Journal of Global Biosciences
ISSN 2320-1355
Volume 4, Number 9, 2015, pp. 3291-3300
Website: www.mutagens.co.in
E-mail: submit@mutagens.co.in
researchsubmission@hotmail.com

Research Paper

APPRAISAL OF ALKALI TREATED MALTING ON PROXIMATE COMPOSITION AND ANTIOXIDANT ACTIVITY OF *Amaranthus cruentus*

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Abstract

Nowadays there has been increased interest in various natural plants as a source of biological active compounds which are sustainable approach to reducing malnutrition among peoples at highest risk. *Amaranthus cruentus* is one of the gluten-free grains and multi-purpose pseudocereal due to its valuable nutritional, functional, and pharmaceutical characteristics. The purpose of this study was to determine the effect of plain water and alkali (2%$NaHCO_3$) treated malting on proximate composition by Association of Official Analytical Chemists standards.The aqueous extract of malted *cruentus* grain flour were investigated employing three different established in vitro methods total phenols, flavonoids and DPPH using gallic acid, quercetin and 2,2-di phenyl-1-picryl hydrazyl radicals. All the analysis was made with the use of UV-Visible Spectrophotometer. The result showed that the significant increased percent observed in protein (36.7%), crude fibre (32.3%) and significant decreased percent was found in iron content (25%) of alkali treated malted flour when compared to unmalted flour at P≤0.05 level. In antioxidant evaluation, total phenol content was significantly higher in alkali treated malted extract; 38.6±1.00 mg GAE/100g followed by plain water malted flour;36.0±0.56 mg GAE/100g which showed significant difference (P≤ 0.05). Similarly, the total flavonoids significant increment was also found maximum in alkali treated flour i.e. 36.0±0.56 (110.5%) (mg QE/100g). Scavenging effect of DPPH free radicals expressed as IC_{50} value and scavenging activity of the alkali treated flour(18 µg/ml**)** was stronger than plain water malted(38 µg/ml) and unprocessed flour (55 µg/ml). Thus, the malting is a practical approach aimed at combating the problem of malnutrition and can be easily adopted at both household levels to produce healthy food to help enhance the nutritional status of people and could be regarded as potential functional foods.

Key words: *Amaranthus cruentus*, Malting, Proximate analysis, Antioxidants.

INTRODUCTION

Today a few plant species among all the varieties available for human nutrition are employed commercially on very large scale. Wheat, maize and rice are the three cereals dominating the nutrition within the world's population, even if appropriate alternatives are available**.** In the International AACC (American Association of Cereal Chemists) has recognized pseudo cereals as grains and could be a good substitute for cereals in allergic persons **(Blaise, *et al.,* 2010).** Although many plants from the family of *Chenopodiaceae* are used for human nutrition (e.g. Spinach, beet), only three plants have gained importance as grains, so called pseudocereals, worldwide. Amaranth (*Amaranthus sp*.), quinoa (Chenpodium quinoa), buckwheat (*Fagopyrum esculentumm* and *Fagopyrum tartaricum*) are assigned to the *dicotyledonae,* which are *monocotylendonae,* but they all produce starch rich seeds that can be used like cereal **(Berghofer and Schoenlechner, 2007)**. In the last years, pseudocereals have gained extensive use not only in the common diet but also in the diet of people with celiac disease or allergies to typical cereals **(Posko *et al .,* 2009)**.Celiac disease is one of the most common lifelong disorders worldwide with as estimated mean prevalence of 1% of the general population. The only acceptable treatment for celiac disease is the strict lifelong elimination of gluten from the diet. The dietary changes required by the celiac patient to begin and maintain a strict gluten free diet are considerable and may have a significant impact on daily life **(Catassi and Fasano, 2008).**

