Evaluation of anti-nutritional factor reduction techniques for pearl millet improved utilization system in Amhara region

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Abstract: *Pearl millet is small grained cereal which becomes a staple food product in Asia and Africa but it is newly introduced to Ethiopia. It provides basically carbohydrate and protein but it is also good sources of minerals. But the presence of tannin and phytate reduce the bioavailability, digestibility and absorption of these nutrients by the body. Anti-nutritional factors exist naturally or may be produced during processing. In these study different anti-nutritional factor reduction techniques (decortication, malting and blanching) were evaluated and reduce tannin and phytate significantly ($p< 0.05$).*
***Key words**: Blanching, decortication, malting and pearl millet*

1. Introduction

Pearl millet is small grained cereals, sometimes referred to as poor man's cereals because of the preference of other cereals by those with a choice. But it becomes a staple food product in Asia and Africa but it is newly introduced in Ethiopia. Basically pearl millet provides carbohydrate and protein but it is also good sources of essential minerals. Pearl millet is a good source of minerals such as calcium, iron, zinc copper and manganese [1] and it's also recommended as a nutritious food especially for children and elderly [2]. They are high in starchy component (61.5-89.14%) and thus serve as an energy food.

The presence of anti-nutritional factors has effective role in reducing the nutritive value of pearl millet due to their ability to bind macro nutrients and make them unavailable, plus reducing digestibility of starch, protein as well as minerals availability [3]. Physical and chemical methods are employed to reduced or remove anti-nutritional factors including soaking, cooking, germination, fermentation, selective extraction, irradiation and enzymes' treatment. Anti-nutritional factors can cause detrimental effects to humans and animal growth and performance by impairing intake, uptake or utilization of other foods and feed components or by causing discomfort and stress to humans and animals.

In Ethiopia pearl millet is grown mainly in sekota zone Aberegela woreda where it's considered as the major cereal crop for population, it's consumed mainly as *Enjera*, *kita* and porridge prepared from the flour. So to improve the utilization of pearl millet there are several methods that need to be employed to improve the nutritional quality of pearl millet by reducing anti-nutritional factors. These processing technologies employed include malting or germination, dehulling or decortications, and blanching. Although these techniques are known to be effective treatments to remove the anti-nutritional factors and enhanced nutritional value. However such information is still scanty in this region. Therefore the aims of the study were carried out to investigate the proximate composition and anti-nutritional factors reduction techniques.

1.1. General objective of the study

- Generating information about the level of anti-nutritional factors in pearl millet so as to improve the nutritional status of and feeding habit of the community

1.2. Specific objectives

- To evaluate the performance of anti-nutritional factor reduction techniques on pearl millet in Amhara region

- To demonstrate improved reduction techniques in rural community for better utilization of pearl millet in Amhara region

2. Material and Method

2.1. Sample collection and preparation

Pearl millet sample were brought from Sekotat Dry Land Agricultural Research Center. The sample was carefully cleaned so as to make the sample free of foreign material as well as broken and shrunken ones at Bahir Dar food science and postharvest handling research center. Finally the cleaned sample was analyzed for its proximate composition, tannin and phytate content based the standard procedure.

2.2. Proximate composition analysis

2.2.1. Moisture content

Moisture content of the sample was determined according to the standard official method of analysis [4]. The moisture content was calculated as follows:-

$$MC\ (\%) = (w1 - w2) \div w1 \times 100$$-------equation (1)

Where:

W1 = original weight of sample and W2 = weight of dried sample

2.2.2. Total crude protein content

Protein was determined by the Kjeldahl method. All nitrogen is converted to ammonia by digestion with a mixture of concentrated sulphuric acid and concentrated orthophosphoric acid containing potassium sulphate as a boiling point raising agent and selenium as a catalyst. Theammonia released after alkalinization with sodium hydroxide is steam distilled into boric acid and titrated with sulphuric acid.

