



## Effect of phytate on bioavailability of select nutrients and simple techniques to reduce phytate in finger millet (*Eleusine coracana*)

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### Abstract

Finger millet, an important dryland crop, is loaded with nutrients. However, it also contains antinutritional factors like phytate. Present study was conducted to determine the effect of various simple processing techniques in reducing phytate. Samples were collected from 15 villages of mountain districts of Uttarakhand, India and evaluated for various parameters. They were subjected to three different treatments – roasting, boiling and malting to evaluate their effects on reducing phytate content and bioavailability of select nutrients. Results indicated that all three treatments were able to reduce phytate content to a varying extent apart from improving the bioavailability, as determined through molar ratios of Fe, Zn, and Ca with phytate. Simple food processing treatments are beneficial to improve the nutritional quality of finger millet for consumption as well as for developing ready-to-eat products.

**Keywords:** finger millet, nutrients, anti-nutrients, phytate, bioavailability

### Introduction

Nutrients are substances that are required by human for growth, cellular activities and overall physiological functions for normal development whereas, the term “anti-nutrients” suggests that they reduce nutritional value of a food product through various means of actions. For the wellbeing, both bio-accessibility and bioavailability of nutrients is very crucial. Bio-accessibility is presence of nutrients in the food so that they are available in the gut for the digestion whereas bioavailability is the fraction of the nutrients that is finally available to the system for physiological functions (Espin *et al.*, 2007) <sup>[9]</sup>. Many food sources are rich in bio-accessibility, yet their bioavailability is negatively affected due to presence of anti-nutrients in them (Soetan and Oyewole, 2009) <sup>[32]</sup>. Anti-nutrients are naturally found in animals and many plant-based foods. In plants, they are compounds designed to protect from bacterial infections and insect infestation (Peumans and Van Damme 1995) <sup>[23]</sup>. Phytate, a phosphorous compound, occurring naturally in many plants is one such compound. Few experts argue against phytate being labelled as anti-nutrient due to certain benefits it has. However, in the current study it has been considered as anti-nutritional compound since it inhibits the bioavailability of certain nutrients. The phosphorus bound to phytate is typically not in the bioavailable form to humans or any non-ruminant animals (Cosgrove 1980). The major concern about the presence of phytate in the diet is its negative effect on mineral uptake. Phytic acid causes the bioavailability of essential minerals to decrease (Desphande and Cheryan, 1984) <sup>[8]</sup>. Minerals of concern in this regard include Zn<sup>2+</sup>, Fe<sup>2+/3+</sup>, Ca<sup>2+</sup>, Mg<sup>2+</sup>, and Cu<sup>2+</sup> (Lönnerdal 2002; Lopez *et al.*, 2002) <sup>[15, 16]</sup>. Mainly due to its chelating property, phytic acid is considered as a most effective anti-nutrient in foods and a cause of mineral ions deficiencies in animal and human nutrition (Grases *et al.* 2017; Bora 2014) <sup>[10, 4]</sup>. Lately, it has been stressed that phytic acid affects the bioavailability of minerals and has a strong effect on infants, pregnant and lactating women when large portions of cereal-based foods are consumed (Chan *et al.* 2007; Al Hasan *et al.* 2016) <sup>[5, 2]</sup>.

Phytate is present in foods at various levels ranging from 0.1 to 6.0% (Gupta *et al.* 2015) <sup>[11]</sup>. In several cases, about 50 to 80% of the total phosphorous in seeds is present as phytates (Lott *et al.* 2000; Raboy 2000) <sup>[17, 26]</sup>. Several strategies have been suggested by researchers to minimise the anti-nutritional effect of food. Whole grains, seeds, legumes, and certain nuts have a high content of phytates that can decrease the absorption of iron, zinc, magnesium and calcium (Schlemmer *et al.* 2009). Several scholars have overviewed plant food anti-nutritional factors (Thakur *et al.* 2019; Samtiya *et al.* 2020) <sup>[28]</sup>. However, to lower the risk of poor absorption of nutrients, it is recommended to avoid eating large quantities of foods containing anti-nutrients at one meal (Stevenson *et al.* 2012) <sup>[33]</sup>.

