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Research Article

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To Study the Germination Effect on Finger Millet, Pearl Millet and Buckwheat: It's Impact on Phytochemical Properties and Their Prebiotic Effect

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Abstract

Effect of germination temperature on the radicle length, phytochemical properties and prebiotic effect of finger millet, pearl millet and buckwheat were investigated. Germination of grain occurred at T1, T2, and T3 with different time range 24 h to 72 h. Results indicated that finger millet and pearl millet showed significantly ($p < 0.05$) better growth at T3 as compare with T1 and T2, while buckwheat had better growth at T1. Total phenolic content significantly ($p < 0.05$) decreased in finger and pearl millet at T3 (28.62 to 13.73 mg GAE/g and 26.88 to 17.85 mg GAE/g) with increase in time from 24 h to 72 h as compared with T1 and T2 (24.17 to 16.72 mg GAE/g, 30.71 to 26.95 mg GAE/g, and 27.17 to 14.46 mg GAE/g, 26.88 to 17.85 mg GAE/g). At 72 h of germination, it increased with germination time for buckwheat. The antioxidant activity of finger and pearl millet significantly ($p < 0.05$) decreased at T1, T2, T3 with an increase in germination time (24 h to 72 h) for finger millet. Therefore, buckwheat shows the higher trend in DPPH (2,2-Diphenyl-1-picrylhydrazyl) value at different germination temperature T1 and time 24 h, 48 h, 72 h. Significantly, ($p < 0.05$) decreased in tannin content of finger millet and buckwheat but increased for pearl millet with increased germination time and temperature. The prebiotic effect increased with germination time for all grains but decreased with increased germination temperature in buckwheat. These findings suggest that germination temperature directly influences the nutritional properties of cereals.

Keywords

Buckwheat, Finger millet, Grain, Germination, Pearl millet

Introduction

Millets are small seeds grasses from the Poaceae grass family which help in diabetes, celiac diseases, digestive health, and blood pressure whereas, *Eleusine coracana* (finger millet) and *Pennisetum glaucum* (pearl millet) are commonly used [1]. Finger millet is an allotetraploid belonging to the Poaceae family and subfamily of chloridoideae which contains potassium, zinc, iron, phosphorus, calcium, and fiber [2]. Pearl millet is a critical small-grain diploid of the Poaceae and Panicoideae subfamily millet contains carbohydrates, minerals, resistant starch, dietary fiber, energy, fat, crude and quality protein, vitamins A and B, and antioxidants [3, 4].

However, finger and pearl millets are excellent sources of phenolic compo-

nents viz., p-coumaric acid and ferulic acid that help to cure breast and colon cancer, hepatocarcinoma, and lipid peroxidation [5, 6]. Pseudocereals are non-grasses grains with starch content like cereal. Buckwheat is one of the pseudocereal cultivated worldwide which is a triangular dicotyledonous kernel with an approximate size of 4 to 9 mm containing hull, spermoderm, endosperm, and embryo [7-9]. There are two closely related species of buckwheat cultivated worldwide, tartary buckwheat (*Fagopyrum tataricum*) and buckwheat (*Fagopyrum esculentum* Moench) [10]. It contains a high crude protein (146.6 g/kg) with a protein digestibility of 68.97 ± 4.42, biological value of 86.33 ± 7.88, protein efficiency ratio 2.69 ± 0.25 and net protein value of 59.77 ± 8.87 [11]. It is also a good source of vitamin-B, such as pantothenic acid, thiamine, niacin, riboflavin, vitamin B6, and C [12]. There are numerous phenolic components, and they are present in a range of plant products. They have significant biological effects and improve life quality [13]. Buckwheat has also been attributed similar properties as compared with cereals such as high phenolic content and high antioxidant activity [14]. The husk has been found to have higher flavonoid content than other parts of the grains [15].

Probiotics word meaning "for life" which are the combination of bacteria and yeasts which is present in digestive system of human beings that claims the good health and provide huge number of benefits such as discomfort, bloating, allergic disorders, non-alcoholic fatty liver disease, breakdown of fat and protein content in digestive system, diarrhea, obesity, type 2 diabetes, and inflammatory bowel disorders in adults [16, 17]. Lactic acid bacteria, *Bifidobacteria*, and yeast *Saccharomyces boulardii* are the most frequently used probiotic strains. *Enterococcus, Streptococcus,* and *Bacillus* also show the potential probiotic value among others [18]. Therefore, finger millet is also considered a good substrate for probiotic bacteria due to higher content of dietary fibre and resistant starch [19]. Dietary fibres in pearl millet are potential prebiotics which promote the growth of probiotic cultures like *Bifidobacterium bifidus* and *Lactobacillus rhamnosus* [20]. Buckwheat is a potential prebiotic due to presence of *Bifidobacterium lactis* which can promote the growth and survival of probiotic lactic acid bacteria [21].