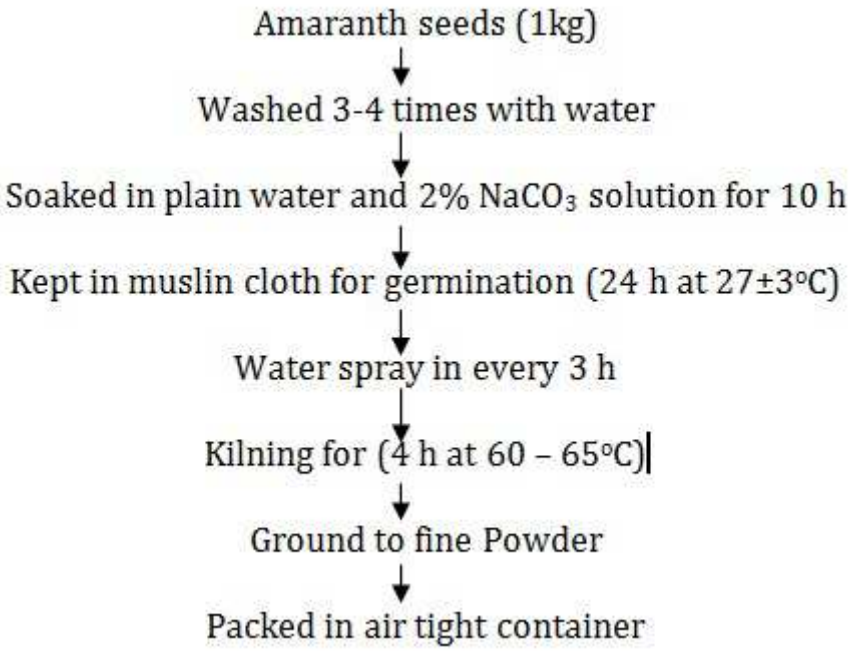
The genus Amaranthus belongs to the family *Amaranthaceae* that includes mainly *cruentus*, *caudatus* and *hypochondriacus* are the essential grain species. Interest in its widespread consumption of *Amaranthus cruentus* for human nutrition has grown recently due to favorable reports of amaranth nutritive value and health benefits. It is highly nutritious pseudocereal known as a dietary source of protein with favorable amino acid composition, vitamins, starch, dietary fiber, essential minerals and has several other protective compounds with high antioxidant, anti carcinogenic **(Grobelnik mlakar *et al.,* 2009)** and considered as potential therapy to treat various chronic diseases such as diabetes, hypercholesterolemia, hypertension, cardiovascular disorders **(Li and Zang, 2001)**.

Several methods have been generally adopted to improve the nutritional qualities antioxidant of cereal-based foods. These include: genetic modification, amino-acid fortification, supplementation or complementation with protein-rich sources and processing techniques which include malting **(Mohammed, *et al.,* 2011).** Malting was suggested to be a suitable scientific approach for improving the nutritional quality of cereals and other seeds **(Gernah *et al.,* 2011).** This is a consequence of enzymes activation and their involvement in the synthesis of a wide range of chemical compounds causing the enhancement of nutritional quality. In addition, malting is a simple tool that allows enhancing the palatability and digestibility **(Taraseviciene *et al.,* 2009).** The aim of this study was to investigate the effect of plain water and alkali malting on proximate nutritional composition as well as antioxidant capacity of *Amaranthus cruentus* which would aid in contributing to the formulation of functional and nutritional enhanced gluten free food products.

MATERIALS AND METHODS

COLLECTION OF GRAIN AND PREPARATION OF FLOUR

Seeds of Amaranth were collected from Indian Agriculture Research Institute (IARI), Delhi. The seeds were cleaned and the broken ones removed by hand. The cleaned seeds were kept in plastic jars and stored at ambient temperature in the laboratory. Then seeds were kept for malting process in which 10 h steeping, 24 h germination and 4 h kilning has been performed to develop a flours.