Digestion: 0.5gram of samples were taken in a tecator tube and 6ml of acid mixture (5parts of concentrated ortho-phosphric acid and 100 parts of concentrated sulfuric acid) was added, mixed- thoroughly and a 3.5ml of 30% hydrogen peroxide was added step by step. As soon as the violent reaction had ceased, the tubes were shaken for a few minutes and placed back into the rack. A 3.00g of the catalyst mixture (ground 0.5g of selenium metal with 100g of potassium sulfate) was added into each tube, and allowed to stand for about 10minute before digestion. When the temperature of the digester reached 370^{o}C, the tubes were lowered into the digester. The digestion was continued until a clear solution was obtained, about 1hour.The tubes in the rack was transferred into the fume hood for cooling, a 15ml of demonized water was added, and shaken to avoid precipitation of sulfate in the solution.

Distillation: A 250ml conical flask containing 25ml of the boric acid-indictor solution was placed under the condenser of the distiller with its tips immersed into the solution. The digested and diluted solution was transferred into the sample compartment of the distiller. The tubes were rinsed with two portions of about 5ml de-ionized water and the rinses were added into the solution. A 25ml of 40% sodium hydroxide solution was added into the compartment and washed down with a small amount of water, Stoppard and the steam switched on. A 100ml solution of the sample was distilled, and then the receiver was lowered so that the tip of the condenser is above the surface of the distiller. The distillation was continued until a total volume of 150ml is collected. The tip was rinsed with a few milliliter of water before the receiver was removed.

Titration: The distilled solution was titrated with 0.1N sulfuric acid to a reddish color.

mg nitrogen in the sample = V*N*14----------equation (2)

g nitrogen sample/100g sample = mg of nitrogen*100/mg of the sample

$$\text{Total nitrogen (\%)} = \frac{[(V-Vb)*N*14]}{W}$$ ----------equation (3)

Crude protein (%) = Total nitrogen (%) * F----------equation (4)

Where:

V=Volume of sulfuric acid consumed to neutralize the test material (ml)

Vb=Volume of the acid consumed to neutralize the blank

F=Conversion factor of total nitrogen to crude protein (6.25)

14=Equivalent weight nitrogen
N= Normality of standard sulfuric acid

2.2.3. Ash content

Ash of the samples was estimated according to the official methods of analysis [4]. Ash content was calculated using the formula:-

$$\text{Ash (\%)} = (w2 \div w1) \times 100 \text{----------equation (5)}$$

Where: W1= Original weight of a sample and W2= Weight of sample after ignition

2.2.4. Crude oil content

Crude oil of pearl millet sample was determined using the official methods of analysis [4] the crude oil was estimated using Soxhlet exractor.

$$\text{CO (\%)} = ((w2 - w1) \div s) \times 100 \text{----------equation (6)}$$

Where:
W1= weight of empty receiver
W2= weight of receiver + oil
S = original weight of dried sample

2.2.5. Crude fiber determination

Crude fiber of the sample was estimated according to the method described by [5]. Two grams of defatted sample were digested in 200 ml boiling 0.255N H_2SO_4 under reflux condenser, for 30 minutes. Then it is filtered under suction using a linen piece as filter medium. The residue was washed with hot water to remove any trace of acid. A second alkali digestion was done using 200 ml boiling 0.344N NaOH for 30 minutes, and then similarly filtered as above. The residue was washed with hot water, dried at 105°C overnight and then reweighed. The (CF) was calculated as:

$$\text{CF (\%)} = ((w1 - w2) \div s) \times 100 \text{----------equation (7)}$$

Where:
W1 = weight of sample before ignition
W2 = weight of sample after ignition
S = original weight of sample

2.2.6. Carbohydrates determination

Total carbohydrates of samples were calculated by subtracting the value of protein, oil, fiber, ash and moisture content from 100.

Total carbohydrates (%) = 100 – [CP% + CF% + CO% + ash% + MC%] --------equation (8)

2.3. Anti-nutritional factor reduction techniques

2.3.1. Malting

Pearl millet sample was soaked for 3hours and allowed to malt for one, two and three days. Then it was sun dried up to moisture content of 12-14 percent.

