Nutritional Communication



Biosci. Biotech. Res. Comm. 12(3): 652-657 (2019)

Effect of natural and synthetic antioxidant on shelf life of different Sudanese *Pennisetum glaucum* L. flour

Mosab Abbas Ahmed Abdalgader¹, Syed Amir Ashraf^{2*}, Amir Mahgoub Awadelkareem², Mohd Wajid Ali Khan³ and Abdelmoniem Ibrahim Mustafa¹

¹Department of Food Sciences, Faculty of Agriculture, University of Khartoum, Sudan ²Department of Clinical Nutrition, College of Applied Medical Sciences, University of Hail, Hail KSA ³Department of Clinical Laboratory Sciences, College of Applied Medical Sciences, University of Hail, Hail KSA

ABSTRACT

Development of rancidity in pearl millet flour even after a shorter period of storage time is the major limitation for its acceptability by the consumers. So, our aim of this study was to improve the shelf life of pearl millet flour obtained from two cultivars, (Ashana and Hreahry) using natural (ascorbic acid) and synthetic (butylated hydroxytoluene) antioxidant. Flour samples were evaluated for free fatty acids (FFA), peroxide value and fat acidity for a time period of 0, 10, 30, 60 and 90 days. We found that, untreated samples had significant increase in FFA compared to samples treated with ascorbic acid and butylated hydroxytoluene. Moreover, peroxide values in butylated hydroxytoluene treated samples were found to be low compared to untreated flour as well as ascorbic acid treated flours for both the cultivars. From our results it was observed that, butylated hydroxytoluene and ascorbic acid treated samples were able to maintain shelf life for 30 days, respectively. However ascorbic acid being a natural antioxidant could be a potential source of preservation and it could provide an effective and natural way for improving the shelf life of pearl millet flour.

KEY WORDS: PEARL MILLET; BHT; ASCORBIC ACID; ANTIOXIDANT; FREE FATTY ACID

ARTICLE INFORMATION:

Corresponding Author: amirashrafy2007@gmail.com Received 23rd July, 2019 Accepted after revision 20th Sep, 2019 BBRC Print ISSN: 0974-6455 Online ISSN: 2321-4007 CODEN: USA BBRCBA Thomson Reuters ISI ESC / Clarivate Analytics USA



NAAS Journal Score 2019: 4.31 SJIF: 4.196 [®] A Society of Science and Nature Publication, Bhopal India 2019. All rights reserved. Online Contents Available at: http://www.bbrc.in/ DOI: 10.21786/bbrc/12.3/15

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INTRODUCTION

Pearl millet (Pennisetum glaucum L.) is one of the oldest cereals known to human being and consumed in various parts of the world as a staple food since hundreds years back (Deepak et al., 2012; Goyal and Chug 2017). It has been one of the major food sources for millions of people, especially those who live in hot, dry regions of the world other than wheat and maize. In contrast, millet is the major food sources of protein for billions of people in Africa and Asia. Millet has been reported to have many nutritional as well as therapeutic properties (Amadou et al., 2013; Sarita and Singh, 2016). Nutritionally pearl millet is on a par or even superior to other cereals such as rice, maize and wheat with respect to energy value, proteins, fat and minerals. Various macronutrients like amino acids, vitamins, minerals, dietary fibers and antioxidants presents in a more balanced ratio in a pearl millet than in other cereals. It makes an important contribution to human diet due to high levels of calcium, iron, zinc, lipids and high quality proteins. The level of stored energy in pearl millet is approximately equal to that of maize. The most prominent feature of pearl millet is relatively higher lipid content, which gives more energetic feed than maize, wheat, or shorgum (Deepak et al., 2012; Devi et al., 2014). Different phytochemicals such as phytic acid, tannins, and phenolic compounds contribute to antioxidant activity, which makes it very important for health, ageing and metabolic diseases (Alghamdi et al., 2108; Ahmed et al 2011, Gull et al, 2015, Kulthe et al 2017 Goyal and Chugh 2017 Al Ghamdi et al 2018).