Flow diagram for the preparation of malted Amaranth flour

PROXIMATE ANALYSIS

Determination of proximate composition was carried out in accordance with **Association of Official Analytical Chemists, 2005**. This constitutes the different classes of nutrients present in the samples such as ash content was determined by weight difference after sample mineralization at 600 **°C** for 6 h. Moisture content was determined by drying in an oven at 85^{0}C to constant weight. Crude protein was determined indirectly from the analysis of total nitrogen (crude protein= amount of nitrogen×6.25) using Kjeldhal method by Kel Plus analyzer (Pelican, Model: KES-061). Crude fat was determined through Socs Plus system (Pelican, Model: SCS-6) by using petroleum ether. Crude fiber content of seeds was determined by digesting dry sample with 1.25% H_2SO_4, followed by 1.25%NaOH solution in Fibra Plus Fiber analyzer (Pelican, Model: FES-4). Vitamin-C was obtained by titrimetric method by using 2-6 dichlorophenol indophenol dye**.** Carbohydrate content was estimated by subtracting the values of protein, moisture, ash, fiber and fat from hundred. The mineral element determined were Iron by Wong's method and Calcium by titration against standard potassium permanganate solution ($KMnO_4$).

PREPARATION OF AQUEOUS EXTRACTS

Sample (50g) plant materials were macerated with 100ml sterile distilled water in a warning blender for 10min. The macerate was first flittered through double-layered muslin cloth and then centrifuged at 4000rpm for 3 min. The supernatant was filtered through wattmann No-1 filter paper and sterilized at 120°C for 30 min. The extract was preserved aseptically in brown battle at 5°C until further use **(Balasundoram, *et al.,* 2006).**

ANTIOXIDANT DETERMINATION

Total phenols

Total phenol content of *A. cruentus* aqueous extract was determined using the Folin-Ciocalteu method of **Jagadish *et al.,* 2009** with slight modification. To 50**µ**L sample were added 250 **µ**L of undiluted Folin-Ciocalteu reagent. After 1min, 750**µ**L of 20 % (w/v) aqueous Na_2CO_3 were added, and the volume was made up to 5.0mL with H_2O. After 2 h incubation at 25ºC, the absorbance was measured at 760nm and compared to a gallic acid calibration curve. Total

phenols were determined as gallic acid equivalents (mg gallic acid/g extract), and the values are presented as means of triplicate analyses**.**

Total flavonoid*s*
Seed extracts (0.5ml of 1:10 g/ml) in water were separately mixed with 1.5 ml of methenol. Add 0.1ml of 10% aluminium chloride. Add 0.1ml of 1M potassium acetate and 2.8ml of distilled water. It remained at room temperature for 30 min. The absorbance of the reaction mixture was measured at 415nm visible spectrophotometer. The calibration curve was prepared by preparing quercetin solution at concentrations 12.5 to 100g ml $^{-1}$ in methanol (**Chang *et al*, 2002).**

DPPH radical scavenging activity
The different concentrations (20 - 200 µg /ml) of *A cruentus* aqueous extracts were dissolved in DMSO (dimethyl sulfoxide) and taken in test tubes in triplicates. Then 5 ml of 0.1m Methanol solution of DPPH (2, 2, Diphenyl-1- Picrylhydrazl) was added to each of the test tubes and were shaken vigorously. They were allowed to stand at 37°C for 20 minutes. The control was prepared without any extracts. Methanol was used for base line corrections in absorbance OD) of sample and measured at 517nm. A radical scavenging activity was expressed as 1% scavenging activity and was calculated by the following formula **(Blois, 1958).**

$$\textbf{Radical scavenging activity (µg/ml)} = \frac{\text{OD control} - \text{OD sample}}{\text{OD control}} \text{x} 100$$

STATISTICAL ANALYSIS
Results were expressed as mean values and standard deviation of three determinations and also statistically analyzed to ascertain its significance. The analytical data obtained for antioxidant activity of malted *Amaranthus cruentus* were subjected to paired *t*-test. The significant difference at ($p \leq 0.05$ level) was estimated.