Despite of its anti-nutritional nature, researchers have also identified many important health benefits of phytate. It has been suggested to play a significant role directly or indirectly in addressing several disease conditions such

as diabetes, cancer, inflammation, heart problems, Parkinson's, Alzheimer's, to mention a few (Irshad *et al* 2017) <sup>[12]</sup>. The medicinal values of phytic acid outweighed its negative impact (Abdulwaliyu 2019) <sup>[11]</sup>. Several strategies have also been suggested to reduce the antinutritional factors in seeds like germination (Nkhata 2018, Laxmi 2015, fermentation (Mohapatra *et al* 2019; Gupta *et al* 2015) <sup>[19, 11]</sup> so that the people are not refrained from consuming otherwise nutritious food grains.

Locally known as *mandua*, finger millet (*Eleusine coracana*) is significant part of traditional cropping system of Uttarakhand apart from other dryland areas. Being primarily rain fed agriculture here the significance of finger millet cannot be undermined, specifically in the present condition of climate changes. It enjoys the place as rice and wheat in the platter in the form of *roti* or *chapatti* (flat bread). Despite its prominent place as calcium-rich grain, the anti-nutritional factors in seeds cannot be ignored. Finger millet contains these anti-nutrients that negatively affect grain palatability and can be a constraint for nutrients bioaccessibility based on the genotypes (Puranik *et al* 2017) <sup>[24]</sup>. There is a wide range of phytate and oxalate content in finger millet (Antony and Chandra, 1999; Makokha *et al.*, 2002) <sup>[3, 18]</sup>. The present study was conducted realizing the need to address the problem of phytate in landraces of Uttarakhand by simple ways of processing techniques and also to improve the availability of select nutrients.



**Fig 1:** Finger millet plant

### Materials and Methods

Samples were collected from various locations in Uttarakhand, India and are summarised in Table 1. Of all the lines collected, few were selected for further profiling based on phenotypic variations. All the lines collected are landraces except the seed collected from Khakra village of Rudraprayag (Sr No 21) that was being distributed as “Pantnagar seed” (after the famous agricultural university’s location – GB Pant university of agriculture and technology, Pantnagar) to the farmers, but the distributor did not know the varietal name. The name mentioned here ‘R-Khakra-Pantnagar’ is the name just for identification as we have given to other lines. It has been used as control for comparison with other landraces.

**Table 1:** Details of finger millet landraces collected

S. No.	District	Block	Village	Sample Name	Selected for Profiling?
1	Almora	Hawalbag	Govindpoor	A-HB-Govindpur	Yes
2	Nainital	Ramgardh	Khairada	N-RG-Khairda	Yes
3	Pithoragardh	Berinag	Udiyari	PT-B-Udiyari	Yes
4	Pithoragardh	Moonakot	Maharkhola	PT-M-Maharkhola	No
5	Bageshwar	Kapkot	Dadim khet	B-K-Dadimkhet	Yes
6	Bageshwar	Garud	Mawaii	B-G-Mawaii	Yes
7	Bageshwar	Bageswar	Chauna	B-B-Chauna	No
8	Uttarkashi	Dunda	Pokhariyal Gaon	U-D-Pokhariyal Gaon	Yes
9	Uttarkashi	Dunda	Chondiyal Gaon	U-D-Chondiyalgaon	No
10	Uttarkashi	Dunda	Saudh	U-D-Saudh	Yes
11	Champawat	Lohaghat	Kali Dhek	CHW-L-Kolidhek	No
12	Chamoli	Karnprayag	Jhirkoti	CH-K-Jhirkoti	Yes
13	Chamoli	Gairsain	Jingooda	CH-G-Jingooda	No

14	Tehari Gadhwal	Pratapnagar	Motana	T-P-Motana	Yes
15	Tehari Gadhwal	Narendra Nagar	Gawad	T-N-Gawad	Yes
16	Tehari Gadhwal	Chamba	Sonekoti	T-CH-Sonekoti	No
17	Tehari Gadhwal	Fakot	Kumali	T-F-Kumali	Yes
18	Tehari Gadhwal	Ghansali	Sayalkund	T-GH-Sayalkund	No
19	Rudraprayag	Rudraprayag	Bachansyu	R-R-Bachansyu	Yes
20	Rudraprayag	August-muni	Rampur	R-AM-Rampur	Yes
21	Rudraprayag	Jakholi	Khakara (seed of Pantnagar)	R-Khakra-Pantnagar	Yes
22	Pauri Gadhwal	Khirsu	Dikholiya	P-Kh-Dikholiya	No
23	Pauri Gadhwal	Khirsu	Takoli	P-Kh-Takoli	Yes