2.3.2. Decortication

The cleaned pearl millet sample was dehulled using mortar and pestle grinder at 25%, 50% and 75%. Finally the decorticated pearl millet sample was winnowed to remove the bran.

2.3.4. Blanching

The blanching was carried out at 80^{o}c for 20 minute, 40 minute and 60 minute. Then it was allowed to dry up to moisture content of 12-14 percent using open sun drying.

2.4. Anti-nutritional content determination

2.4.1. Determination of tannin content

Tannin content (TC) of pearl millet samples was estimated using modified Vanillin-HCL in methanol as described by [6]. About 0.2g of ground sample was placed in l00 ml conical flask. Ten milliliters of 1% HCL in methanol (v/v.) were added. The contents were mechanically shaken for 20 minutes and centrifuged at 2500 rpm for 5 minutes. One milliliter of supernatant was pipetted into a test tube and 5 milliliters of vanalin-HCl

reagent (mixing equal volume of 8% concentrated HCl in methanol and 1%vanillin in methanol) were added. The optical density was read using a colorimeter. At 500nm after 20 minutes incubation at 30°c, a blank sample was carried out with each run of samples. A standard curve was prepared expressing the result as catechin equivalents, i.e. amount of catechin (mg per ml) which gives color intensity equivalent to that given by tannin after correcting for blank.

$$TC\ (\%) = ((c \times 10) \div 200) \times 100$$----------equation (9)

Where:

c = concentration corresponding to optical density

10 = volume of extract in ml

200 = sample weight in mg

2.4.2. Determination of phytic acid content

Phytate of each sample was determined according to the method described by [7]. One gram of finely ground sample was weighed into a 125 ml conical flask, extracted with 50 ml 3% TCA (W/V) for 3 hours with mechanical shaking. Then the suspension was centrifuged at 3000 rpm for 10-15 minutes. Ten milliliters of aliquots supernatant were transferred into a 50 ml boiling tubes and then 4 mls of $FeCl_3$ (2 mg Ferric iron (Fe^{+3}) per ml 3% TCA), were added. The tube was heated in boiling water bath for 45 minutes and one or two drops of 3% sodium sulphate (Na_2SO_4) in 3% TCA were added. The tube was cooled and centrifuged at 3000 rpm for 10 - 15 minutes and the clear supernatant was decanted carefully. The precipitate was then washed twice by dispersing well into 25 ml 3% TCA and heated in boiling water bath for 5-10 minutes and centrifuged. The supernatant was decanted and then washed once with 25 ml of distilled water. The precipitate was cautiously dispersed in a few ml of distilled water enriched with 3 ml 1.5N NaOH with mixing. The volume was made approximately to 30 ml with distilled water and heated in boiling water bath for 30 minutes. The contents of the boiling tube were filtrate (hot filtered) through filter paper (Whatman No.1). The filtered was discarded and the precipitate was dissolved in 40 ml 3.2N HNO_3 (hot) into a 100 ml volumetric flask. The precipitate was washed with distilled water. The flask contents were cooled to room temperature (28-32ºC) and diluted to volume with distilled water. Then 0.5 ml was transferred to a gradual test tube, and 2 mls of 1.5 M potassium thiocyanate (KSCN) were added and the volume was completed to 10 ml with distilled water. The density of color was read at 480 nm (within one minute) in spectrophotometer (corning, 259). A standard curve of different Fe $(NO_3)_3$ concentrations was plotted to calculate the ferric ion concentration. The phytate phosphorus was calculated from the ferric ion concentration assuming 4:6 (iron: phosphorus) molar ratio.

$$Phytate\ (mg/100g) = (6/4(A \times C \times 20 \times 10 \times 50)) \div (1000 \times S) \times 100$$-------equation (10)

Where:

A = optical density

C = concentration corresponding to optical density

S = weight of sample

3. Result and discussion

3.1. Proximate composition

The proximate composition of pearl millet is shown in table one below. The moisture content of pearl millet sample collected was found 8%. The moisture content found was in the pearl millet sample was greater than other researcher analysis quantification [8]. The crude protein content and crude oil were found 12.2% and 0.6% respectively. In case of protein the results obtained were lower than reported in the same study [9] but it is in a good agreement with the report of another studies [10] [11]. The total ash and carbohydrate of pearl millet were found 1.7% and 75.36% respectively. The result obtained in case of total ash content of pearl millet sample was similar with finding in pearl millet composition analysis [10] while the total carbohydrate content of pearl millet result was found to be much greater than the range reported for proximate composition analysis for different cereals [12]. The difference is expected to arise majorly from postharvest handing practices and to a lesser extent due to differences in agronomic practice.