Germination is a complex process that involves activation of various enzymes and metabolic pathways within the seed. This causes various physiological changes to support growth, such as the activation of enzymes involved in the degradation of storage reserves, including starch and proteins, to provide energy and nutrients for the growing plant [22]. Germination can also stimulate the synthesis of secondary metabolites, such as phenolics, which are known to have various health-promoting effects [23]. Germination is a simple and cost-effective method to enhance the grains and cereals nutritional quality. Germination has been used for many years to enhance nutritional value by increasing nutrient digestibility and bioavailability and reducing antinutrients such as phytic acid [24]. Germination can also decrease the levels of antinutritional compounds such as tannins, phytic acid, and oxalates due to activation of enzyme [25]. Thakur et al. [26] reported in research that the crude fibre and protein, antioxidant activity, phenolic components, and mineral content of buckwheat significantly increased after germination, while anti-nutrients like tannin and phytic acid significantly decreased by 59.91 and 17.42%, respectively. Many studies have found that germination process can increase the availability of antioxidants and phenolic compounds in certain grains; some studies have also reported a decrease in the levels of phenolic compounds during germination. Antinutrients like phytic acid and oxalates tend to decrease upon germination, improving nutrient bioavailability [27]. The effect of germination may vary with the grain species and requires more research to conclude. Germination has also been found to increase the prebiotic effect of barley and finger millet, and moth bean when inoculated with *Lactobacillus acidophilus* compared to the ungerminated grains in the form of a drink [28].

This study explores the effect of germination of buckwheat, finger millet, and pearl millet with different germination temperatures by varying germination time and observes the impact on phytochemical properties of germinated grains and their prebiotic effect.

Materials and Methods

Materials

The buckwheat, finger millet, and pearl millet were procured from the local market of Phagwara, Punjab, India. The chemicals and reagents were obtained from Lovely Professional University, Phagwara, Punjab, India.

Germination of grains

The germination of grains (finger millet, pearl millet, and buckwheat) was occurring at different temperatures such as T1, T2, and T3 and time (24 h, 48 h, and 72 h). Whereas T1, T2, and T3 represent the 22 °C, 26 °C, and 30 °C. The grains were germinated in a container covered with filter paper and wet cotton to maintain the humidity. Tray dryer (PPI FiniX72) was used to dry the germinated grains at 60 °C. The grains were ground and stored in airtight packages for further analysis.

Radicle length

The radicle length of the germinating grains was measured after different time intervals such as 24 h, 48 h, and 72 h of germination in mm.

Total phenolic content

Sample extracts were prepared using 10 ml of 80% methanol and mixed thoroughly for 30 min. at room temperature. After that centrifugation process occurred at 4000 rpm for 15 min and supernatant was used for further estimation [29]. Total phenolic content was estimated calorimetrically by using Folin-Ciocalteu assay from the methanolic extract of the sample [30]. 100 µl of the sample extract was taken in test tube, and then added 1 ml of Folin-Denis reagent and 2 ml of 7.5% sodium carbonate. After 30 min of incubation was done and absorbance was taken at 765 nm in a UV-spectrophotometer (UV-1800 Shimadzu UV-spectrophotometer). The calibration curve was prepared with a standard gallic acid, and results are expressed as gallic acid equivalent (GAE).

DPPH assay

DPPH radical scavenging activity was used to estimate the antioxidant activity [31]. In 10 µl of methanolic extract, 3.990 ml of 0.1 mM solution was added and incubated for 30 min in dark room. Then absorbance was taken at 515 nm in UV-spectrophotometer. The results were expressed as a percentage of inhibition of the DPPH radical.

$$\% \text{ inhibition of DPPH assay} = \frac{\text{Absorbance of control} - \text{Absorbance of sample}}{\text{Absorbance of control}} \times 100$$

Tannin content

Folin-Denis reagent with a standard curve of tannic acid was used to estimate the tannin content [32]. 1 ml of sample extract was taken and 0.5 ml of Folin-Denis reagent was added. After that 1 ml of 35% sodium carbonate was added and mixed thoroughly. Absorbance was taken at 760 nm after 30 min of incubation. The tannin contents were expressed as tannic acid equivalent from the standard curve.

