwilting." (Rastogi and Shukla, 2013) [2]. It is also known as *Rajagro* (Gujarati), *Rajgeera* (Marathi), *Cheera* (Malayalam), *Keerai* (Tamil), and *Ramdana* ('seed sent by God') in Bihar (Raiger and Jajoriya, 2023). Grain amaranth is a highly nutritious crop and an outstanding source of high-quality protein, containing between 14.5 and 15.1 percent. Its protein content closely matches the levels recommended by FAO/WHO for a balanced diet. The protein efficiency ratio (PER) of grain amaranth ranges from 1.5 to 2.0, compared to 2.5 for casein. Additionally, when cooked, amaranth grains exhibit high protein digestibility, averaging around 90% (Das, 2016) [3]. It has 3.1 to 5 percent dietary fibre, 2.5 to 4.4 percent ash, 5.6 to 10.9 percent crude fat and abundant supplies of minerals, including calcium, magnesium, copper, zinc, iron, potassium, and phosphorus (Gebreil *et al*., 2020) [4]. In order to improve the nutritional composition of amaranth grain and products to be made from them, the current study was designed to examine the nutritional composition of amaranth grain as well as the impact of soaking and germination processing treatments on nutritional and mineral components. The utilization of these underutilized grains may be encouraged by the application of certain processing techniques for amaranth.

2. Materials and Methods
2.1 Materials
Amaranth grains (Swastik brand) were procured from the local farmer of village Undel, Khambhat, Anand, Gujarat, India.

2.2 Methods

The soaking and germination of amaranth grains were carried out as per the method given by Egli *et al*. (2002) [5]. In this, soaking was done at ambient temperature with grain to water ratio 1:5 w/v, while germination was carried out by keeping grains tightly packed in a muslin cloth and maintaining temperature and RH to 25 °C and 90 percent respectively in BOD incubator, followed by kilning at 60 °C till moisture content reaches to below 10 percent. The experimental matrix showing the list of runs conducted for the RSM design is tabulated in Table 1. Standard AOAC (2019) [6] method were followed for analysis of moisture content, fat content, crude fibre content, protein content and total ash content of raw amaranth and processed amaranth, while Mineral content was determined using Inductively Coupled Plasma-Optical Emission Spectrometry (ICP-OES).

Table 1: Experimental RSM design for standardization of germination process of amaranth grains

Run No.	Soaking period (h) (A)	Germination period (h) (B)
1	18	32
2	24	32
3	18	48
4	22	43
5	14	21
6	12	32
7	14	43
8	18	16
9	22	21
10	18	32

2.3 Statistical analysis

All the experiments were carried out in triplicate. The data were analyzed using Central Composite Rotatable Design (CCRD) in Response Surface Methodology (RSM) with 5% level of significance.

3. Results and Discussion

Different combinations of soaking and germination periods have a substantial impact on proximate composition of amaranth grains shown in Table 2. There were significant changes observed in fat, fibre, protein, ash, and carbohydrate contents of the germinated amaranth grain as compared to raw grains. The results for those are tabulated on dry basis in Table 2.

The fat content of germinated samples decreased significantly from an initial 6.64 percent in raw grains to 3.40 percent (run no. 3) and 3.53 percent (run no. 4) as shown in Table 2. It is due to lipase activity during germination, which hydrolyses lipids into free fatty acids and glycerol. There may also be reduction in some water-soluble fatty acids, liquid fats and triglycerides due to leaching in water during soaking and washing the grains (Chauhan *et al*., 2015 and Xu *et al*., 2017) [9, 10]. Thakur *et al*. (2021) [8] and Siwatch *et al*. (2019) [7] also observed a similar decreased trend in fat content of amaranth grain from 5.35 to 2.58 percent and 7.33 to 6.51 percent respectively, after the soaking and germination.

The fibre content of germinated samples increased significantly from an initial 5.27 percent in raw grains to 12.87 percent (run no. 3) and 12.53 percent (run no. 4). This happens because of the protein-carbohydrate interaction is disrupted and the polysaccharides in the seeds' cell wall undergo structural modification (Jan *et al*., 2017) [11]. Thakur *et al*. (2021) [8] and Siwatch *et al*. (2019) [7] also found increasing trend in fibre content from 3.83 to 6.69 percent and 2.04 to 2.19 percent respectively, after soaking and germination of amaranth grains.

