IMPACT OF DECORTICATION METHODS ON TOTAL PHENOLIC CONTENT, ANTIOXIDANT ACTIVITY AND OXIDATIVE STABILITY OF TWO PEARL MILLET CULTIVARS DURING STORAGE

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Abstract
This study aims to assess the effect of decortication methods on the antioxidant activity (AA), total phenolic content (TPC), peroxide value (PV), acid value (AV), free fatty acid (FFA) and total acidity (TA) of two pearl millet cultivars (White and Green) grown in Sudan during 6-month storage. Modern decortication method was carried out by using Tangential Abrasive Dehuller while the grains were decorticated in a traditional way by stone dehuller. The above measurements were determined using standard analytical procedure. Decortication raised the TPC and AA in both cultivars as compared to untreated cultivars. The TPC and AA of modern decorticated cultivars were higher than that treated with traditional decorticated method. Green cultivars exhibited higher TPC than white cultivars. Storage of the grains gradually reduced the TPC of treated and untreated white and green cultivars. Significantly higher PV, AV and FFA was observed in modern decorticated cultivars (particularly Green cultivar) as compared with traditional decorticated and untreated cultivars. At 45th day of storage, a significant rise in PV was found in treated and untreated cultivars. Storage of the grain resulted in gradual increase in AV, FFA and TA with modern decorticated samples having significantly higher values in both cultivars at the end of storage period.

Keywords: Pearl millet; Decortication methods; Antioxidant activity; Peroxide value; Acid value; Free fatty acid

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1. INTRODUCTION

According to US Department of Agriculture and US Department of Health and Human services, cereals play an essential part in human diet with a recommended daily consumption of 42 g of whole grain products (USDA, 2005). In terms of world agriculture production, millet is regarded as the 6th cereal and most vital drought-resistant crop. Among various millet types, pearl millet is the most highly cultivated millet grown widely in Asia and Africa (Devi *et al.,* 2011).Therefore, most developing countries now focus specific attention to millet grains for use as food of health benefit and in production of biofuels by some developed countries (Li *et al.*, 2008). Previous studies have shown that millet grains are good source of phenolic compounds and antioxidant (Chandrasekara and Shahidi, 2010, 2011). These phenolic content and antioxidant activity of cereal grains have been reported to be greatly affected by processing method employed (Shahidi, 2009). Different traditional processing techniques such as fermentation (Hassan et al., 2006), germination (Abdelrahamanet al., 2007) and malting (Samiaet al., 2005) are employed to improve the edible, nutritional and sensory attributes of millet grains before consumption as food. The main anatomical components of millet grain include pericarp, germ and endosperm. Generally, traditional decortication of millet grains involves the use of wooden mortar and pestle and the wastes of the decorticated grains are known to be a potential source of natural antioxidants. The parts of the cereal grains like the pericarp, seed coat and aleurone layer are

rich in polyphenols and phytates (Awika *et al.*, 2005). However, these polyphenols and phytic acid content in cereals like pearl millet are reduced after decortication (Monawar, 1983). Researches have shown that traditional decortication of pearl millet by hand-pounding or using a mechanical device adversely affect the total phenolic content and antioxidant capacity of the grains but no significant effect was reported in the protein and fat contents (Hama *et al.*, 2011; Lestienne *et al.*, 2005; Bagdia *et al.*, 2011). The reduction was attributed to the fact that the peripheral parts of the grain rich in all these components were removed during the traditional decortication technique (Hama *et al.*, 2011). Therefore, there is need for innovative decortication technique that can be applied to large amount of pearl millet grains in a short time while retaining most of its health beneficial properties and with minimal effect on storage quality. Also variation in the amount of phenolic compounds and antioxidant present in the grains depends on several factors such as species, cultivar, growing location, environmental conditions, among others (Adom*et al.*, 2003; Bonoli*et al.*, 2004; Zielinski and Kozlowska, 2000).Therefore, the aim of this study is to assess the effect of modern decortication method on the antioxidant activity, total phenolic content and oxidative stability of two pearl millet cultivars during storage.