Prebiotic effect

Budhwar et al. [33] method was used to study the prebiotic effect. 0.5 g of ground sample was mixed with 10 ml of distilled water. After that autoclaved at 121 °C, 15 psi for 15 min and cooled the mixture. Then the cooled mixture was inoculated with the probiotic bacteria *L. acidophilus* and *Lactobacillus plantarum* at 10^6 dilutions. After inoculation sample was incubated at 37 °C for 24 h. The bacterial count was enumerated with Lactobacillus deMan–Rogosa–Sharpe (MRS) agar medium. The fermented samples were serial diluted up to 10^7 and pour plated. Then incubated the petri plates at 37 °C for 24 h and counted the number of colonies forming units.

Results and Discussion

Radicle length

In the study, it observed finger millet and pearl millet depicted better growth at germination temperature T3 as compared with T1 and T2 as shown in figure 1a and 1b. Similarly, buckwheat had better growth at T1 as shown in figure 1c. The initial growth of radicals in finger millet starts at 48 h with germination temperature T1 (2.0 mm), similarly with increase in time radical length was significantly ($p < 0.05$) increased (3.6 mm). However, with increase in temperature T2 radical growth of finger millet start at 24 h (2.8 mm) which significantly ($p < 0.05$) increased up to 72 h (19.2 mm). Therefore, at T3 temperature significantly ($p < 0.005$) maximum growth of radical of finger millet was observed (29.2 mm). In pearl millet the growth of radical was start grow at 24 h (0.4 mm, 4.0 mm, and 8.8 mm) at different temperature T1, T2, and T3 which significantly ($p < 0.005$) increased up to 72 h (4 mm, 14.2 mm, and 19.6 mm). However, the initial growth of buckwheat significantly ($p < 0.005$) increased with increase in temperature of germination T1, T2, and T3 at 24 h (no growth, no growth, 0.3 mm), 48 h (1.2 mm, 1.6 mm, and 2.6 mm), and 72 h (5.0 mm, 4.8 mm, and 4.2 mm). The specific reason behind this was the temperature-induced inhibition of enzyme activity that inhibits germination. The inhibition of germination has been observed in study with both increases in time and temperature [34].

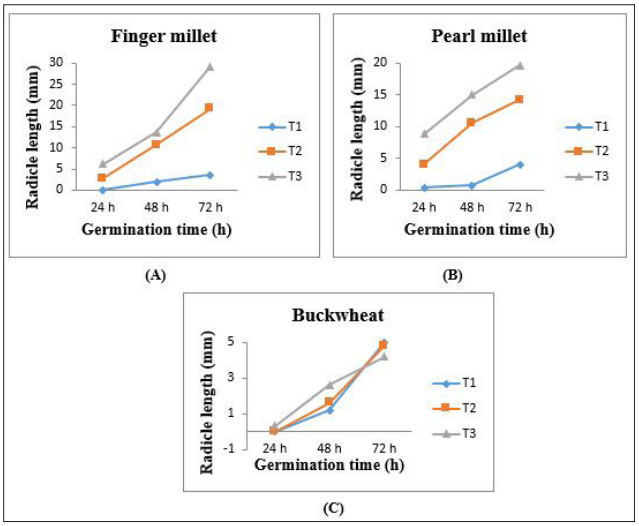

Figure 1: Radicle length of the germinated finger millet, pearl millet, and buckwheat grains.

Generally, higher temperatures promote seed germination by breaking dormancy, while cooler temperatures can suppress germination. However, different plant species have different optimal temperatures for seed germination shall vary with the species, and some crops have a higher percentage of germination at lower temperatures [35]. The prolonged exposure to high temperatures can also negatively impact seed germination. The high temperatures can lead to increased respiration rates and decreased metabolic activity in the embryo, reducing the energy available for germination. However, high temperatures can cause water stress, and further impeding seed germination [36].