RESULTS AND DISCUSSION
Table1: Proximate Composition of Unprocessed and Malted Flours of *Amaranthus cruentus*

Proximate Parameters	*Amaranthus cruentus (*Amaranth) flours		
	Unprocessed	Malted	
		Plain Water	Alkaline medium (2%$NaHCO_3$)
Moisture(g/100g)	9.9±0.15	8.2±0.10* (17.1↓)	8.9±0.10ns (10.1↓)
Ash(g/100g)	2.4±0.25	1.8±0.21* (25↓)	1.6±0.21* (33.3↓)
Fat(g/100g)	7.4±0.36	6.1±0.30* (17.5↓)	6.0±0.43* (18.9↓)
Crude Fibre(g/100g)	6.5±0.37	4.5±0.43* (30.76↓)	5±0.15* (23%↓)
Protein(g/100g)	16.6±0.26	20.5±0.40* (23.5↑)	22.7±0.15* (36.7↑)
Carbohydrate(g/100g)	57.3±0.43	56.4±0.43^{ns} (1.6↓)	52.4±0.43^{ns} (8.5↓)
Iron(mg/100g)	12.4±0.35	11.7±0.26^{ns} (5.6↓)	9.3±0.20* (25↓)
Calcium(mg/100g)	80.7±0.60	84.2±0.15^{ns} (4.3↑)	81.6±0.30^{ns} (1.1↑)

% Dry values are expressed as means of 3 determination ±SD *= significant, ns= no significant difference

Proximate analysis

The moisture content of Amaranth Unmalted Flour (AUF) was 9.9±0.15g/100g and after malting, the moisture content of Amaranth Malted Flour (AMF) was 8.2±0.10 g/100g in plain water and 8.9±0.10g/100g with alkaline ($NaHCO_3$) solution. This showed that plain water and alkali AMF was significantly decreased ($P \geq 0.05$) by 17% and 10.1% respectively when compared with (AUF). Similar results were obtained to those of barley malting where the moisture content decreased from 19.3 to 10.5% **(Schuster and Grünewald, 1997).**

Maximum significant decreased in ash content was observed in alkali malted amaranth flour by 33.3% when compared to AUF (2.4±0.25g/100g). This result was agreement with **Afify *et al.,* 2012** who found that ash content of germinated white sorghum was 1.20g/100g(17.24%) decreased when compared to the unprocessed seed flour (1.45±0.01g/100g).There was removal of seed coat during malting, which contributes towards the reduction in the total mineral content of malted samples **(MacMasters *et al.,* 1971).**

The result data (Table1) indicated that the fat content of AUF was7.4±0.36 g/100g, with steeping 10h and germination 24h, maximum decreased was seen in alkali malted 19% and minimum in plain water malted 17.33% amaranth flour, there were significant differences in fat content with regard to steeping and germination time. This data was comparable in fat content with a values 8.6 ± 0.17g/100g and 8.7 ± 0.01 g/100g in unprocessed foxtail millets (yellow and purple variety) according to **Choudhury, and Baroova (2011)** and after malting lower fat content was observed (53.3% to 59.7%). Hydrolysis of lipid and oxidation of fatty acids take place during germination of seeds. The hydrolyzed products do not accumulate in the seed, but the glycerol becomes a part of carbohydrate pool and the fatty acids are oxidized through α and β oxidation, resulting in decrease in fat on malting **(Mayer and Mayber, 1963).**

The fibre content of both malted amaranth flour (plain water and alkali) was significantly lower by 33% and 21.0% respectively at $p \leq 0.05$. Although there were reports of much higher reduction (72.0%) in crude fibre content of refined malted millets **(Malleshi and Desikachar, 1981).** Similar results obtained by **Malleshi and Klopfenstein, (1998)** that 8.8, 36.0 and 51.5% reduction in malt flours of sorghum, pearl and finger millet. This extent of reduction differences might be due to the type of grain and degree of removal of the seed coat. It was reported that a significant decrease in neutral detergent fibre on malting occurred due to cell wall degradation during sprouting process **(Aisien 1982; Glennie,1984).** In addition, the exclusion of rootlets and shoots of sprouts also appears to influence the fibre content of malted cereals **(Chavan and Kadam, 1989).**

Protein content of AUF was 16.6±0.26g/100g.After malting the result revealed that protein content of alkali ($NaHCO_3$) amaranth malted flour showed highest value i.e 22.7±0.15g/100g when compared to both unmalted flour and plain water treated malted flour. Although there was significant reduction was observed in both malted flours by 23.5% in plain water and alkali treated AMF by 36.7%**.** These were comparable with the results of **Kanensi** and his coworkers that the crude protein content of *Amaranthus cruentus* seed was 15.4 ± 0.9g/100g which was increased by 16.6 ± 0.2g/100g (8%). **Mbithi-mwikya** reported that there was a slight but significant increase in protein content of finger millet at each sampling time, from 6.1% in ungerminated seeds to 7.9% during the 96 hours of germination. He attributed the increases in protein content to be due to dry matter loss particularly through carbohydrates through respiration causing an apparent increase in other nutrients such as proteins.