2. MATERIALS AND METHODS

2.1. Materials

Pearl millet cultivars (White and Green) were procured from Department of Agronomy, Faculty of Agriculture, University of Khartoum, cleaned and used for the study. All chemicals used were of analytical grade.

2.2. Traditional and modern decortication

The grains of the two cultivars were carefully cleaned, freed from foreign material as well as broken and shrunken kernels. The seeds were divided into three portions; one portion was milled as whole grains into fine powder to passed through a 0.4 mm mesh. The other two portions were either dehulled (decorticated) using modern; Tangential Abrasive Dehuller Device (TADD) or traditionally (stone dehuller) and after dehulling the grains were milled to fine flour to pass a 0.4 mm screen. The flour was then kept into plastic bottles and stored for different period of time at room temperature (25^{o}C).

2.3. Extracts preparation

Exactly, 1 g of treated and untreated samples was extracted for polyphenolic estimation and antioxidant activity successively with 60% methanol (50 mL) for 1 h in a magnetic stirrer at room temperature. The extraction was repeated with the residue and the extracts were pooled and filtered through Whatman No 1 filter paper. The methanolic extracts were stored at 20 ºC till further use.

2.4. Total phenolic content (TPC) determination

The TPC of treated and untreated samples were analyzed using the Folin Ciocalteu method with some modifications (Singleton and Rossi, 1965).Exactly, 200 µL appropriately diluted sample or a standard solution of varying concentrations was mixed with 400 µL of Folin–Ciocalteu reagent. Deionized water was used for dilution and control. The solution was diluted with deionized water to a total volume of 4.6 mL and then thoroughly mixed. After incubation for 10 min at room temperature, 1 mL of 10% Na_2CO_3 solution was added, then immediately mixed, and incubated for 2 h. The absorbance was read at 765 nm on a spectrophotometer (Apel, Saitama, PD-303UV, Japan). Measurements were recorded in triplicate. The gallic acid of 1 mg/mL was used as the standard, and the total phenolic compounds of the samples were expressed in milligram gallic acid equivalent (GAE) per 100 mL extracts (mg GAE/100 mL extracts).

2.5. Antioxidant activity determination

The radical scavenging activity of the extract was determined using 1,1-diphenyl-2-picrylhydrazyl (DPPH) assay (Ghafoor *et al.*, 2012). The extract sample (1 mL) was mixed with 2 mL of DPPH radicals' solution

(prepared by dissolving 1 mg DPPH in 100 mL of methanol). After thorough mixing and 5 min incubation at room temperature, the absorbance values (Δ517 nm) were obtained. The control consisted of 1 mL of distilled water in 2 mL of DPPH solution and the free radical scavenging activities (RSA) were calculated using equation below:

$$RSA\ (\%) = \frac{Abs_{control} - Abs_{extract}}{Abs_{control}} \times 100$$

2.6. Peroxide value (PV), Acid value (AV), Free fatty acid (FFA) and Total acidity (TA)

The FFA, AV and TA of the treated and untreated samples during storage were determined by titrating the free fatty acids with alkali in presence of ethyl alcohol as solvent [Ca 5a-40]. The peroxide value (PV) was estimated by using sodium thiosulfate solution as titrating agent against the evolved iodine in the sample, after reacting the peroxides present in the sample with salt of iodine (KI) [Cd 8-53] as reported in AOCS (2004).

2.7. Statistical analysis

All experiments were carried out in triplicate, and data were assessed using ANOVA described by Snedecor and Cochran (1987). Differences between the treatment means were separated using Duncan's multiple range tests. Significance was accepted at $p < 0.05$.

