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EXPLORING THE INFLUENCE OF PROCESSING TECHNIQUES ON THE BIOACTIVE PROPERTIES OF PEARL MILLET (PENNISETUM GLAUCUM)

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ABSTRACT :
The objective of this study was to evaluate the antioxidant, anti-nutritional, functional properties, and microbial load of pearl millet. The millet was soaked for varying periods of 8, 12, 16, 20, and 24 hours, and germination was tested at 24, 48, and 72-hour intervals. The results showed that longer soaking periods improved water absorption capacity and antioxidant activity, while microbial load remained low, suggesting an enhanced shelf life. Additionally, fermentation-induced starch hydrolysis led to increased water absorption capacity (WAC) and decreased oil absorption capacity (OAC). These findings provide insights for optimizing processing techniques to improve the functional and nutritional quality of pearl millet
Key-words: Pearl millet; germination; WAC; OAC; functional properties; microbial load;

INTRODUCTION:

Millets were historically a crucial part of our ancestors' diet, playing a vital role in their nutrition and livelihoods. However, the Green Revolution shifted the focus to wheat and rice, reducing the prominence of millets despite their significant nutritional benefits. Characterized by small grains and low water requirements, millets are rich in nutrients and are gaining recognition as sustainable future foods[1]. The United Nations General Assembly declared the year 2023 as the International Year of Millet to highlight their importance in leading a healthy life. In India, various millet types are cultivated, with Pearl millet *(Pennisetum glaucum)* being a key staple in Asia and Africa, known locally as Kambu, Bajra, Sajje, and Sajjalu[2].

Pearl millet is vital for addressing food security and nutritional needs in developing nations[3]. Indian research shows that gluten-free pearl millet is more nutrient-dense than maize and rice, offering a high-energy source with carbohydrates, fats, and proteins of high biological value, along with B complex vitamins, Vitamin A, and essential minerals like calcium and iron[4]. Processing methods such as milling, malting, and fermentation can reduce anti-nutritional factors and enhance digestibility[5]. Techniques like soaking improve texture and reduce anti-nutritional properties, while germination enhances the grain's nutritional profile by activating endogenous enzymes[6].

With rising gluten sensitivity, the demand for gluten-free foods has increased[7]. Pearl millet, a gluten-free grain, is versatile and used in porridge, flour, and various traditional dishes[8]. Current research explores the chemical, functional, and nutraceutical properties of pearl millet flour processed through soaking and germination[7]. In the context of diabetes, pearl millet offers hypoglycemic benefits by inhibiting enzymes like α-amylase and α-glucosidase, and germination further enhances its polyphenol content. Increasing awareness of millet's benefits underscores the need for improved processing techniques to maximize their nutritional potential, and this study aims to determine optimal soaking and germination periods for preparing nutrient-rich pearl millet flour.

OBJECTIVES:

- To investigate the effects of germination on pearl millet soaked for various intervals.
- To germinate the soaked samples for varying durations to prepare dehydrated powder.

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- To analyze the antioxidant activity, anti-nutritional, functional and anti-diabetic properties of the germinated pearl millet flour.
- To assess the microbial load in pearl millet flour soaked and germinated at different intervals.

MATERIALS AND METHODS:

Soaking of Pearl Millet at Various Intervals:

Pearl millet grains (icmv2) were purchased from the local market of Virudhunagar district located in southern parts of INDIA. It was cleaned and divided into five portions. Then it is soaked in water for 8, 12, 16, 20, and 24 hours.

Determination and Comparison of Antioxidant Activity:

To evaluate antioxidant activity, 10g of germinated pearl millet flour was dissolved in 100ml ethanol and centrifuged at 3000rpm for 15 minutes. The supernatant was then subjected to the DPPH assay to measure free radical reduction[9].

Determination of Anti-Nutritional Factors:

Anti-nutritional factors (Tannin and Phytic acid) are natural compounds in foods that can hinder nutrient absorption. To evaluate these in germinated pearl millet flour, ethanol extracts were analyzed using Ayoola, G.A.'s [10]method. This assessment reveals how germination reduces these inhibitors, enhancing the millet's nutritional value.

Analysis of Functional Properties:

Functional properties such as water absorption, oil absorption, and swelling capacity were analyzed in pearl millet flour germinated for 24, 48, and 72 hours, using standard procedures.

ANTI-DIABETIC ACTIVITY:

α-amylase inhibition test

Various concentrations of the sample extract were mixed with 100 µL of 0.02 M sodium phosphate buffer (pH 6.9) and 100 µL of α-amylase solution (4.5 U/mL) and preincubated at 25°C for 10 minutes[9]. Afterward, 100 µL of a 1% starch solution was added, and the mixture was incubated at 25°C for an additional 30 minutes. The reaction was stopped by adding 1.0 mL of dinitrosalicylic acid reagent. The test tubes were then placed in a boiling water bath for 5 minutes, followed by cooling to room temperature. The reaction mixture was diluted tenfold with distilled water, and absorbance was measured at 540 nm. Results were compared to a control, which contained buffer instead of extract, and the percentage inhibition of α-amylase activity was calculated.