### Methodology

Elemental analysis was performed using Inductively coupled plasma mass spectrometry, ICP-MS (Make: Agilent Technologies, Model: 7800) as per AOAC 2015.01 Using a calibrated analytical balance,  $0.1 \pm 0.005$  g of ground sample was accurately weighed and transferred into a clean, dry teflon digestion vessel. Subsequently, 5-ml of suprapure concentrated Nitric acid, 1-ml of hydrogen peroxide and 3-ml of demineralized water was added. After about 30 minutes, digestion vessels were closed with caps and loaded into the microwave digestion carousel (Make: Anton Paar, Model: Multiwave GO) and digested using a temperature-time program as given in Table 2. After completion of the digestion program, teflon vessels were cooled to room temperature, removed from the carousel and slowly unscrewed to release the pressure. The solution was filtered through Whatmann No. 42 filter paper into a 10 ml calibrated volumetric flask. Filter paper was rinsed with demineralized water and quantitatively transferred to the volumetric flask to make-up to the volume. On each day of the analysis, ICP-MS was tuned prior to initiation of analysis. Stock standards were prepared using individual standards of Iron, Zinc and Calcium. Appropriate dilutions were made to prepare working standard, which was used for preparing seven calibration standards ranging in concentration from 0.005 to 0.5 mg/l (ppm) using 2% nitric acid as the diluent. Upon acceptable calibration, digestates were aspirated to determine concentration of elements of interest based on their mass – Iron (mass 56), Zinc (mass 66), and Calcium (mass 40). ICP-MS conditions set for the elemental analysis are given in Table 3. Samples were diluted using acidified ultrapure water whenever the concentration in the digestate exceeded calibration range.

**Table 2:** Microwave digestion program

Step	Power (W)	Temp (°C)	Ramp (°C/Min)	Hold Time (Min)	Fan level
1	650	100	10	15	1
2	650	140	10	15	1
3	650	180	10	15	2

**Table 3:** ICP-MS analytical conditions

Parameter	Value
RF Power (W)	1550
RF matching (V)	1.75
Sample Depth (mm)	8.0
Torch –H (mm)	0.0
Torch-V (mm)	0.3
Carrier Gas flow (L/min)	1.03
Nebulizer (rps)	0.1
Sample pump (rps)	0.5

For Phytate determination, 10ml of 2.4% HCl was added to 0.2g of finely ground sample. A control sample which contained all the reagents but without the sample was processed along with each batch and underwent all the analytical steps. The mixture was kept on boiling water bath for 5 min and immediately transferred onto shaker incubator for 55 min at 37°C and 250 rpm. The reaction mixture was transferred to a 15 ml BD vial and centrifuged at 1000g for 5 min. Supernatant was transferred to another 15 ml BD vial containing 1g of NaCl; mixed well by keeping in shaker incubator at 250 rpm and 37°C for 20 min followed by 60-minute storage in refrigerator at 4°C. Mixture was centrifuged again at 1000 g for 10 min. From the supernatant 1ml was taken and added to 9 ml of distilled water (using glass pipettes). 3-ml of aliquots from each 10ml volume (Control, std and samples) were taken in Ria vials in duplicate. To each 3-ml of aliquot, 1ml of Wade reagent was added. The reaction mixture was shaken properly and centrifuged at 1000g for 10 min. Supernatants were used to measure absorbance at 500nm using a UV-Vis Spectrophotometer (Make: Agilent Technologies, Model: Cary 60). Sodium salt of phytic acid was used to prepare calibration standards.

Molar ratios, the mole of phytate and minerals, was determined as per the method described by Queiroz *et al.* (2011) [25] and Norhaizan and Faizadatul Ain (2009) [22]. Molar ratios were calculated by dividing the weight of phytate and minerals with its atomic weight. Phytic acid/Min (Phy/Min) molar ratio is calculated according to the equation,

$$MR = (\text{Phy} / \text{MW Phy}) / (\text{Min} / \text{AW Min})$$

Where, MR = molar ratio; Phy = phytic acid in the sample (mg/kg); MW Phy = phytic acid molecular weight (660 Da); Min = Fe, Zn or Ca in the sample (mg/kg); AW Min = Fe (56 Da), Zn (65 Da) or Ca (40 Da) atomic weight.

## Results and discussion

### Effect on reducing phytate content

Analytical results of various forms of phosphorous including phytate in control samples as well as treated samples is shown in Table 4. R-Khakra-Pantnagar is an established variety specifically released for hilly regions of Uttarakhand. Results indicate that there exists a wide variability for all the parameters studied amongst the lines. Only three lines (PT-B-Udiyari, P-Kh-Takoli, and N-RG-Khairda) showed higher phytate content than R-Khakra-Pantnagar indicating that there are many other lines that are nutritionally better in terms of their phytate content compared to released variety. Hence, these can be considered in further breeding programs and varietal development / improvement. However, before being considered for such varietal improvement programs, other parameters of interest must be evaluated.