Total phenolic content

Total phenolic content of germinated buckwheat, finger and pearl millet was observed at different temperature T1, T2, and T3 at germination time 24 h, 48 h, and 72 h as shown in table 1. Total phenolic content at T1, T2, and T3 in finger millet was significantly ($p < 0.05$) decreases with increase in time 24 h (4.17 mg GAE/g, 27.17 mg GAE/g, and 28.62 mg GAE/g), 48 h (17.48 mg GAE/g, 21.11 mg GAE/g, and 21.89 mg GAE/g) and 72 h (16.72 mg GAE/g, 14.46 mg GAE/g, and 13.73 mg GAE/g). Similarly, it observed total phenolic content in T1 significantly ($p < 0.05$) lower (24.17 mg GAE/g and 17.48 mg GAE/g) as compared with T2 (27.17 mg GAE/g and 21.11 mg GAE/g) and T3 (28.62 mg GAE/g and 21.89 mg GAE/g) at 24 h and 48 h. According to previous studies, the TPC of finger millet significantly ($p < 0.05$) decreased from 1.80 ± 0.11 to 0.38 ± 0.02 g/100 g [37]. Hithamani and Srinivasan, reported total phenolic content had a significant ($p < 0.05$) reduction of 50% was observed after germination of 48 h [38].

However, at T1 total phenolic content of pearl millet and buckwheat showed significantly ($p < 0.05$) decrease trend at 24 h whereas, at 48 h pearl millet at T1 (29.66 mg GAE/g) showed non-significantly ($p < 0.05$) decrease as compared with T2 (22.16 mg GAE/g) and T3 (21.71 mg GAE/g). Similarly, buckwheat at T1 and T2 (55.68 mg GAE/g and 54.62 mg GAE/g) showed non-significantly ($p < 0.05$) difference

Table 1: Total phenolic content of the germinated finger millet, pearl millet, and buckwheat grains in mg GAE/g sample.

Total phenolic content (mg GAE/g)									
Germinated sample	Finger millet			Pearl millet			Buckwheat		
Germination time (h)	Germination temperature (°C)								
	T1	T2	T3	T1	T2	T3	T1	T2	T3
24 h	24.17±0.44^{cA}	27.17±0.44^{cB}	28.62±0.02^{cB}	30.71±0.33^{cC}	26.88±0.02^{cB}	24.17±0.06^{cA}	52.16±0.41^{cC}	50.53±0.07^{cB}	27.65±0.07^{cA}
48 h	17.48±0.07^{bA}	21.11±0.20^{bB}	21.89±0.02^{bB}	29.66±0.04^{bB}	22.16±0.041^{bA}	21.71±0.02^{bA}	54.02±0.02^{bB}	53.09±0.02^{bB}	41.42±0.12^{bA}
72 h	16.72±0.03^{aB}	14.46±0.02^{aA}	13.73±0.06^{aA}	26.95±0.14^{aC}	17.85±0.021^{aB}	15.60±0.06^{aA}	55.68±0.02^{aB}	54.62±0.11^{aB}	42.84±0.07^{aA}

Note: Data are expressed as mean ± SD (n=3). $^{a-c}$Mean values within rows with lower superscript do vary significantly ($p < 0.05$) difference from each other. $^{A-C}$Mean within column with upper superscript in column do vary significantly ($p < 0.05$) from each other.

with each other at 48 h. At 72 h pearl millet showed significantly ($p < 0.05$) decline trends in total phenolic content at T1, T2, and T3 (26.95 mg GAE/g, 17.85 mg GAE/g, and 15.60 mg GAE/g). Therefore, in buckwheat at 72 h total phenolic content was decreased at T3 (42.84 mg GAE/g) as compared with T2 and T1 (54.62 mg GAE/g and 55.68 mg GAE/g). Bhati et al. [39] investigated that the total phenolic content of pearl millet decreased with germination. The highest decrease in phenolic content was at 30 °C (15.43 ± 0.02 mg GAE/100 g) at 72 h of germination. Unlike in finger millet, the reduction was consistent with the germination temperature and time increase. Obadina et al. [40] found a significant decline trend in total phenolic content from 169.90 to 130.20 mg/100 g sample after 96 h of germination at 25 °C. Terpinc et al. [41] investigated the total phenolic content of buckwheat significantly ($p < 0.05$) increased with the germination time.

Živković et al. [22] also found a similar trend of decreasing phenolic content after 24 h and a gradual increase. The total phenolic content of foxtail millet and proso millet has also been found to increase with germination [42]. The specific mechanism behind the reduction in total phenolic content is due to polyphenol oxidase enzyme which degrades the free polyphenols by the oxidation process [43]. The findings of Singh et al. noted a decrease in the phenolic content in pearl millet which could be due to the leaching of polyphenols at the soaking period and an increase in enzymatic activity during germination [44]. The activity of polyphenol oxidase and hydrolytic enzymes during germination process can contribute to the loss of phenolic compounds [45]. Similarly, total phenolic content was increased in buckwheat due to release of polyphenols from the polysaccharides cell wall during the soaking treatment, which led to the softening of tissue structure of grain [43].