Table-1: Proximate composition of pearl millet

Nutrients	Values
Moisture	8%
Crude protein	12.2%
Crude oil	0.6%
Total ash	1.7%
Total carbohydrate	75.36%
Crude fiber	2.12%

3.2. Tannin and phytate composition

The tannin and phytate content of pearl millet is presented in table two below. Tannin content of pearl millet sample was found 337.09mg/100g. The tannin content obtained in this study was greater than other similar studies in Sudan [13].

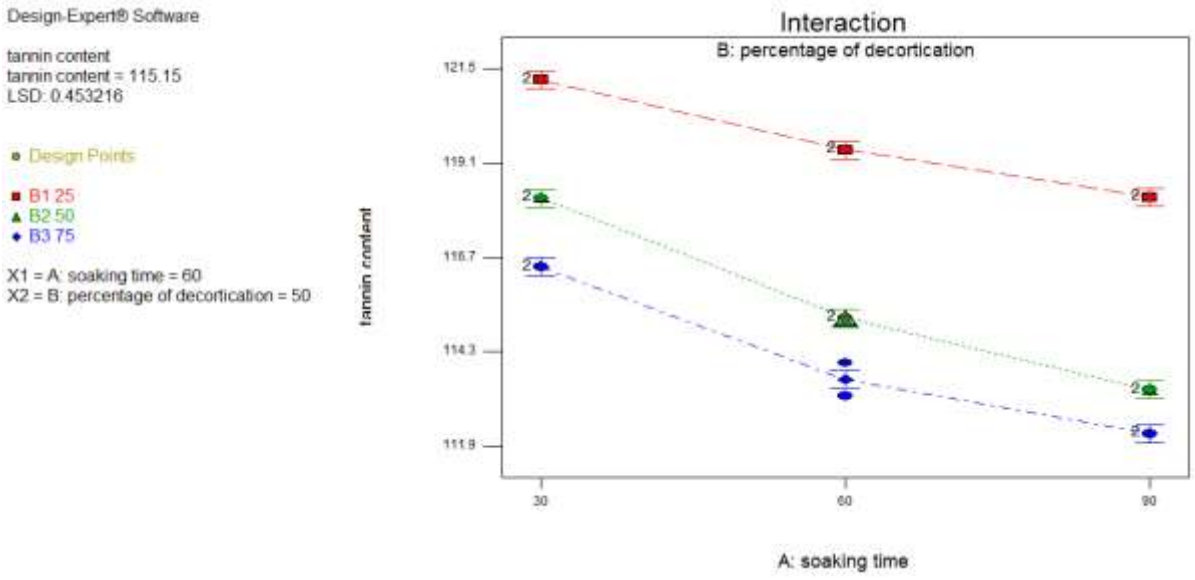
The phytate content was 442.12mg/100g which is in good agreement report [10]. In both cases the quantity naturally existing anti-nutritional factors in pearl millet are much higher where it has the potential to reduce bioavailability, digestibility and absorption of nutrients in general [2].

Table-2: Tannin and phytate content of pearl millet

Anti-nutritional factor	Quantity
Tannin	337.09mg/100g
Phytate	442.12mg/100g

3.2.1. Effect of decortication on tannin content

Percentage of decortications and soaking time has significant effect on tannin content reduction ($p<0.05$). Maximum reduction in tannin was attained at 75 percent decortication at 90minute soaking time where the reduction was from 337.09mg/100g to 111.9mg/100g. In general term as soaking time and percentage of decortications increases the tannin content also reduces significantly. The study result coincides with earlier investigations on different varieties of pearl millet [14] [15].