Antioxidants are substances that protect cells from damage caused by unstable molecules known as free radicals. Antioxidants acts by retarding autoxidation of triglycerides. The amounts of protection provided by antioxidant depend on its concentration, its reactivity towards the particular reactive oxygen species being considered, and the status of the antioxidants with which it interacts (Odusola et al., 2013; Pushparaj & Urooj, 2014). Few antioxidants such as butylated hydroxyl toluene (BHT) and ascorbic acid acts by scavenging for oxygen or chelating pro-oxidant metal ions. But the use of synthetic chemicals such as BHT has also been met with skepticism from consumers; because of this many of us today demand food products without synthetic additives (Eskin and Przybylski, 2001). When pearl millet is grinded into flour, the resulting flour tends to become rancid due to oxygen exposure as well as high moisture content. This is attributed to the deterioration of its triglycerides through lipolysis and subsequent oxidation of de-esterified unsaturated fatty acids. These chemical changes manifest themselves as off-odors and off-taste of the flour (Palande et al., 1996; Yadav et al., 2012; Ashraf *et al.*, 2016). Thus, it becomes unsuitable for the consumers to store these flours for longer period of time. There are various reports, which indicate storing of millet flour for even few days causes rapid rancidity and produces off-flavor and bitter taste. In order to minimize losses occurring during storage, the chemical conventional treatment could emerge as an alternative method of storage (Mohamed *et al.*, 2010; Mohamed *et al.*, 2011, Al Ghamdi et al 2018).

Moreover, various research studies were carried out for proximate composition and mineral accessibility, but information on effect of using natural and synthetic antioxidant activity on the keeping quality in pearl millet is limited. Our objective was to extend the shelf life of pearl millet flour using natural and synthetic antioxidants.

MATERIAL AND METHODS

Material: Two Sudanese pearl millet cultivars (Ashana, Hreahry), were obtained from the local market of Elobied, North Kordofan State, Sudan. Polyethylene bags, butylated hydroxytoluene (BHT) and ascorbic acid were obtained from a local chemical supplier in Khartoum, Sudan.

Treatments of pearl millet flour: The grains of both the cultivars were cleaned and milled using traditional stone mill. Pearl millet flours were divided into three groups of about 5 kg for each one of the three treatments. One group was left untreated and considered as control, the second group was treated with BHT (0.02%) and the third group was treated with ascorbic acid (0.5%). The required quantity of antioxidants was first mixed by hand in a small portion of flour sample. The mixture was then added to the bulk flour and mixed well to ensure uniform distribution. The treated and untreated flour samples were stored for 90 days at prevailing room temperature (37 ± 4 °C) in polyethylene bags. The samples were periodically tested for 0, 10, 30, 60 and 90 days (Kapoor and Kapoor *et al.*, 1990).

Proximate analysis: The determination of moisture, crude fiber and ash were carried out according to the AACC (2008) standard methods, while crude fat and crude protein were determined according to the AOAC (2005) standard methods (Ibrahim *et al.*, 2018).

Determination of Free fatty acid, fat acidity and Peroxide value: Free fatty acid (as oleic acid) was determined according to the (Majid *et al.*, 2015). Fat acidity was measured according to the standard method of the AACC (2008). The peroxide value was determined according to the standard method (Bashir *et al.*, 2015).

Statistical analysis: The analysis of variance (ANOVA) was performed to examine significant effect in all

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Table 1. Proximate composition of pearl millet (Ashana and Hreahry) cultivars							
Cultivar	Moisture (%)	Ash (%)	Crude Proteins (%)	Crude Fibre (%)	Lipid (%)	Available carbohydrate (%)	
Ashana	4.69 ± 0.44^{b}	2.28 ± 0.46^{b}	12.84 ± 0.58^{b}	1.52 ± 0.70^{a}	5.12 ± 0.32^{a}	73.55 ± 2.14 ^a	
Hraehry	6.28 ± 0.07^{a}	2.80 ± 0.74^{a}	14.99 ± 0.22^{a}	1.48 ± 0.03^{a}	5.43 ± 0.40^{a}	69.02 ± 0.40^{a}	
Means (s) values (\pm SD) having different superscripts in the same column are not significantly different (P \leq 0.05); n.s: not significant							

parameters measured. Duncan Multiple Range Test was used to separate the means (Montgomery, 2001).