DPPH assay

Table 2 represents the % inhibition of DPPH assay of the germinated finger millet, pearl millet and buckwheat grains. In present investigation, antioxidant activity observed in germinated finger millet at T1 (23.06%) which showed significantly ($p < 0.05$) higher as compared with T2 and T3 (18.31% and 14.58%) at 24 h. Similarly, it observed with increase in temperature T3 % inhibition of DPPH activity (13.68% and 12.78%) showed non-significantly ($p < 0.05$) decline trend at 48 h and 72 h as compared with T1 (16.73% and 14.69%) and T2 (15.40% and 14.32%). However, antioxidant activity in pearl millet T1 (20.88%) showed significantly ($p < 0.05$) higher as compared with T2 and T3 (19.61% and 19.21%) at 24 h. Therefore, at 48 h and 72 h germinated pearl millet showed non-significantly ($p < 0.05$) difference with each other at T1 (19.69% and 17.91%), T2 (18.89% and 17.65%), and T3 (18.52% and 17.14%). Germinated buckwheat at 24 h showed significantly ($p < 0.05$) difference with each other at

Table 2: % inhibition of DPPH assay of the germinated finger millet, pearl millet and buckwheat grains.

% inhibition of DPPH assay									
Germinated sample	Finger millet			Pearl millet			Buckwheat		
Germination time (h)	Germination temperature (°C)								
	T1	T2	T3	T1	T2	T3	T1	T2	T3
24 h	23.06±0.07^{cC}	18.31±0.05^{cB}	14.58±0.03^{cA}	20.88±0.12^{cB}	19.61±0.03^{cA}	19.21±0.10^{cA}	28.24±0.03^{aC}	24.73±0.09^{aB}	17.30±0.05^{aA}
48 h	16.73±0.07^{bB}	15.40±0.05^{bB}	13.68±0.03^{bA}	19.69±0.18^{bA}	18.89±0.16^{bA}	18.52±0.39^{bA}	42.24±0.03^{bB}	41.07±0.09^{bB}	26.14±0.06^{bA}
72 h	14.69±0.07^{aB}	14.32±0.05^{aB}	12.78±0.03^{aA}	17.91±0.07^{aA}	17.65±0.07^{aA}	17.14±0.10^{aA}	53.50±0.03^{cB}	53.32±0.46^{cB}	34.27±0.03^{cA}

Note: Data are expressed as mean ± SD (n = 3). $^{a-c}$Mean values within rows with lower superscript do vary significantly ($p < 0.05$) difference from each other. $^{A-C}$Mean within column with upper superscript in column do vary significantly ($p < 0.05$) from each other.

T1 (28.24%), T2 (24.73%), and T3 (17.30%). Similarly, at 48 h and 72 h germinated buckwheat showed non-significantly ($p < 0.05$) difference in antioxidant activity with each other at T1 (42.24% and 41.07%) and T2 (53.50% and 53.32%). Whereas T3 (26.14% and 34.27%) at 24 h and 72 h showed significantly ($p < 0.05$) lower DPPH value as compared with T1 (42.24% and 41.07%) and T2 (53.50% and 53.32%).

Karki and Kharel [46] showed significantly ($p < 0.05$) decrease in DPPH with germination of finger millet. The lowest antioxidant activity was found grains germinated at 30 °C of 3.97%, 1.51% and 1.27% inhibition of DPPH after 24 h, 48 h and 72 h. Beitane et al. [47] reported antioxidant activity of buckwheat was decrease after 24 h of germination, which increased after 48 h. Singh et al. [44] investigated in research that antioxidant activity of finger millet and pearl millet decreased corresponding to the decrease of the total phenolic content. The decline in antioxidant activity of finger millet and pearl millet was higher with an increase in the germination temperature. This could be attributed to the increase in germination, which causes enzymatic activity that reduces the antioxidant components, such as the total phenols. The antioxidant activity was activity decreased with an increase in the germination temperature. This trend was like that of the total phenolic content had an initial decrease after 24 h and an increase in DPPH inhibition from there on [22]. The phenolic compound synthesis increased the antioxidant activity during the germination process. The specific reason behind the reduction of DPPH value is due to degradation of flavonoid compounds during the germination process [43].