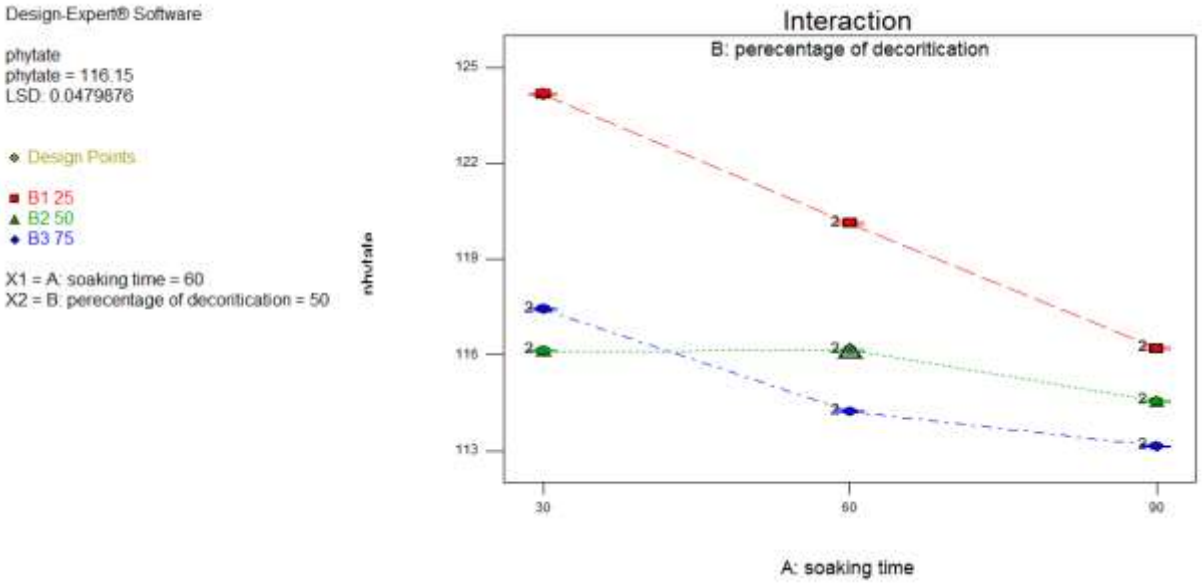


- *Soaking time is in minute*
- *Decortication is in percentage*

Figure-1: Effect of decortication on tannin content

3.2.2. Effect of decortication on phytate content

As it is observed in the figure-two below phytate content reduces significantly ($p < 0.05$) during soaking and decortications which means no increment in all conditions. In case of soaking for 90minute and decortications at 75% the phytate content reduces from 442.12mg/100g to 113mg/100g. According to [9], soaking and decortication reduce phytate of pearl millet at significant level. As it was reported in studying phytate content reduction on faba and kidney beans [16], soaking in water significantly reduced phytic acid content. The reduction in phytic acid during soaking could be attributed to leaching out of phytate by soaking water under concentration gradient [17].