3. RESULTS AND DISCUSSION

3.1. Total phenolic content (TPC) of modern and traditional decorticated pearl millet cultivars during storage

The effect of decortication methods on the TPC and antioxidant activity of pearl millet cultivars during storage is presented in Table 1. Decortication affected the TPC of the millet cultivars. The result showed that decortication significantly ($p < 0.05$) reduced the TPC in both white and green cultivars as compared with their whole samples throughout the storage period. Treatment of the millet with modern decortication technique significantly ($p < 0.05$) raised the TPC of the milletas compared with traditionally treated millet. The TPC of the green cultivar were significantly ($p < 0.05$) higher than that of white cultivar. Storage of the grains gradually reduced the TPC of treated and untreated white and green cultivars.

Similarly, Chandrasekara *et al.*(2012) reported variation in TPC among different millet cultivars after processing such as decortication and cooking. The decrease in TPC after decortication of millet grains could be attributed to the removal of the outer layers of the grain. In accordance with the present results, the TPC of pearl millet was found to reduce after dehulling of the grains (Hag *et al.,* 2002). Thus, the results of this study lend further support to the fact that phenolic compounds of cereal grains are mainly concentrated in the outer layers of the grain (Awika *et al.,* 2005; Zielinski and Kozlowska, 2000). The higher TPC observed in millet cultivars treated with modern decortication method could be attributed to lesser amount of phenolic compounds removed during the decortication process as compared with traditional method. In addition, the reduced TPC in millet treated with tradition method could be due to the degradation of phenolics by the heat applied during the process or leaching into the endosperm to form complexes with proteins and other macromolecules, thus making phenolics less extractable. The difference in the TPC among the two millet cultivars studied after decortication could be due to variable distribution of polypheolics in different grain layers, namely testa, aleurone layer and pericarp. This was also in agreement with the findings of TPC of millet grain influenced by dehulling as reported by Chandrasekara *et al.* (2012).

3.2. Antioxidant activity of modern and traditional decorticated pearl millet cultivars during storage

A contrasting trend was observed in the antioxidant activity of the treated and untreated millet cultivars during storage (Table 1) as compared to that of their TPC. Decortication of the millet significantly ($p < 0.05$) enhanced the antioxidant activity of the millet in the two cultivars with those treated with modern method having higher value at day 0 of storage.

Table 1. Total phenolics (mg/100g) and antioxidant activity (%) of millet seeds as affected by decortication method and storage period

Cultivar	Decortication method	Storage period (days)				
		0	15	30	45	60
Total phenolics						
	Whole	299.50±0.71	286.10±0.12	285.80±0.17	270.80±0.24	273.80±0.87
White	Modern	194.10±0.06	178.50±0.06	181.60±0.12	167.70±0.09	142.80±0.14
	Traditional	168.60±0.06	133.00±0.04	174.00±0.10	150.70±0.74	121.50±0.10
	Whole	835.30±0.52	678.80±1.00	794.20±0.71	534.00±0.04	408.70±0.64
Green	Modern	434.90±0.05	403.60±0.08	374.70±0.15	299.00±0.08	308.20±0.09
	Traditional	272.20±0.15	268.40±0.13	223.70±0.27	188.80±0.55	207.70±0.15
$LSD_{0.05}$				75.79*		
SE±				26.79		
Antioxidant activity						
White	Whole	17.54±1.18	19.30±0.45	23.17±0.65	26.77±1.32	36.76±1.48
	Modern	39.04±2.14	39.97±0.71	44.76±1.05	39.84±0.59	57.56±0.00
	Traditional	33.90±0.37	37.31±0.22	43.07±1.20	49.20±0.84	57.83±1.00
Green	Whole	16.36±1.34	20.16±0.64	24.21±0.57	26.75±0.59	38.05±0.38
	Modern	36.71±1.65	29.09±0.49	46.59±0.82	49.00±1.05	58.24±0.41
	Traditional	35.54±1.27	37.06±0.37	45.29±0.25	49.05±0.54	58.90±0.66
$LSD_{0.05}$				6.960*		
SE±				2.424		

Values are means ±SD. Mean(s) having different superscript(s) in columns and rows are significantly different (P≤0.05) according to DMRT.