Tannin content

Table 3 represents the tannin content of the germinated finger millet and pearl millet and buckwheat. In present study it observed in finger millet tannin content was significantly ($p < 0.05$) decrease with increase in temperature from 24 h to 72 h at T1 (136.01 mg TA/g, 106.38 mg TA/g, and 60.40 mg TA/g), T2 (100.51 mg TA/g, 81.35 mg TA/g, and 77.01 mg TA/g), and T3 (80.84 mg TA/g, 31.03 mg TA/g, and 14.17 mg TA/g). Similarly, at 24 h, 48 h, and 72 h in germinated pearl millet tannin content show the increased tend at T1 (39.71 mg TA/g, 56.32 mg TA/g, and 81.09 mg TA/g), T2 (72.41 mg TA/g, 136.52 mg TA/g, and 151.85 mg TA/g), and T3 (121.96 mg TA/g, 140.61 mg TA/g, and 187.35 mg TA/g). However, the tannin content in germinated buckwheat at T3 (7.37 mg TA/g and 4.91 mg TA/g) showed significantly ($p < 0.05$) lower as compared with T1 (10.92 mg TA/g and 6.39 (10.92 mg TA/g), and T2 (9.51 mg TA/g and 5.67 (10.92 mg TA/g) at 24 h and 72 h. Therefore, at 48 h germinated buckwheat show the significantly ($p < 0.05$) decline in tannin content from T1 to T3 (9.51 mg TA/g, 7.52 mg TA/g, and 5.63 mg TA/g).

In a previous study, the tannin content of finger millet was found to decrease significantly ($p < 0.05$) after germination, while it increased in pearl millet [48]. Similarly, the tannin content of finger millet decreased with germination time, and the tannin content of pearl millet increased with germination. The tannin content was found to have a significant ($p < 0.05$) reduction of about 30% after germination of 48 h [38]. This could be due to the enzymatic hydrolysis of polyphenols and tannins induced by the germination process [46, 48]. The tannin content of pearl millet is more concentrated in its seed coat and is not involved in the germination process, thereby increasing the tannin content [49]. The increase in the tannin content also increased with the germination temperature. Buckwheat has been found to have a high decrease in tannin content with germination [50]. The reduction of tannin content in finger millet and buckwheat may be due to the binding of protein by tannin in the grain, leaching of tannin during soaking, and other metabolic enzymes of germination [51].

Prebiotic effect

The prebiotic effect of germinated cereals is higher with increased bacterial cell count than the non-germinated cereal due to the presence of components that are suitable for optimal growth of bacteria that are synthesized by enzymatic reaction during germination [33]. With *L. acidophilus* and *L. plantarum* the prebiotic effect increased with an increase in germination time. At 30 °C finger millet germinated for 72 h had shown the highest prebiotic effect of 9.56 Log CFU/g when inoculated with *L. acidophilus* and *L. plantarum*. The pearl millet inoculated with *L. plantarum* had the highest prebiotic effect of 9.23 CFU/g, at 30 °C during germination process for 72 h. While pearl millet inoculated with *L. acidophilus* had the highest prebiotic effect of 8.47 Log CFU/g at the same condition. The highest prebiotic effect of buckwheat inoculated with both *L. acidophilus* and *L. plantarum*, was observed for grains germinated at 22 °C for 72 h of 8.6 and 9.8 Log CFU/g, respectively. Though the prebiotic effect increases with the germination temperature for pearl millet and finger

Table 3: Tannin content of the germinated finger millet, pearl millet and buckwheat grains.

Tannin content (mg TA/g)									
Germinated sample	**Finger millet**			**Pearl millet**			**Buckwheat**		
Germination time (h)	**Germination temperature (°C)**								
	T1	**T2**	**T3**	**T1**	**T2**	**T3**	**T1**	**T2**	**T3**
24 h	136.01 ± 0.03^{cC}	100.51 ± 0.25^{cB}	80.84 ± 0.44^{cA}	39.71 ± 1.27^{aA}	72.41 ± 0.02^{aB}	121.96 ± 0.25^{aC}	10.92 ± 0.44^{cB}	9.51 ± 0.25^{cB}	7.37 ± 0.25^{cA}
48 h	106.38 ± 0.25^{bC}	81.35 ± 0.25^{bB}	31.03 ± 0.42^{bA}	56.32 ± 0.76^{bA}	136.52 ± 0.25^{bB}	140.61 ± 0.44^{bC}	9.51 ± 0.25^{bC}	7.52 ± 0.25^{bB}	5.63 ± 0.25^{bA}
72 h	60.40 ± 0.25^{aB}	77.01 ± 0.44^{aC}	14.17 ± 0.44^{aA}	81.09 ± 1.78^{cA}	151.85 ± 0.25^{cB}	187.35 ± 0.07^{cC}	6.39 ± 0.04^{aB}	5.67 ± 0.25^{aA}	4.91 ± 0.25^{aA}