The result showed that the carbohydrate content was decreased with in steeping and germination period. However, the values were not significant different at $p \leq 0.05$. A maximum carbohydrate loss was found in alkaline malted amaranth flour (8.5%) while a minimum was in plain water malted amaranth flour (1.6%) with comparison with unprocessed flour. At the same time comparing to the study of **Frank *et al.,* 2009** that the carbohydrate content was also decreased after germination in African yam bean 60.16±0.01g/100g (4%). Similarly, decreased data was found in starch content of finger millet by 33.85% after malting was reported through **Nirmala *et al .,* 2000**. This decreased data might be due to increase in alpha-amylase activity (**Lasekan 1996**). The alpha-amylase breaks down complex carbohydrates to simpler and more

absorbable sugars which are utilized by the growing seedlings during the early stages of germination **(Elkhier and Hamid, 2008).**

Unmalted Amaranth flour had an iron content 12.4±0.35mg/100g. By the data it was seen that the iron content was 5.6% decreased in plain water malted flour and 25% in amaranth alkali treated malted flour when compared to unmalted flour. Micro-elements content were decreased after malting treatments reported by **Lestienne *et al.,* (2005)** that up to 40% of Fe content of sorghum grain. The calcium content of plain water and alkali treated malted amaranth flour was 84.2±0.15and 81.6±0.30 mg/100g respectively. This shows that there was no significant increased in all malted flours at (P ≥ 0.05) level. The increased in calcium content after malting is supported by **Desai *et al.,* 2010** who observed 20%increment in calcium value during malting of ragi flour. It was noticed from the data that the malting process was useful to increase the calcium content. Malting of finger millet improves digestibility and bioavailability of nutrients, improves sensory and nutritional quality **(Malleshi and Desikachar, 1986).**

Table 2: Antioxidant Activity of Unprocessed and Malted Flours of *Amaranthus cruentus*

Malted Flours	Total Phenol (mg GAE/100g)	Flavonoids (mg QE/100g)	DPPH scavenging capacity IC50 (µg/ml)
AUF	24.8±0.75	17.1±0.72	55
APMF	32.8±1.19 (32.2↑)	21.9±0.40 (28↑)	38
AAMF	**38.6±1.00 (55.6↑)**	**36.0±0.56(110.5↑)**	**18**