All the three food processing techniques—Roasting, Boiling, and Malting were able to reduce the phytate content to a varying extent. Percent reduction in phytate content of various lines due to the employed techniques is shown in Table 5. Highest reduction (36%) in phytate content was observed due to malting in line, A-HB-Govindpur while lowest reduction of 0.8% was observed in B-K-Dadimkhet due to boiling. Roasting has resulted in an average reduction of 20% across lines with maximum reduction of 35% in P-Kh-Takoli while R-R-Bachansyu showed a reduction of only 2.9%. Similarly, malting resulted in an average reduction of 19% across lines with maximum of 36% reduction in A-HB-Govindpur and minimum of 1.3% in T-F-Kumali. Boiling showed lowest reduction amongst all the treatments at 16% across lines with a maximum reduction of 32% in A-HB-Govindpur while 0.8% was observed in B-K-Dadimkhet. Reductions in phytate content for all lines studied across treatments are graphically represented in Figure 2. Application of processing techniques like boiling and malting have been found to be effective in reducing phytate content earlier also (Shigihalli *et al.* 2018, Wadikar *et al.* 2006) [30, 36]. Similarly, roasting was also found effective in decreasing the phytate content significantly in millets and this degradation of phytates increases with the increase in the temperature (Salif *et al.* 2019) [27]. However, current study didn't vary treatment temperatures. Findings of Tiwari *et al.* (2014) [34] and Chauhan *et al.* (2015) [6] in pearl millet are also in agreement with our findings.

### Effect on bioavailability of select nutrients

Bioavailability of select nutrients (Iron, Zinc and Calcium) was studied using indirect method by determining the molar ratios between phytate and nutrient of interest. This is much easier and economical method compared to enzymatic method and can easily be deployed by researchers for mass screening of germplasm. R-AM-Rampur and A-HB-Govindpur were found to have much lower Phytate:Iron molar ratio compared to other lines and are significantly lower than the local variety, R-Khakra-Pantnagar while R-AM-Rampur was found to be lowest in Phytate: Zinc molar ratios (Table 6). However, Phytate:Calcium molar ratios were very low for all the lines studied. Results of molar ratios are summarised in Table 6. In all the lines, treatments have improved the bioavailability to varying extents as shown in Figures 3 through 5 indicating that simple treatments can improve the absorption of minerals resulting in improved nutritional status.

However, considering the phytate: Calcium molar ratio > 0.24 being detrimental for Calcium absorption as suggested by Morris and Ellis (1985), these ratios in finger millet obtained (0.26-1.14 in treated lines compared to 0.40-1.28 in control) in current study are far higher even after treatments. For phytate: Zinc molar ratio, the value ranged from 67 to 160 post-treatment compared to 79 - 162 in control. Turnlund *et al.* (1984) [35] found that when the molar ratios exceeded 15, zinc absorption will be affected negatively. All the lines showed very high phytate: zinc molar ratios thereby indicating that increased consumption of finger millet may potentially hinder zinc absorption. The molar ratio of phytate to iron ranged from 16 - 74 in control whereas in treated lines it varied from 14 - 72. When the phytate: iron molar ratio of >1, as suggested by Hallberg *et al.* (1989) [13] is used as the critical value, the phytate content in majority of the samples seems to affect the iron absorption negatively. Hence, studied lines of finger millet including the released variety, are far exceeding the suggested molar ratios for increased bioavailability. However, when included in diet along with other diversified foods, such molar ratios may decrease resulting in increased absorption. It is suggested that more research would be needed to quantify the effect on molar ratios when a combination of treatments is used. Apart, various time – temperature combinations can also be studied which may result in desirable molar ratios.