α-glucosidase inhibition test

Different concentrations of the sample extract were combined with 100 µL of 0.1 M phosphate buffer (pH 6.9) and 100 µL of α-glucosidase solution (1 U/mL) and preincubated at 25°C for 5 minutes. Next, 100 µL of 5 mM *p*-nitrophenyl-α-D-glucopyranoside was added, and the mixture was incubated at 25°C for 10 minutes. Following incubation, absorbance was measured at 405 nm and compared with a control sample containing 100 µL of buffer in place of the extract. Results were calculated and expressed as a percentage.

ANALYSIS OF MICROBIAL LOAD:

The microbial load was carried out to find out the shelf-life of selected germinated pearl millet flour by using standard plate count method for 15 days and 30 days intervals.

RESULTS AND DISCUSSION:

Determination and Comparison of Anti-Oxidant Activity

Anti-oxidant is a substance which inhibits the process of excessive oxidation and diminishes the presence of free radicals. It may prevent or delay cell damage (Fig.1).

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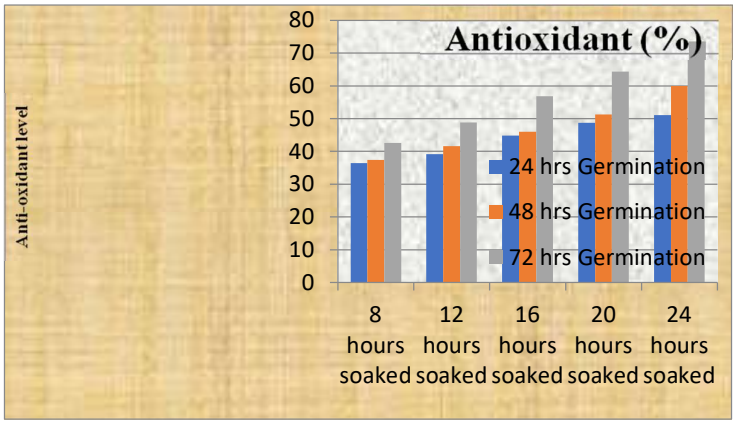

Figure 1 – Anti-oxidant activity of germinated pearl millet flour

DPPH assay was conducted on pearl millet grains soaked and germinated for varying durations and then dried in a hot air oven at 45°C. The highest antioxidant activity, with a value of 73.7±0.02 and a low coefficient of variation, was observed in grains soaked for 24 hours and germinated for 72 hours (Fig 1). The results demonstrated a gradual increase in antioxidant activity with longer germination periods, likely due to increased biosynthesis of antioxidant compounds. These findings are consistent with the study by Bhuvaneshwari[11].

DETERMINATION OF ANTI-NUTRITIONAL FACTORS:

The anti-nutritional factors are the substances generated in natural food substances by the normal metabolism of species and by different mechanisms which interfere with the absorption of nutrients.

Table 1: Qualitative analysis of anti-nutritional factors

QUALITATIVE SCREENING OF ANTI-NUTRIENTS						
Soaking interval (Hours)	Germination interval (Hours)					
	24		48		72	
	Tannin	Phytic acid	Tannin	Phytic acid	Tannin	Phytic acid
8	(+) (+) (+) (+) (+)	(+) (+) (+) (+) (+)	(+) (+) (+) (+) (+)	(+) (+) (+) (+) (+)	(+) (+) (+) (+) (+)	(+) (+) (+) (+) (+)
12	(+) (+) (+) (+)	(+) (+) (+) (+)	(+) (+) (+) (+)	(+) (+) (+) (+)	(+) (+) (+) (+)	(+) (+) (+) (+)
16	(+) (+) (+)	(+) (+) (+)	(+) (+) (+)	(+) (+) (+)	(+) (+) (+)	(+) (+) (+)
20	(+) (+)	(+) (+)	(+) (+)	(+) (+)	(+) (+)	(+) (+)
24	(+)	(+)	(+)	(+)	(+)	(+)

Table 1 shows that the qualitative analysis of anti-nutritional factors present in pearl millet flour subjected to different soaking and germinating intervals. The results obtained exhibited that the increase in soaking and sprouting time significantly reduces the anti-nutritional compounds such as tannin and phytic acid. This phenomenon was found in the soaking treatments attributed to the leaching of polyphenols as suggested by Jood[12]. It was found that the activity of the enzyme and phytase increases with the duration of soaking. Also, it was observed that the phytic acid reduces in samples of pearl millet during soaking and sprouting, by the increased activity of phytase degrading

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the phytic acid. Report on phytic acid reduction from soaking, germination and/or fermentation of cereals as documented by Nkama and Gbenyi[13]. Since the value of anti-nutritional factors reduced significantly in 24 hours of soaking and 48 hours germination.