RESULTS AND DISCUSSION

Proximate analysis of raw material: The moisture contents of Ashana and Hreahry cultivars were found to be 4.69% and 6.28 %, respectively as shown in table 1. Statistical analysis of the results showed a significant difference ($p \le 0.05$) in moisture contents between Ashana and Hreahry cultivars. Previous studies reported that, moisture content of pearl millet varies from 5.4 % and 6.48 % (Eltayeb, 2006), which was in consistent with the previous studies. Moreover, ash contents for the Ashana and Hreahry cultivars were found to be 2.28 % and 2.80 % respectively. Statistical analysis of the ash % showed significant difference ($p \le 0.05$) between the two cultivars. These results were higher than the reported values 0.73% by Gull et al., 2015. Crude protein content of Ashana and Hreahry cultivars were found to be 12.84 % and 14.99 % respectively. However, Amadou et al. (2013) reported 14.8% protein content, which was in comparable with Hreahry cultivars. Ashana cultivar flours were found to be having less protein content than Hreahry cultivars. Crude fiber of Ashana and Hreahry cultivars were found to be 1.52 % and 1.48 % respectively. Percentage of crude fiber was found to be lower than the range of 2.4% and 8.6% as reported by Eltinay *et al.* (2005). Nambiar *et al.* (2011) reported that, the lipid content of pearl millet varied from 2.4% to 5.0%. While our investigation found that, 5.12% and 5.43% lipid content for Ashana and Hreahry cultivars respectively. Carbohydrate content in Ashana and Hreahry cultivars were found to be 73.55% and 69.02% respectively. These values of the available carbohydrates were higher than 67.67% and 68.55% reported by Eltayeb, (2006).

Stability studies: Shelf life study for pearl millet flour of two cultivars were carried out in which three parameters were selected and checked viz, Free fatty acid (FFA), peroxide value and total acidity. FFA of both the cultivars was presented in table 2. Which shows that, Ashana cultivar had significantly increased (P \leq 0.05) in the untreated flour sample at the storage of 0, 10, 30, 60 and 90 days and values recorded were 0.465, 0.640, 1.170, 1.700 and 3.030 %, respectively (Table 2). On the other hand, ascorbic acid treatment indicated lower value of FFA and significantly different than untreated flour at same days of analysis, were found to be 0.42, 0.54 0.79, 0.93 and 1.70 %, respectively. Treatment with BHT recorded lowest value compared with untreated flour and ascorbic acid treatment, whereas values obtained were 0.43, 0.52, 0.77, 0.90 and 1.09 %, respectively. While, Hreahry cultivar flour results were presented in Table 2. Which showed that FFA were significantly increased $(P \le 0.05)$ in the untreated flour sample at the 0, 10, 30, 60 and 90 days, were found to be 0.35, 0.71, 1.38, 1.53

Table 2. FFA (% oleic acid) of Hreahry cultivar flour as affected by the addition of antioxidants						
Storage period (days)	0	10	30	60	90	
Treatment						
	Hreahry cultivar flour					
Control	0.35 ± 0.02^{p}	0.71 ± 0.04^{lm}	1.38 ± 0.08^{f}	1.53 ± 0.13^{de}	3.17 ± 0.06^{a}	
BHT	$0.42 \pm 0.06^{\circ p}$	$0.45 \pm 0.04^{\text{op}}$	0.79 ± 0.08^{jkl}	$0.88\pm0.08^{\rm hijk}$	1.53 ± 0.08^{de}	
Ascorbic acid	$0.46 \pm 0.00^{\circ p}$	0.52 ± 0.01^{no}	$0.74 \pm .08^{\text{klm}}$	$0.96 \pm 0.01^{\text{hi}}$	$1.64 \pm 0.14^{\circ}$	
	Ashana cultivar flour					
Control	0.47 ± 0.02^{op}	0.64 ± 0.03^{lm}	$1.17 \pm 0.30^{\text{ef}}$	1.70 ± 0.06^{de}	3.03 ± 0.13^{a}	
BHT	$0.43 \pm 0.06^{\circ p}$	$0.52 \pm 0.01^{\text{op}}$	0.77 ± 0.06^{jk}	0.90 ± 0.01^{hijk}	1.09 ± 0.16^{de}	
Ascorbic acid	$0.42 \pm 0.06^{\circ p}$	0.54 ± 0.07^{no}	0.79 ± 0.07^{jkl}	0.93 ± 0.04^{hi}	$1.70 \pm 0.14^{\circ}$	
Mean values \pm SD. sharing same superscript(s) are not significantly different (P \leq 0.05)						