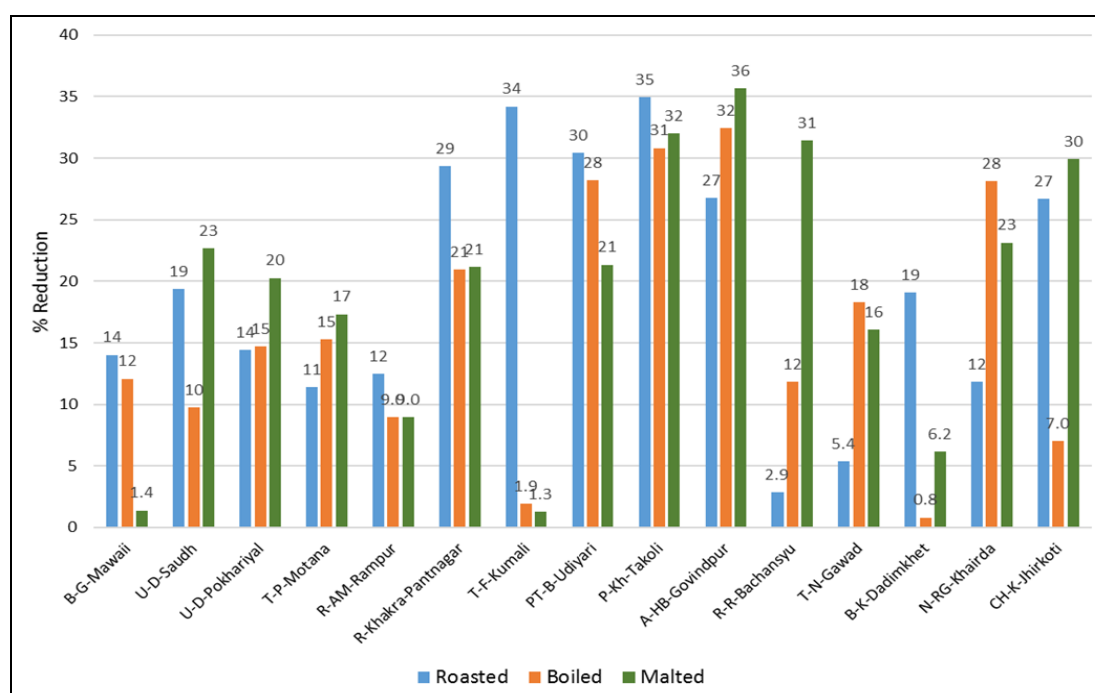
**Table 4:** Results of phosphorous forms in treated and control lines

Line	Treatment															
	Control				Roasting				Boiling				Malting			
	Total P	Ext. P	Bound P	Phytate P	Total P	Ext. P	Bound P	Phytate P	Total P	Ext. P	Bound P	Phytate P	Total P	Ext. P	Bound P	Phytate P
B-G-Mawaii	275	208	67	1493	275	189	87	1284	275	191	84	1312	275	168	107	1472
U-D-Saudh	298	227	71	1593	298	174	124	1284	298	196	102	1438	298	213	85	1232
U-D-Pokhariyal	268	218	50	1703	268	207	61	1458	268	170	98	1453	268	225	43	1358
T-P-Motana	312	257	55	1737	312	203	109	1539	312	192	120	1472	312	205	107	1436
R-AM-Rampur	273	210	63	1725	273	191	82	1511	273	211	62	1570	273	199	74	1571
R-Khakra-Pantnagar	267	155	112	1805	267	195	71	1276	267	228	39	1427	267	201	66	1423
T-F-Kumali	263	180	82	1782	263	210	53	1173	263	217	45	1748	263	181	81	1759
PT-B-Udiyari	313	282	31	1821	313	203	111	1266	313	192	121	1307	313	200	113	1432
P-Kh-Takoli	290	209	82	1978	290	203	87	1287	290	196	94	1368	290	221	69	1344
A-HB-Govindpur	289	233	56	1801	289	212	77	1319	289	201	88	1217	289	233	56	1158
R-R-Bachansyu	276	230	46	1715	276	202	73	1666	276	197	78	1511	276	203	72	1176
T-N-Gawad	228	148	80	1757	228	207	20	1663	228	192	35	1436	228	210	18	1475
B-K-Dadimkhet	247	227	20	1531	247	222	25	1240	247	218	29	1519	247	229	18	1437
N-RG-Khairda	251	225	25	1901	251	194	57	1676	251	225	25	1366	251	244	7	1461
CH-K-Jhirkoti	247	225	21	1644	247	192	54	1205	247	171	75	1529	247	228	19	1153