Note: Data are expressed as mean ± SD (n = 3). $^{a-c}$Mean values within rows with lower superscript do vary significantly ($p < 0.05$) difference from each other. $^{A-C}$Mean within column with upper superscript in column do vary significantly ($p < 0.05$) from each other.

millet, similarly the prebiotic effect decreases with germination temperature in buckwheat. Arora et al. [52] found that barley germination increased the prebiotic effect of the grains significantly from 7.75 ± 0.05 to 8.88 ± 0.05 Log CFU/g. Figure 2 represents the prebiotic effect of the germinated finger millet, pearl millet and buckwheat inoculated with *L. acidophilus* and *L. plantarum* in Log CFU/g sample.

Conclusion

In present research authors conclude that germination conditions can significantly ($p < 0.05$) impact their nutritional and functional properties. Finger millet and pearl millet showed better growth at higher germination temperatures, while buckwheat had better growth at lower germination temperatures. Therefore, total phenolic content significantly ($p < 0.05$) decreased in finger millet and pearl millet and similarly, increased in buckwheat with an increase in germination time. The antioxidant activity significantly ($p < 0.05$) decreased in finger millet and pearl millet, while the tannin content also significantly ($p < 0.05$) decreased in finger millet and increased in pearl millet with an increase in germination temperature. All three grains showed a significantly ($p < 0.05$) increase in prebiotic effect with an increase in germination time. These results could have significant implications for enhancing these grains nutritional and functional value by optimizing the germination conditions. This could also aid in the formulation of finger millet, pearl millet, and buckwheat-based foods.

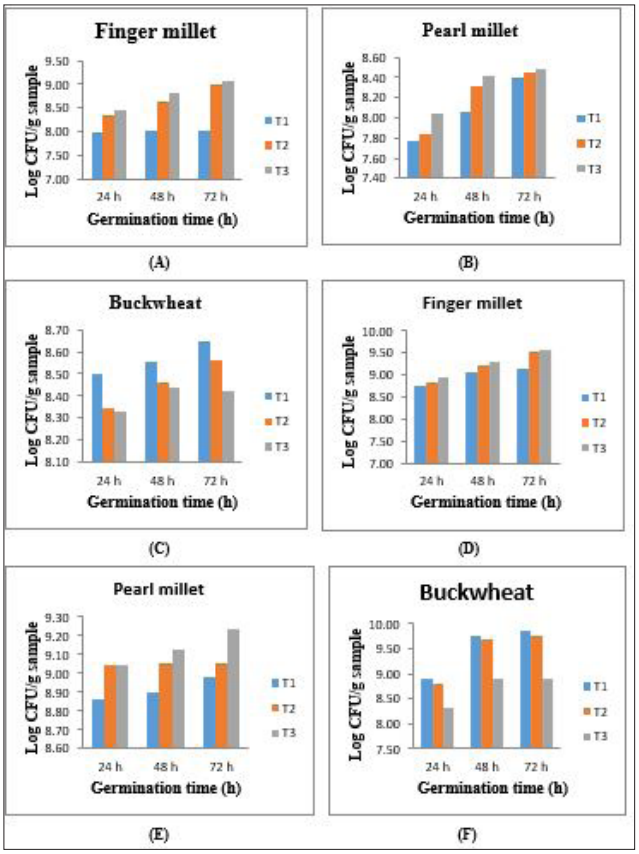

Figure 2: Prebiotic effect of the germinated **(A)** Finger millet **(B)** Pearl millet and **(C)** Buckwheat inoculated with *L. acidophilus* in Log CFU/g sample **(D)** Finger millet **(E)** Pearl millet and **(F)** Buckwheat inoculated with *L. plantarum* in Log CFU/g sample.

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Conflict of Interest

None.

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