Values are expressed as means of three determination ±SD

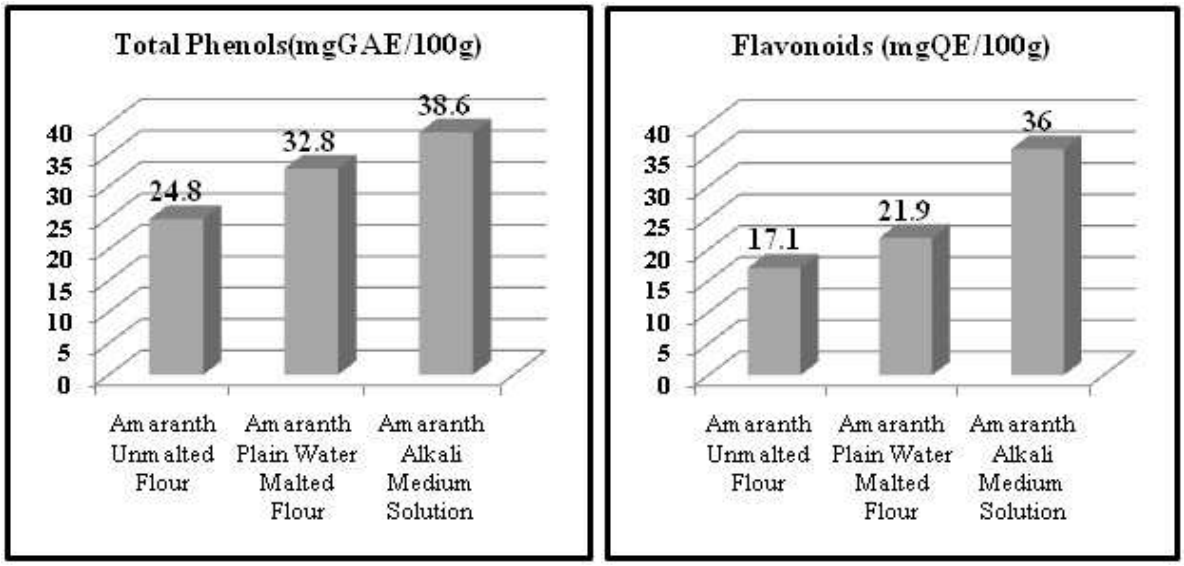


Figure 1a&b: Total Phenolic Content (mg GAE/100g) and Flavonoids Content (mgQE/100g) of Unmalted and Malted Flours of *Amaranthus cruentus*

Antioxidant activity analysis

The total phenols and flavonoids content results are shown in Table2 and Figure 1a&b. The total phenols content in AUF was 24.8±0.75mg Gallic acid equivalent (GAE)/100g. Both malted flours differed greatly and found to be significant highest in alkali treated ($NaHCO_3$) malted flour 38.6±1.00 mg GAE/100g.Therefore, in the alkaline treatment, the cellulose enzyme actively play an important role in release of phenols with free hydroxyl groups, which can improve the

antioxidant activity. Total phenolic content increased on malting may be due to enzymatic release of bound phenolic compounds during seed germination **(Maillard and Berset, 1995).** In the recent years, polyphenols gained much attention due to its biological properties **(John *et al.,* 2006).** In extract from alkali treated amaranth malted flour, the flavonoids content 36.0±0.56 mg QE/100g was significantly (p≥0.05) higher than unprocessed (17.1±0.72 mg QE/100g) and plain water 21.9±0.40 mg QE/100g malt flour. Moreover, malting seeds to improve the nutritional compounds in seedlings is effective, too. Research proved that the content of total flavonoids was distinctly elevated during seed germination. Flavonoids are thought to be able to play critical role in those healing activity, and the main functional molecule are rutin, quercitin, hyperin and so on **(Watanabe *et al.,* 1997).**

Table 3: Free Radical Scavenging Activity (µg/ml) of Unprocessed and Malted Flours of *Amaranthus cruentus*.

Concentration	Ascorbic Acid	AUF	APMF	AAMF
20	87.44	36.8	43.6	45.0
40	88.35	42.2	47.2	66.8
60	89.72	48.9	57.5	67.2
80	90.6	49.1	58.1	69.0
100	91.09	49.3	62.0	70.4
120	92.46	50.1	62.7	71.0
140	93.8	51.2	63.1	72.0
160	94.2	57.4	63.4	72.7
180	95.2	58.1	64.0	74.2
200	97.03	62.4	64.7	77.1

AUF= Amaranth Unmalted flour, APMF= Amaranth Plain Water malted Flour AAMF= Amaranth Alkali Malted Flour