All results reported on dry weight basis

**Table 5:** Reduction on phytate content due to various treatments across select lines

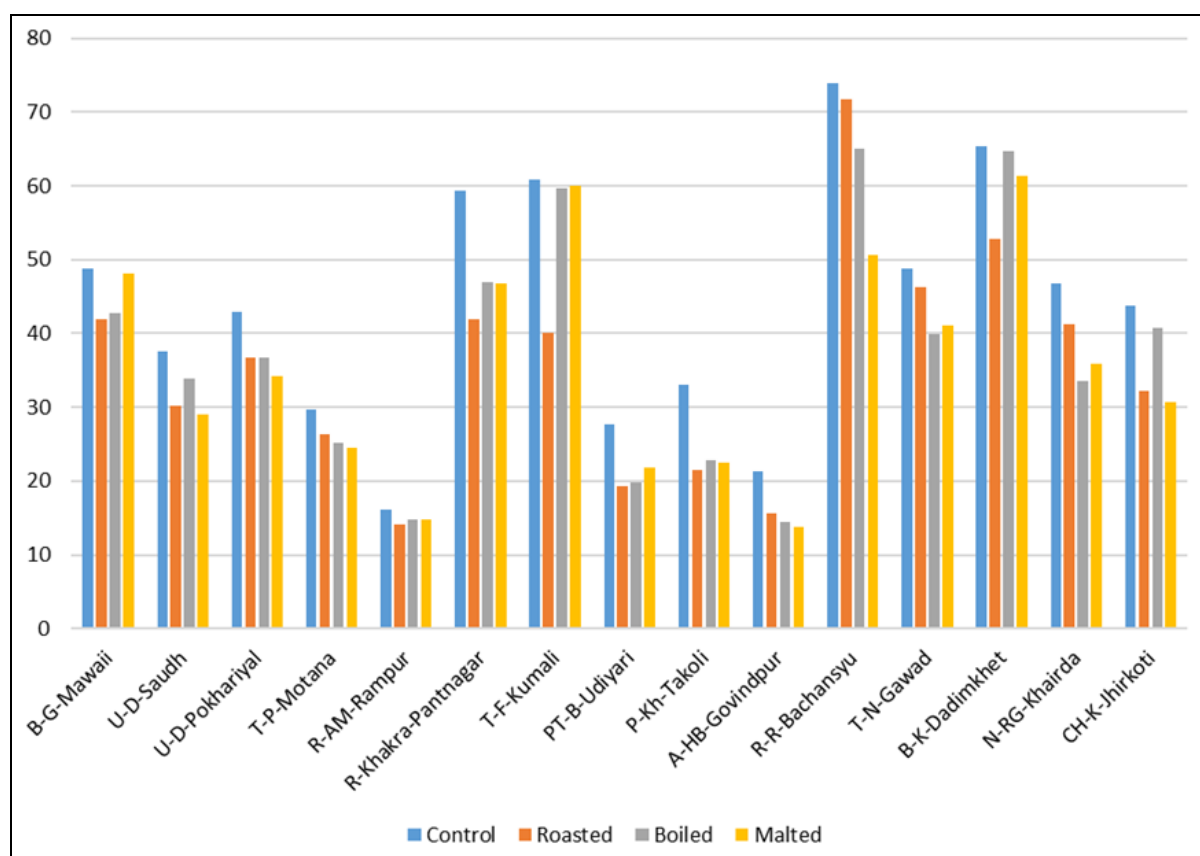
% Reduction in phytate content			
Variety	Roasted	Boiled	Malted
B-G-Mawaii	14	12	1.4
U-D-Saudh	19	10	23
U-D-Pokhariyal	14	15	20
T-P-Motana	11	15	17
R-AM-Rampur	12	9.0	9.0
R-Khakra-Pantnagar	29	21	21
T-F-Kumali	34	1.9	1.3
PT-B-Udiyari	30	28	21
P-Kh-Takoli	35	31	32
A-HB-Govindpur	27	32	36
R-R-Bachansyu	2.9	12	31
T-N-Gawad	5.4	18	16
B-K-Dadimkhet	19	0.8	6.2
N-RG-Khairda	12	28	23
CH-K-Jhirkoti	27	7.0	30