Cultivar type has no significant effect on the antioxidant activity of the millet. As storage period increased, the antioxidant activity increased with decorticated treated samples having significantly ($p < 0.05$) higher value than whole samples in both cultivars. However, the method of decortication employed has no significant effect on the antioxidant activity of the cultivars during storage.

The results of higher antioxidant activity of decorticated millet cultivars as compared to the untreated millet found in this study contradicts previous report where decortication reduced the antioxidant activities of grain (Lestienne *et al.*, 2005; Bagdia *et al.*, 2011; Hama *et al.*, 2011). It has been reported that phenolic compounds are mainly located in the peripheral parts of the grains (pericarp and aleurone layer) and removal of the pericarp during decortication may reduce their contents (Hama *et al.*, 2011). However, it has been reported that antioxidant activity of phenolic compounds depends on the nature of free radicals employed in assay in addition to their concentration and structural characteristics as well as synergistics interactions with other compounds present (Chandrasekara *et al.*, 2012). The enhanced antioxidant activity in decorticated millet, particularly modern decorticated millet cultivars, could be due to effective removal of the outer layer with minimal degradation of the phenolic compounds and also not forming complexes with macromolecules such as protein in the endosperm, thus improving the extractability of the phenolics with increase antioxidant activity. Therefore, the applicability of pearly millets as functional food may be enhanced by processing with modern decortication method.

3.3. Peroxide value (PV), acid value (AV), free fatty acid (FFA) and total acidity (TA) of modern and traditional decorticated pearl millet cultivars during storage

As shown in Table 2, at day 0 of storage, treatment of the cultivars with modern decortication significantly ($p < 0.05$) increased the PV of the millet as compared with traditional and whole samples with green cultivar having higher value.

Table 2. Peroxide value, acid value and free fatty acids (%) of millet seeds as affected by decortication method and storage period

Cultivar	Decortication method	Storage period (days)				
		0	15	30	45	60
Peroxide value						
	Whole	3.32±0.14	7.17±0.33	8.31±1.13	9.55±0.27	9.65±0.15
White	Modern	5.66±0.25	8.54±0.32	9.85±0.11	11.20±0.20	11.37±0.07
	Traditional	3.25±0.55	7.42±0.32	9.00±0.28	10.20±0.19	10.78±0.03
	Whole	3.20±0.75	6.33±0.49	8.12±0.61	9.68±0.31	9.38±0.09
Green	Modern	7.82±0.46	7.75±0.11	9.29±0.55	10.35±0.06	10.96±0.25
	Traditional	5.76±1.01	7.47±0.30	9.64±0.22	10.08±0.09	10.62±0.55
$LSD_{0.05}$				0.7231*		
SE±				0.2556		
Acid value						
White	Whole	0.5440±0.04	0.5609±0.02	0.7340±0.03	0.7449±0.06	0.7570±0.04
	Modern	0.5713±0.01	0.7371±0.15	0.9342±0.05	0.9744±0.21	1.560±0.01
	Traditional	0.5460±0.03	0.6603±0.03	0.7956±0.05	0.9133±0.01	0.9799±0.09
Green	Whole	0.4510±0.03	0.5492±0.06	0.6985±0.08	0.7733±0.03	0.8133±0.04
	Modern	0.6620±0.02	0.6788±0.04	0.8507±0.03	1.005±0.02	1.267±0.06
	Traditional	0.5383±0.10	0.6878±0.08	0.9581±0.03	0.9750±0.12	1.115±0.01
$LSD_{0.05}$				0.1033*		
SE±				0.03651		
Free fatty acids						
White	Whole	0.2720±0.02	0.2805±0.01	0.2720±0.02	0.3622±0.04	0.3799±0.02
	Modern	0.2857±0.01	0.3707±0.07	0.4652±0.02	0.5776±0.10	0.4871±0.01
	Traditional	0.2857±0.01	0.3301±0.01	0.4176±0.05	0.4600±0.00	0.4867±0.04
Green	Whole	0.2255±0.02	0.2753±0.03	0.3727±0.02	0.4266±0.01	0.4453±0.02
	Modern	0.3310±0.01	0.3394±0.02	0.4443±0.02	0.5023±0.01	0.6501±0.03
	Traditional	0.2342[j]±0.05	0.3438±0.04	0.4767±0.02	0.4875±0.06	0.5894±0.11
$LSD_{0.05}$				0.05165*		
SE±				0.01826		
Total acidity						
White	Whole	1.0880±0.04	1.1218±0.02	1.4680±0.03	1.4595±0.06	1.5140±0.04
	Modern	1.1426±0.01	1.4742±0.15	1.8684±0.05	1.9488±0.21	3.1200±0.01
	Traditional	1.0920±0.03	1.3206±0.03	1.5912±0.05	1.8266±0.01	1.9598±0.09
Green	Whole	0.9020±0.03	1.0984±0.06	1.3970±0.08	1.5466±0.03	1.6266±0.04
	Modern	1.3240±0.02	1.3576±0.04	1.7014±0.03	2.0100±0.02	2.5340±0.06
	Traditional	1.0766±0.10	1.3756±0.08	1.9162±0.03	1.9500±0.12	2.2300±0.01
$LSD_{0.05}$				0.1033*		
SE±				0.03651		