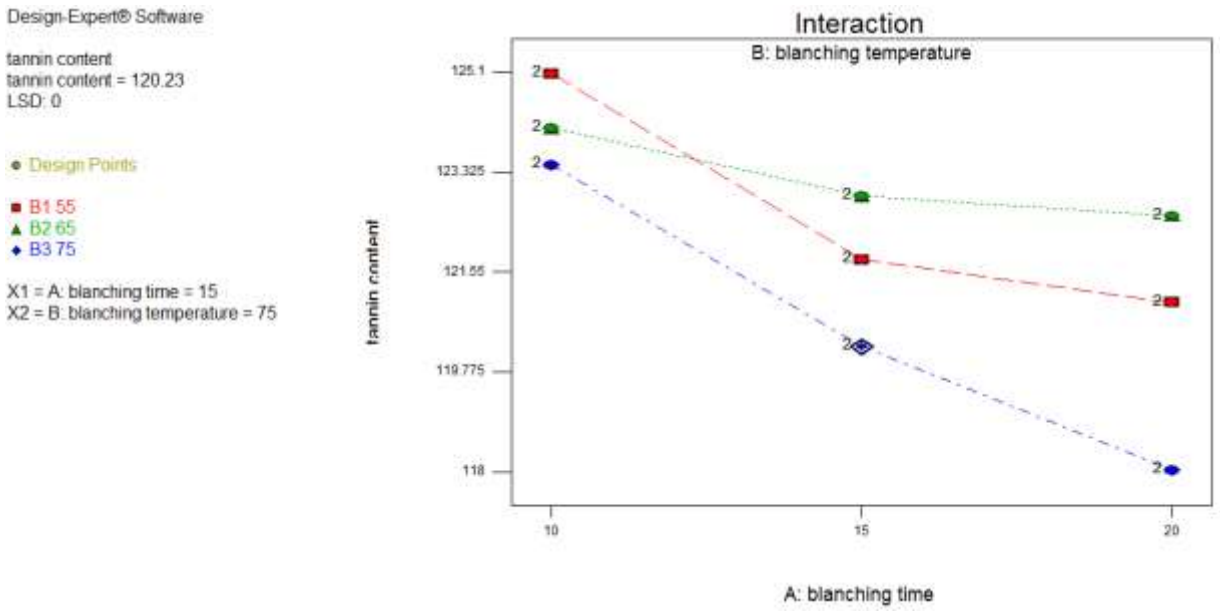


- *Soaking time is in minute*
- *Decortication is in percentage*

Figure-2: Effect of decortication on phytate content

3.2.3. Effect of blanching on tannin content

As it is seen the figure-three below blanching reduces tannin content significantly ($P < 0.05$). Tannin content of pearl millet reduced as the blanching temperature and time increase. Blanching of pearl millet at 75^{o}c for 20 minute reduced tannin from 337.09mg/100g to 118mg/100g.