The protein content of germinated samples increased significantly from an initial 15.07 percent in raw grains to 17.02 percent (run no. 3) and 16.92 percent (run no. 4). This increase might result from concentrating protein fractions, a relative decrease in dry matter content, and proteolytic enzyme activity (Hejazi and Orsat, 2016) [12]. Thakur *et al*. (2021) [8] and Siwatch *et al*. (2019) [7] reported a similar increased trend in protein content of amaranth grain from 17.40 to 18.62 percent and 15.27 to 16.19 percent respectively, after the soaking and germination.

There were minimal variations shown in the ash content after the germination of amaranth grain (Table 2), all the runs showing a slight decrease from the raw amaranth grains (2.66 percent). This reduction might be due to leaching out of some water-sensitive soluble minerals during soaking (Uwaegbute *et al*., 2000 and Chinma *et al*., 2009) [13, 14]. Thakur *et al*. (2021) [8] and Siwatch *et al*. (2019) [7] reported reduction in ash content from 2.32 to 2.09 percent and 2.93 to 2.20 percent respectively.

The carbohydrates content of germinated samples increased significantly from an initial 75.62 percent in raw grains to 77.25 percent (run no. 3) and 77.20 percent (run no. 4). During soaking and germination, the complex carbohydrates converted into simple sugars by enzymes like α-amylase resulting in improved digestibility (Oghbaei & Prakash, 2016) [15].

Table 2: Effect of soaking and germination period on proximate composition of amaranth flour

Run No.	Soaking period (h)	Germination period (h)	Moisture (%)	Fat (%) d.b.	Fibre (%) d.b.	Protein (%) d.b.	Ash (%) d.b.	Carbohydrates (%) d.b.
Raw amaranth	-	-	10.3±0.15^{e}	6.64±0.15^{e}	5.27±0.13^{a}	15.07±0.09^{a}	2.66±0.01^{h}	75.62±0.22^{d}
1	18	32	7.44±0.18^{c}	4.57±0.17^{c}	9.52±0.05^{f}	16.26±0.37^{cd}	2.44±0.12^{d}	76.71±0.24^{abc}
2	24	32	6.82±0.20^{a}	4.22±0.05^{b}	10.59±0.09^{g}	16.49±0.23^{cde}	2.40±0.04^{c}	76.88±0.19^{ab}
3	18	48	7.29±0.20^{bc}	3.40±0.14^{a}	12.87±0.07^{j}	17.02±0.26^{e}	2.31±0.01^{a}	77.25±0.36^{a}
4	22	43	6.96±0.07^{a}	3.53±0.18^{a}	12.53±0.09^{i}	16.92±0.08^{e}	2.33±0.01^{a}	77.20±0.27^{a}
5	14	21	7.11±0.11^{ab}	5.67±0.16^{d}	6.06±0.06^{c}	15.49±0.39^{ab}	2.51±0.08^{f}	76.32±0.31^{bc}
6	12	32	7.47±0.17^{c}	5.16±0.13^{d}	7.94±0.13^{e}	15.96±0.43^{bc}	2.48±0.01^{e}	76.39±0.54^{bc}
7	14	43	6.91±0.23^{a}	4.15±0.16^{b}	11.18±0.15^{h}	16.66±0.16^{e}	2.38±0.00^{b}	76.80±0.33^{abc}
8	18	16	7.59±0.08^{cd}	5.80±0.23^{d}	5.58±0.12^{b}	15.39±0.34^{ab}	2.54±0.01^{g}	76.25±0.22^{c}
9	22	21	7.81±0.13^{d}	5.27±0.09^{d}	7.64±0.06^{d}	15.93±0.48^{bc}	2.49±0.01^{ef}	76.29±0.41^{bc}
10	18	32	7.05±0.16^{ab}	4.59±0.10^{c}	9.42±0.08^{f}	16.28±0.24^{cd}	2.43±0.01^{d}	76.68±0.25^{abc}
S.Em.			0.10	0.26	0.79	0.18	0.02	0.19
C.D.			0.23	0.59	1.80	0.40	0.05	0.54
C.V. (%)			4.51	18.00	27.05	3.42	3.14	0.42