Values are means ±SD. Mean(s) having different superscript(s) in columns and rows are significantly different (P≤0.05) according to DMRT.

As storage period progresses up to 45 days a significant ($p < 0.05$) increase was observed in the treated and untreated millet of both cultivars. At the end of the storage period (60 days), the PV of decorticated samples were higher than the whole sample win both cultivars with modern decorticated sample having significantly ($p < 0.05$) greater value than traditional decorticated sample in the white cultivar. The AV, FFA and TA followed the same trend. The result at day 0 (Table 2) revealed that decortication process of the white cultivar had no significant effect on the AV and FFA as compared with that of whole sample. However, the use of modern decortication method significantly raised the TA of both white and green cultivars throughout the storage period. Also, a significantly greater AV

and FFA was observed in the decorticated green cultivar with modern decorticated sample exhibiting the highest value. Storage of the grain resulted in gradual increase in AV, FFA and TA with modern decorticated samples having significantly ($p < 0.05$) higher values in both cultivars at the end of storage period.

According to Lai and Varriano-Marston (1980), the unsaturated fatty acids (oleic and linoleic) accounts for more than 85% of the total fatty acid present in both whole and decorticated pearl millet. Furthermore, decortication of millet has been reported to have no significant effect on the fatty acids of millet cultivars probably due to the varying distribution of fatty acids in the grain from the outer layers into the endosperm (Liu, 2011). Despite the high TPC of millet grains treated with modern decortication method, the grain still possessed higher PV as compared with other sample and this could be due to high amount of fat still retained in the grain after modern decortication as compared with traditional decortication. This fat that is rich in unsaturated fatty acids could undergo rancidity during storage thereby increasing the PV of the decorticated grain. It has been reported that decortication lowered fat content in millet due to the separation of the germ during decortication (Dendy, 1995). Also the germ has high palmitic acid and incomplete removal during decortication process may also result in increased AV, FFA and TA of the grain during storage. Therefore, the use of modern decortication to process millet grains can affect the storage quality and sensory properties of the grains.

4. CONCLUSION

It can be concluded that decortication increased the antioxidant potential of both pearl millet cultivars with highest values obtained in modern decorticated grains. Also, modern decortication significantly raised the oxidative stability of the cultivars during storage, particularly green pearl millet cultivar.

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