Table 3. Peroxide value (mEq/kg) of Ashana cultivar flour as affected by the addition of antioxidants						
Storage period (days)	0	10	30	60	90	
Treatment						
	Hreahry cultivar flour					
Control	$3.28 \pm 0.15^{\text{m}}$	5.24 ± 0.11^{jk}	7.95 ± 0.09 ^g	10.76 ± 0.39^{d}	17.07 ± 0.30^{a}	
BHT	3.63 ± 0.26^{m}	3.77 ± 0.21 ^m	5.72 ± 0.13 ^{ij}	8.55 ± 0.01^{f}	10.59 ± 0.14^{d}	
Ascorbic acid	3.49 ± 0.38^{m}	4.52 ± 0.05^{1}	6.16 ± 0.21 ⁱ	$8.78 \pm 0.07^{\rm f}$	12.88 ± 0.15°	
	Ashana cultivar flour					
Control	3.22 ± 0.09^{p}	4.95 ± 0.11 ⁿ	8.30 ± 0.16^{hi}	10.74 ± 0.35^{d}	16.69 ± 0.23^{a}	
BHT	3.14 ± 0.05^{p}	3.18 ± 0.14°	5.49 ± 0.45^{m}	7.99 ± 0.40^{ij}	10.72 ± 0.28^{d}	
Ascorbic acid	3.15 ± 0.04 ^p	4.07 ± 0.06°	6.15 ± 0.23 ⁿ	8.22 ± 0.28 ^{hi}	$12.06 \pm 0.00^{\circ}$	
Mean values \pm SD. sharing same superscript(s) are not significantly different (P \leq 0.05)						

and 3.17 %, respectively. On the other hand, ascorbic acid treatment indicated lower value and significantly different from the untreated flour at the same days of analysis, were found to be 0.46, 0.52, 0.74, 0.96 and 1.64 %, BHT treatment recorded lowest value compared with untreated flour and ascorbic acid treatment, were found to be 0.42, 0.45, 0.79, 0.88 and 1.53 %, respectively. A high FFA value is mainly due to hydrolytic changes associated with the action of lipolytic enzymes. Further, an increase in lipase activity during storage may have led to a significantly higher FFA value in control than the treated flour (Yadav et al., 2012).

Peroxide Value: Ashana cultivar flour were found to be increased significantly ($P \le 0.05$) in the untreated flour sample at the storage of 0, 10, 30, 60 and 90 days and values recorded were 3.22, 4.95, 8.30, 10.74 and 16.69 mEq/kg respectively as presented in table 3. On the other hand, ascorbic acid treatment indicated lower value of PV than untreated flour at same days of analysis, and it was observed 3.14, 3.18, 5.49, 7.99 and 10.72 mEq/kg, respectively. Treatment with BHT recorded lowest value compared with untreated flour and ascorbic acid treatment, whereas values obtained were 3.15, 4.07, 6.15, 8.22 and 12.06 mEq/kg, respectively. While, Hreahry cultivar flour results were mentioned in table 3. Which showed that, PV were significantly increased ($P \le 0.05$) in the untreated flour sample at the 0, 10, 30, 60 and 90 days, were found to be 3.27, 5.23, 7.94, 10.76 and 17.07 mEq/kg, respectively. Additionally, BHT treatment indicated significantly different from the untreated flour at the same days of analysis, and found to be 3.49, 4.51, 6.16, 8.77 and 12.88 mEq/kg, respectively. Furthermore, ascorbic acid treatment recorded lowest value compared with untreated flour and ascorbic acid treatment, were found to be3.62, 3.76, 5.71, 8.55 and 10.59 mEq/ kg respectively. PV increased significantly (P \leq 0.05) in control flour from 3.22 to 17.08 mEq/kg fat after 90 days of storage. The increasing trend of PV during storage was in agreement with the observation of Chaudhary and Kapoor, (1984).