Analysis of Functional Properties

Table 2 - Functional properties of pearl millet flour

Soaking interval (Hours)	WATER ABSORPTION CAPACITY (ml/g)			OIL ABSORPTION CAPACITY (ml/g)			SWELLING CAPACITY (%)		
	Germination Interval (Hours)								
	24	48	72	24	48	72	24	48	72
8	1.35	1.44	1.55	1.80	1.73	1.64	15.7	20.2	22.9
12	1.45	1.59	1.67	1.71	1.62	1.52	19.6	23.8	26.7
16	1.7	1.76	1.79	1.64	1.51	1.47	26.7	30.1	34.2
20	1.83	1.96	1.97	1.53	1.45	1.36	29.4	32.7	37.8
24	2.02	2.047	2.42	1.42	1.36	1.29	33.2	35.3	39.1

Table 2 illustrates the functional properties of pearl millet flour at different soaking and germination intervals, showing notable variations in water absorption capacity (WAC), oil absorption capacity (OAC), and swelling capacity (SC). The results indicated that longer soaking and germination times significantly improved WAC, reaching its highest value (2.42 ml/g) after 24 hours of soaking and 72 hours of germination. This improvement is likely due to better hydration and enzymatic activity, as supported by Tomar[14]. On the other hand, OAC decreased with extended processing, with the lowest value (1.29 ml/g) observed under the same conditions, suggesting a breakdown of hydrophobic components during germination. Additionally, SC increased substantially, peaking at 39.1% after 24 hours of soaking and 72 hours of germination, likely due to changes in the flour's structure, such as the expansion of starch granules and increased water absorption. These findings highlight the importance of soaking and germination in enhancing the functional properties of pearl millet flour for diverse food applications.

ANTI-DIABETIC ACTIVITY

α-amylase inhibition test

The results indicate that the germinated pearl millet extract (after 72 hours) demonstrates a significantly higher α-amylase inhibition (IC50 = 402 mg) compared to the raw extract (IC50 = 363 mg). This suggests that germination enhances the production of bioactive compounds, such as phenolics and flavonoids, which play a key role in inhibiting α-amylase activity. Additionally, the inhibition effect is concentration-dependent, with higher extract concentrations leading to greater inhibition. This suggests that increasing levels of these bioactive compounds enhance their ability to bind to and inhibit α-amylase. Such properties highlight germinated pearl millet's potential role in dietary approaches for managing post-meal blood glucose levels.

α-glucosidase inhibition test

The test showed that germinated pearl millet exhibited higher α-glucosidase inhibition (IC50 = 375 mg) compared to its raw counterpart (IC50 = 342 mg), which is likely attributed to the biochemical changes that occur during germination. This process activates bioactive compounds such as polyphenols and flavonoids, which increase in concentration, enhancing the millet's enzyme-inhibiting properties. The results also showed a concentration-dependent effect: higher extract concentrations led to stronger enzyme inhibition as more active compounds interacted with α-

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glucosidase. This significant inhibition suggests that germinated millet may aid in blood glucose management by slowing carbohydrate digestion, potentially benefiting diabetics and those controlling postprandial glucose levels.

Analysis of Microbial Load

Table 3 - Estimation of microbial load in pearl millet flour

Microbial load in pearl millet flour(*10^{-9} CFU/g)							
Soaking interval (Hours)	**Initial colony count**	**Germination interval (Hours)**					
		24		**48**		**72**	
		15 days	**30 days**	**15 days**	**30 days**	**15 days**	**30 days**
8	-	32 TFTC	124 TFTC	29 TFTC	127 TFTC	37 TFTC	132 TFTC
12	-	23 TFTC	129 TFTC	34 TFTC	131 TFTC	39 TFTC	153 TFTC
16	-	44 TFTC	138 TFTC	46 TFTC	135 TFTC	62 TFTC	138 TFTC
20	-	47 TFTC	132 TFTC	51 TFTC	141 TFTC	49 TFTC	132 TFTC
24	-	51 TFTC	136 TFTC	55 TFTC	149 TFTC	56 TFTC	158 TFTC

Table 3 presents the evaluation of the microbial load in pearl millet flour, which was soaked and germinated at various intervals and then dried in a hot air oven at 45°C. Colony counts were recorded at 15-day and 30-day intervals. The results indicated that pearl millet flour possesses excellent shelf stability, with its shelf life significantly extended through the germination process[15]. On average, the flour's shelf life is approximately six months. For optimal preservation, it should be stored in an airtight container. This ensures minimal exposure to moisture and air, which helps maintain its quality and prolongs its shelf life.

CONCLUSION:

In conclusion, this study highlights the significant influence of processing techniques, such as varying soaking durations and germination intervals, on the antioxidant, anti-nutritional, functional, and anti-diabetic properties of pearl millet (Pennisetum glaucum). The findings reveal that prolonged soaking enhances water absorption capacity and antioxidant activity while maintaining low microbial load, contributing to better shelf life. Fermentation-induced starch hydrolysis improved water absorption capacity but reduced oil absorption capacity, affecting the functional attributes of the flour. These results underscore the importance of optimizing processing methods to enhance the functional and nutritional quality of pearl millet, thereby promoting its potential as a nutritious and functional food ingredient.

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