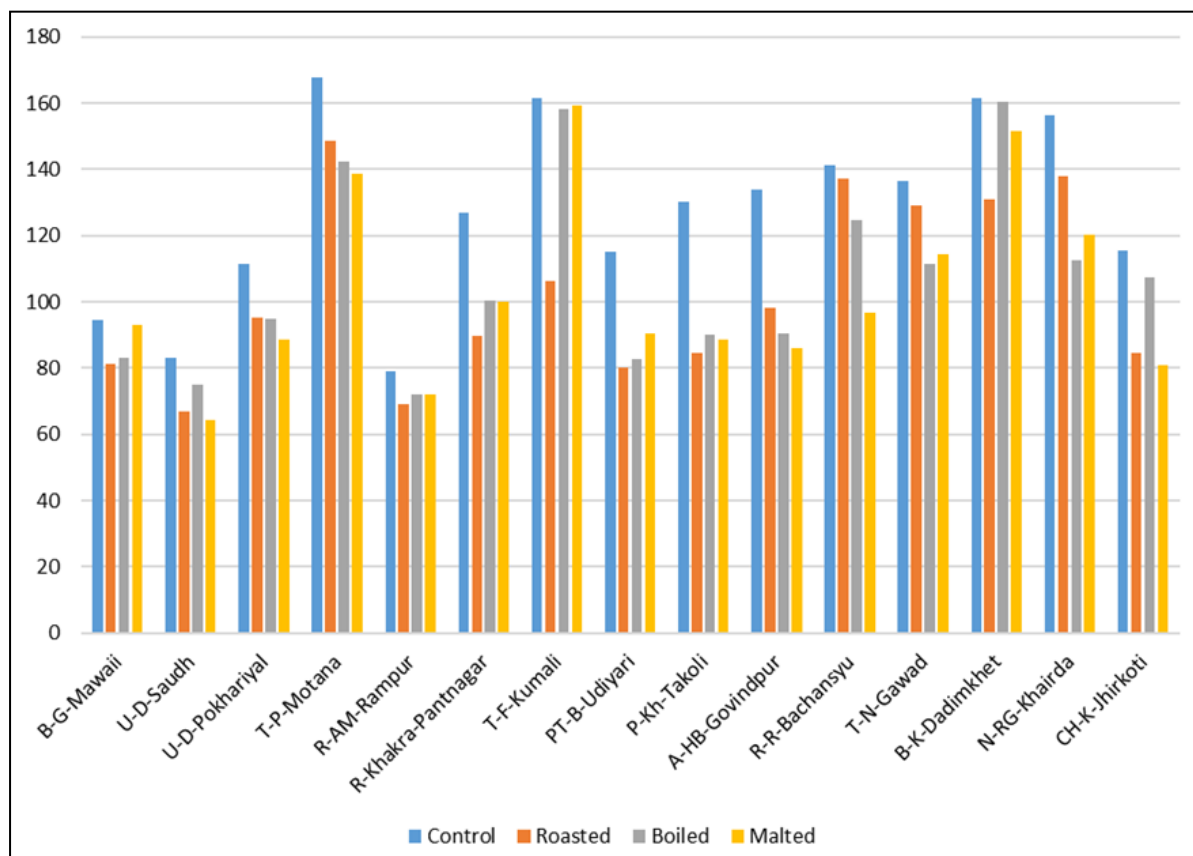
**Fig 2:** Percent reduction in phytate content due to treatments