Fat Acidity: In table 4 fat acidity changes during storage of the pearl millet flour at ambient temperature (37

Table 4. Fat acidity (mg KOH/ 100g) of Ashana cultivar flour as affected by the addition of antioxidants						
Storage period (days) Treatment	0	10	30	60	90	
	Hreahry cultivar flour					
Control	26.92± 0.29 ⁿ	$63.64 \pm 6.40^{\text{ghi}}$	$88.93 \pm 6.55^{\text{f}}$	125.20±18.46°	183.40±13.04 ^a	
ВНТ	21.99 ± 0.26^{n}	40.72 ± 0.78^{m}	51.22 ± 0.13^{jkl}	$67.71 \pm 0.84^{\text{gh}}$	111.10 ± 1.40^{d}	
Ascorbic acid	26.72± 0.57 ⁿ	48.72 ± 0.81^{klm}	53.05 ± 0.10^{jkl}	68.63 ± 0.71^{g}	127.80 ± 3.44°	
	Ashana cultivar flour					
Control	22.42 ± 0.40^{n}	$37.06 \pm 0.49^{\text{ghi}}$	95.41 ± 4.65^{f}	115.60±21.91°	186.50 ± 8.92^{a}	
BHT	24.01±2.98 ^m	29.57 ± 0.63^{m}	51.53±0.32 ^{jkl}	$65.51 \pm 1.12^{\text{gh}}$	118.30±5.52 ^{ac}	
Ascorbic acid	24.45± 3.03 ^m	34.79 ± 0.88^{klm}	58.17 ± 4.32^{lm}	72.54 ± 2.70^{hi}	125.10±4.26 ^{ad}	
Mean values \pm SD. sharing same superscript(s) are not significantly different (P \leq 0.05)						

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 \pm 4°C). A gradual, yet significant (P \leq 0.05) increase was observed in the fat acidity of the three treatments. The mean values of fat acidity at the 10 days of storage for untreated, BHT, ascorbic acid treatments were found to be 37.06, 29.57 and 34.79 mg KOH/ 100g, respectively and no significant differences ($P \le 0.05$) were found. At 60 days of storage, the values of fat acidity were found to be 115.60, 65.51 and 72.54 mg KOH/ 100g for untreated, BHT, ascorbic acid treatments, respectively and significant difference ($P \le 0.05$) were found between them. Untreated flour reported the highest values at all time during storage period, followed by ascorbic acid treatment and the BHT treatment recorded the lowest value. Similarly, Table 4 shows the changes in fat acidity during storage of the pearl millet flour at ambient temperature (37 \pm 4 °C).

Results at the begging of the storage were 26.92, 21.99 and 26.72 mg KOH/ 100g for untreated, BHT and ascorbic acid treatments, respectively and indicated no significant difference (P \leq 0.05). A gradual, yet significant (P \leq 0.05) increase was observed in the fat acidity of the three treatments. The mean values of fat acidity at the 30 days storage of the untreated, BHT, ascorbic acid treatments were 84.18, 44.82 and 48.46 mg KOH/ 100g, respectively and significant differences ($P \le 0.05$) were found between them, at 60 days of storage, the values of fat acidity were 125.20, 67.71 and 68.63 mg KOH/ 100g for untreated, BHT, ascorbic acid treatments, respectively and significant difference ($P \le 0.05$) were found between them. Untreated flour reported the highest values at all time during storage period, followed by ascorbic acid treatment and the BHT treatment recorded the lowest value. Jalgaonkar et al. (2016) reported that fat acidity of flour from the untreated grain increased from 30.3 to 123.7 mg KOH/100g. Additionally, Tiwari et al. (2014) found that fat acidity was above 30 mg KOH/100g in the untreated pearl millet flour.

CONCLUSION

Pearl millet flour has been well known staple food source which is not only providing major nutrients like protein, carbohydrate and fat but also have important vitamins and minerals. However, due to development of rancidity in pearl millet flours it has been not well accepted from the consumers. Based upon our results, we found that BHT and ascorbic acid treatments were able to reduce both hydrolytic and oxidative reaction. Furthermore BHT was found to be better than ascorbic acid treatment, in retardation of the lipid degradation in pearl millet flour. The BHT and ascorbic acid treatment was able to maintain the keeping quality of flour up to 30 and 30 days respectively. Our study would be useful for the scientist; miller, retail seller, as well as consumer, as utilization of this antioxidant will help to keep the quality of millet flour for longer duration. In addition to that, it would also encourage utilization of pearl millet grains, which is still untapped despite its various nutritious and therapeutic benefits.

ACKNOWLEDGEMENTS

We are grateful to the Department of Food Sciences, Faculty of Agriculture, University of Khartoum, Sudan and Department of Clinical Nutrition, College of Applied Medical Sciences, Hail University, Saudi Arabia for providing facilities to carrying out the present study.

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