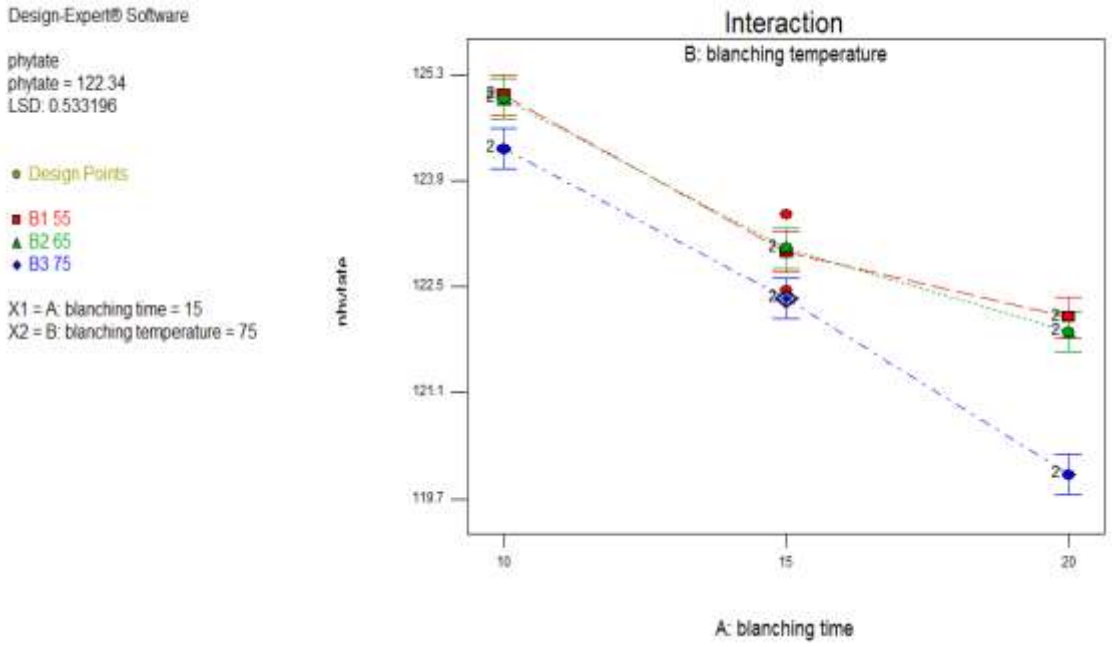


- *Blanching time is in minute*
- *Blanching temperature is in ^{o}c*

Figure-3: Effect of blanching on tannin content

3.2.4. Effect of blanching on phytate content

In the figure-four below the phytate content of pearl millet sample was reduced significantly (P<0.05). In all cases the phytate content reduced but in the case of blanching temperature 75^{o}c and time 20 minute maximum reduction of phytate observed. At blanching temperature 75^{o}c and time 20 minute the phytate content was reduced from 442.12mg/100g to 119.7mg/100g.

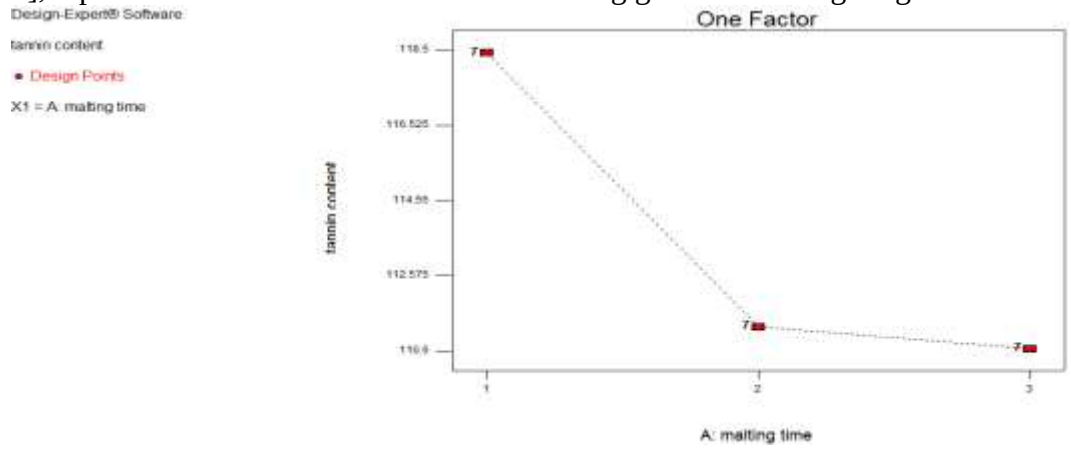


- *Blanching time is in minute*
- *Blanching temperature is in ^{o}c*

Figure-4: Effect of blanching on phytate content

3.3.5. Effect of malting on tannin content

In figure-five below germinating or malting reduces the tannin content of pearl millet significantly (P<0.05). At malting for three days the maximum reduction of tannin was found which 110.8mg/100g. The result of the study was found lower than similar studies on pearl millet [18] which was around 29.2 mg/100g. In contrast to our finding [19], reported an increase in tannin content during germination in guar grains.



- *Malting time is in days*

Figure-5: Effect of malting on tannin content

4. Conclusion and recommendation

Blanching, malting and decortication reduce tannin and phytate content of pearl millet significantly (p<0.05). For improved utilization of pearl millet employing these anti-nutritional factor reduction methods would be mandatory so as to enhance the nutritional status of the society of Amhara region especially Sekota districts. From all these reduction techniques malting reduces tannin more effectively than the rest two reduction techniques which also reported [20]. In all cases the increment of tannin and phytate was not observed during processing which was in contradict with other studies [19] report on other grains. Sensory attributes and baking quality of food product from pearl millet triggered us not to recommend the reduction techniques which reduced maximum anti-nutritional factor. So in case of malting and blanching are 2day and 15minute at 75^{o}c were the recommended treatments while in decortications 75% is highly preferable. For weaning food preparation malting at 3 day is much more recommendable than the rest of the reduction techniques.

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