**Table 6:** Treatment induced bioavailability changes

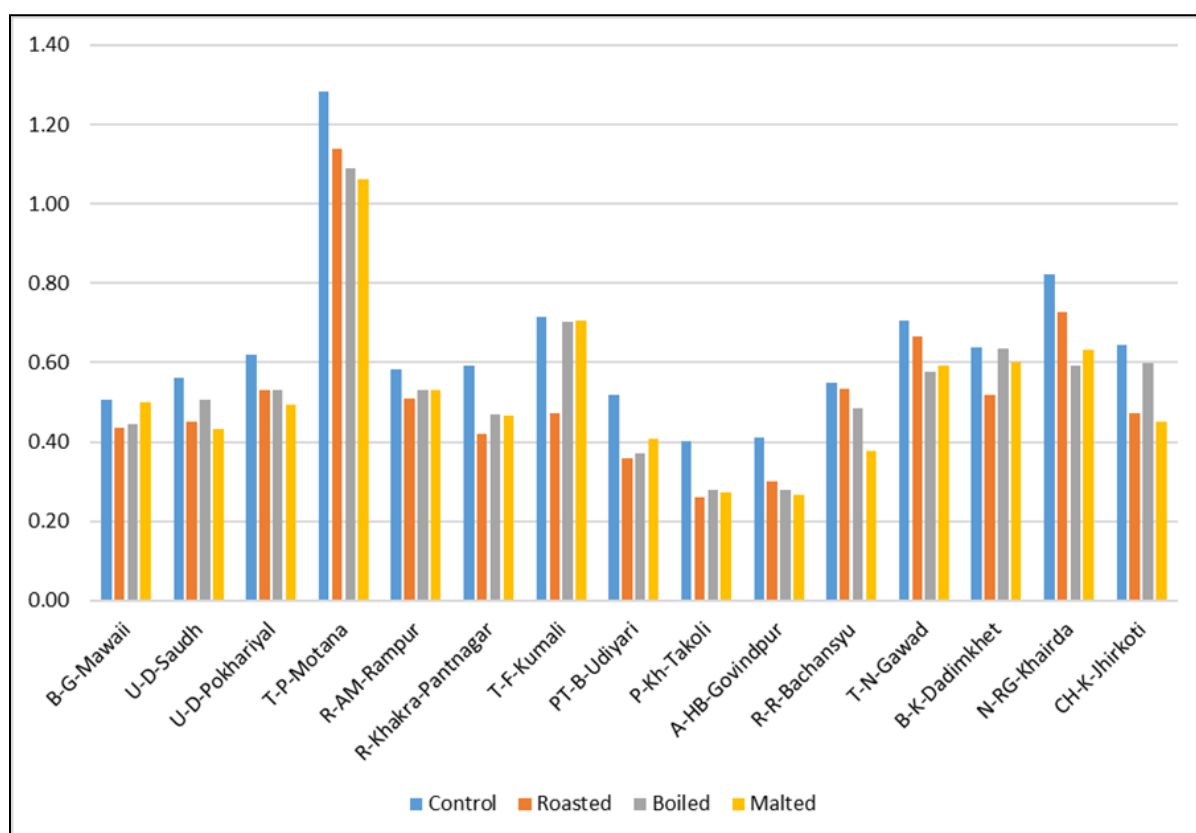
Line	Phy: Fe MR				Phy: Zn MR				Phy: Ca MR			
	Control	Roasted	Boiled	Malted	Control	Roasted	Boiled	Malted	Control	Roasted	Boiled	Malted
B-G-Mawaii	49	42	43	48	94	81	83	93	0.51	0.44	0.45	0.50
U-D-Saudh	38	30	34	29	83	67	75	64	0.56	0.45	0.51	0.43
U-D-Pokhariyal	43	37	37	34	111	95	95	89	0.62	0.53	0.53	0.49
T-P-Motana	30	26	25	25	168	149	142	139	1.28	1.14	1.09	1.06
R-AM-Rampur	16	14	15	15	79	69	72	72	0.58	0.51	0.53	0.53
R-Khakra-Pantnagar	59	42	47	47	127	90	100	100	0.59	0.42	0.47	0.47
T-F-Kumali	61	40	60	60	161	106	158	159	0.72	0.47	0.70	0.71
PT-B-Udiyari	28	19	20	22	115	80	83	90	0.52	0.36	0.37	0.41
P-Kh-Takoli	33	22	23	23	130	85	90	89	0.40	0.26	0.28	0.27
A-HB-Govindpur	21	16	14	14	134	98	91	86	0.41	0.30	0.28	0.27
R-R-Bachansyu	74	72	65	51	141	137	125	97	0.55	0.53	0.48	0.38
T-N-Gawad	49	46	40	41	136	129	112	115	0.70	0.67	0.58	0.59
B-K-Dadimkhet	65	53	65	61	162	131	160	152	0.64	0.52	0.63	0.60
N-RG-Khairda	47	41	34	36	156	138	112	120	0.82	0.73	0.59	0.63
CH-K-Jhirkoti	44	32	41	31	116	85	107	81	0.64	0.47	0.60	0.45

All results reported on dry weight basis

**Fig 3:** Changes in Phytate: Iron Molar ratios



**Fig 4:** Changes in Phytate: Zinc Molar ratios



**Fig 5:** Changes in Phytate: Calcium Molar ratios

Historically, Uttarakhand has been a millet growing state until green revolution replaced them. However, with changing climatic conditions, erratic rainfall, reduced irrigational facilities it is imperative to have climate resilient crops such as millets back into cultivation for sustainable agriculture. Also, growing demand across the world for millets and its products, will help farmers in increasing their incomes. But the flip side is, presence of

anti-nutritional factors in millets including finger millet. The results manifest that with the implementation of simple processing techniques the anti-nutritional factors can be minimized. If such factors can be minimized in finger millet-based products, its consumption and demand can be easily increased resulting in not only economic improvement of farmers but improvement in nutritional status leading to nutritional security. Small-scale entrepreneurship in production of millet-based products will create employment opportunities for youth and women of hilly areas.

### Conclusions

The research led to the conclusions that simple food processing techniques could reduce the undesirable phytate content in finger millet lines thereby increasing the bioavailability of studied nutrients. Thus, these simple techniques can improve the nutritional content. Such techniques, in theory, can also be applied to other millets, for achieving similar results. However, the same should be established through scientific evidence. However, more research is needed to study the effect of combination treatments.

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