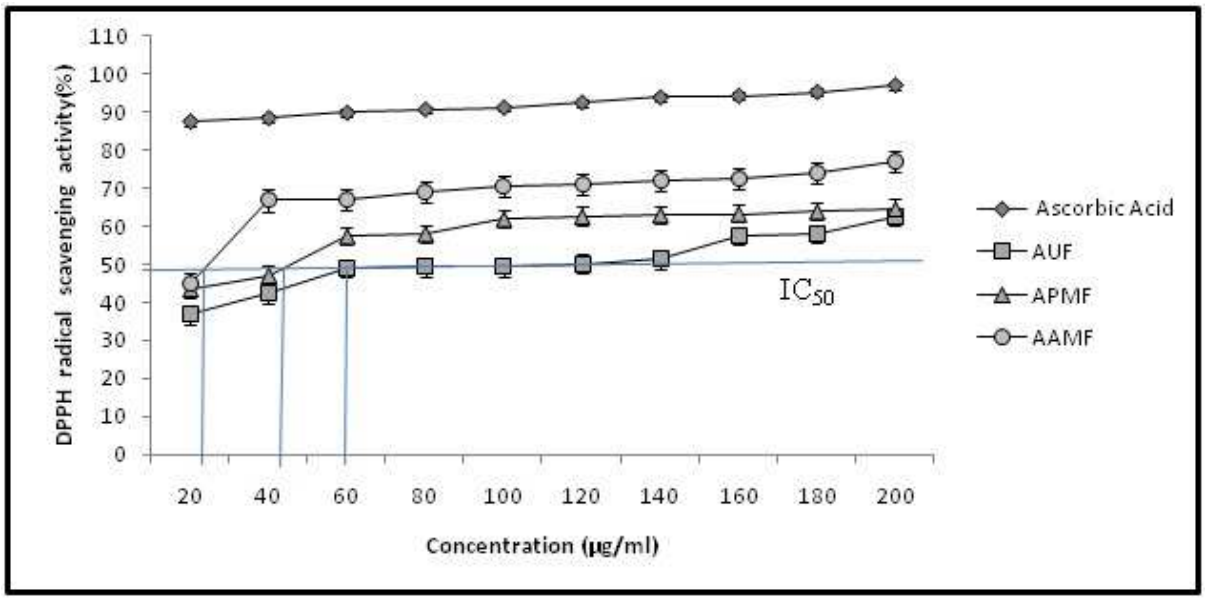


Figure 2: Free radical scavenging activity (µg/ml) of Unprocessed and Malted Flours of *Amaranthus cruentus*

The model of scavenging the stable DPPH radical is a widely used method to evaluate the free radical scavenging ability of various samples **(Ebrahimzadeh *et al.,* 2008).** DPPH is a stable

nitrogen-centered free radical the color of which changes from violet to yellow upon reduction by either the process of hydrogen- or electron- donation. Substances which are able to perform this reaction can be considered as antioxidants and therefore radical scavengers **(Dehpour *et al.,* 2009).** It was found that the radical-scavenging activities of both the extracts increased with increasing concentration. IC^{50} for DPPH radical-scavenging activity were reported in Table3 and Figure2 .The DPPH radical scavenging was expressed as IC_{50} value. The antioxidant activity of alkali treated ($NaHCO_3$) malt flour was stronger, than those treated with plain water malted and unprocessed flours. The strongest antioxidant activity (IC_{50}) was 18µg/ml in AAMF followed by plain water AMF (38 µg/ml) when compared to AUF (55 µg/ml).

CONCLUSION

Pseudocereals have considerable scope to be utilized as nutritional and functional food because it has better nutritional quality as compared to other cereals in many aspects. The results obtained in the present study showed that alkali treated malted flour was found to be most effective when compared to plain water malted flour in retention of the nutrients (protein, crude fibre and calcium). The alkali treated amaranth flour extract had good antioxidant properties including total phenols, flavonoids and DDPH scavenging radical activities when compared to plain water malt flour. As we know, processing like malting (steeping, germination and kilning) brings out the hydrolysis of carbohydrates and protein to certain extent of food grains, thus result in reduced cooking time, improve digestibility, protein efficiency ratio of the grain and improve the bioavailability of some essential nutrients. It also shows antioxidants potential which have been associated with protection from chronic diseases which are prevalent in our community. Thus, alkali malted amaranth extract obtained as the outcome of the present study will be useful in developing low cost dietary formulations for human beings and easily available to be consumed for increasing the nutrition as well as safeguarding against possible deficiencies.

ACKNOWLEDGEMENT

Authors are thankful to Prof. Aditya Shastri, Vice chancellor, University Dist- Tonk, Rajasthan. 304022, India for providing facilities for the successful completion of the study.

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