Table 3: Effect of soaking and germination period on mineral composition of amaranth flour

Run No.	Soaking period (h)	Germination period (h)	Fe (mg/100 g)	Mn (mg/100 g)	Zn (mg/100 g)	Cu (mg/100 g)	Ca (mg/100 g)	Mg (mg/100 g)
Raw grain	-	-	6.67±0.10^{h}	1.77±0.06^{d}	3.02±0.08^{d}	0.87±0.01^{e}	130.3±01.51^{h}	215.9±0.65^{j}
1	18	32	7.82±0.15d^{e}	1.97±0.07^{bcd}	3.42±0.11^{bc}	0.96±0.04^{d}	148.8±1.08^{d}	251.2±0.62^{e}
2	24	32	8.07±0.09^{d}	2.02±0.09^{bc}	3.47±0.12^{bc}	1.08±0.02^{c}	151.1±1.53^{c}	252.9±0.43^{d}
3	18	48	12.53±0.18^{a}	2.27±0.09^{a}	3.72±0.20^{a}	1.28±0.06^{a}	162.2±0.72^{a}	276.6±0.43^{a}
4	22	43	10.17±0.15^{b}	2.17±0.12^{ab}	3.60±0.08^{ab}	1.19±0.04^{b}	162.0±0.81^{a}	274.3±0.52^{b}
5	14	21	7.32±0.13^{fg}	1.91±0.09^{cd}	3.30±0.16^{c}	0.88±0.03^{e}	141.7±0.85^{f}	244.0±0.51^{h}
6	12	32	7.77±0.16^{e}	1.97±0.10^{bcd}	3.37±0.03^{c}	0.96±0.05^{d}	145.2±2.70^{e}	246.5±0.43^{g}
7	14	43	8.47±0.18^{c}	2.07±0.14^{abc}	3.50±0.12^{bc}	1.08±0.02^{c}	158.4±1.04^{b}	264.2±0.60^{c}
8	18	16	7.06±0.09^{g}	1.90±0.15^{cd}	3.30±0.07^{c}	0.86±0.04^{e}	136.7±0.98^{g}	233.0±0.45^{i}
9	22	21	7.57±0.23^{ef}	1.95±0.18^{bcd}	3.37±0.09^{c}	0.90±0.03^{de}	143.5±0.85^{ef}	246.0±0.60 g
10	18	32	7.86±0.19^{de}	1.97±0.18^{bcd}	3.42±0.07^{bc}	0.96±0.04^{d}	148.4±0.95^{d}	250.1±0.45^{f}
S.Em.			0.09	0.07	0.06	0.09	0.02	0.75
C.D.			0.27	0.21	0.19	0.27	0.06	2.21
C.V. (%)			1.93	6.28	3.41	1.93	4.00	0.88

As shown in Table 3, the mineral content increased significantly ($p \leq 0.05$) after the soaking and germination process of amaranth grains. The iron content increased from 6.67 to 12.53 mg/100 g, similarly manganese from 1.77 to 2.27 mg/100 g, zinc from 3.02 to 3.72 mg/100 g, copper from 0.87 to 1.28, calcium content from 130.3 to 162.2 mg/100 g and magnesium from 215.9 to 276.6 mg/100 g. Thakur *et al*. (2021) [8] was also reported similar increasing trend in mineral content after soaking and germination process of amaranth grains. Guardianelli *et al*. (2019) [16] observed 17.65, 7.45, and 5.26 percent increase in Zn, Cu, and Mn contents after soaking and germination of amaranth grains, respectively.

4. Conclusions

Amaranth shown to be rich suppliers of proteins, crude fibres and mineral components after a chemical analysis. Processing methods like soaking and germination are easy ways to enhance the nutritional qualities without the use of complicated equipment's. The fat content of germinated grains decreases as a result of the hydrolysis of lipids brought on by the enhanced lipolytic activity during germination. Protein, crude fibre and minerals compounds are all abundant in germinated amaranth grain. As a result, processing methods can improve the nutritional content and usability of these underutilised grains, making them the ideal diet for those with coeliac disease or other gluten-related conditions.

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