



King Saud University

Journal of the Saudi Society of Agricultural Sciences

www.ksu.edu.sa
www.sciencedirect.com

ORIGINAL ARTICLE

Effect of traditional fermentation process on the nutrient and antinutrient contents of pearl millet during preparation of Lohoh

Magdi A. Osman

Food Science and Nutrition Dept., College of Food and Agricultural Sciences, King Saud University, P.O. Box 2460, Riyadh 11451, Saudi Arabia

Received 3 April 2010; accepted 5 June 2010

Available online 17 December 2010

KEYWORDS

Pearl millet;
Fermentation;
Antinutritional factors;
Amino acid;
Proximate composition;
Lohoh

Abstract Effect of traditional fermentation on the proximate composition, soluble sugars, amino acids, enzymes inhibitor activities, phytic acid, and tannins were investigated in the pearl millet flour during preparation of lohoh bread. During 24 h fermentation, protein and lipid contents were not significantly ($P > 0.05$) changed. Carbohydrate content significantly ($P > 0.05$) decreased with parallel increase in soluble sugars. Amino acid analysis revealed that fermentation significantly ($P > 0.05$) decreased glycine, lysine and arginine contents. Fermentation was found to cause significant reduction in trypsin and amylase inhibitors activities and the phytic acid content. Tannin content of the pearl millet showed significant ($P < 0.05$) increase after fermentation.

© 2010 King Saud University. Production and hosting by Elsevier B.V. All rights reserved.

1. Introduction

Pearl millet (*Pennisetum glaucum*) is a major food staple in semi-arid and arid lands of Africa and Asia. Pearl millet is well adapted to drought and sandy acid soil of low fertility. The

E-mail address: magdios@ksu.edu.sa

1658-077X © 2010 King Saud University. Production and hosting by Elsevier B.V. All rights reserved.

Peer review under responsibility of King Saud University.

doi:10.1016/j.jssas.2010.06.001



Production and hosting by Elsevier

crop ranks as world's fourth most tropical food cereal. The nutrient composition of pearl millet indicates that it is a good source of energy and protein (Gassem and Osman, 2008; Ouattara Cheik et al., 2006; Sawaya et al., 1984). Essential amino acid profile revealed that pearl millet is 40% richer in lysine and methionine and 30% richer in threonine than in protein of corn (Burton et al., 1972). Nutritional studies indicated that metabolized energy of pearl millet for non-ruminant animals is approximately equal to that of maize (Fancher et al., 1987). As with other cereals certain nutritional inhibitors, such as enzyme inhibitors, phytic acid and tannins are associated with pearl millet. These factors affect the nutritional value of the grain by inhibiting protein and starch digestibility and mineral bioavailability. Several methods have been employed to improve the nutritional quality of cereals. Fermentation is one of the oldest methods and widely used process of

cereals such as sorghum and millets. Many investigators have reported that fermentation can be effectively used for improving nutritional quality of cereal grain by increasing protein content and digestibility (Inyang and Zakari, 2008; Ali et al., 2003; El Hag et al., 2002) and available lysine content and relative nutritive value (Hamad and Fields, 1979). Fermentation was also found to decrease trypsin inhibitory activity (TIA), amylase inhibitor, activity, phytic acid, and tannins (Osman, 2004; Ejigui et al., 2005; Eltayeb et al., 2007; Abdel-Haleem et al., 2008).

In Saudi Arabia, the pearl millet is grown in South-West region of Jazan. The millet is used by locals to prepare fermented bread known as Lohoh. Lohoh is thick pancake-like livened bread that is similar to Injera a traditional Ethiopian bread made from sorghum. There is no information available regarding the nutritional quality of the bread. Therefore, the objective of this work was to investigate the effect of fermentation on proximate composition, soluble sugars, antinutrients content and amino acids of pearl millet flour used in the preparation of lohoh bread.

2. Materials and methods

2.1. Experiment materials

Pearl millet seeds were obtained from local grain market in Abu-Arish (Jazan region, Saudi Arabia). The seeds were carefully cleaned and freed from foreign materials.

2.1.1. Lohoh preparation

The fermentation was carried out in a traditional way used by housewives. Pearl millet flour was mixed with water in the ratio of 1:2 to make a dough (ajeen) and incubated at 30 °C for 24 h in a sterile covered flask, usually by this time the dough would have a good consistency and sour taste. The dough was fermented by adding 5% inoculate (starter) from previously fermented dough to start each subsequent batch. Fermentation was performed in duplicate and sampled every 4 h during the fermentation period. For chemical analysis and antinutrient factor, samples were dried in a vacuum oven at 50 °C [Heraucus LBS-Co]. The dried samples were milled to fine powder using a coffee miller then passed through a 60 mesh sieve and kept at 4 °C.

2.2. Methods

2.2.1. Chemical composition

Moisture, crude protein, fat, and ash of the samples were determined according to AOAC (1995) methods. Carbohydrates was calculated by difference. Glucose, fructose and maltose were determined using chromatographic procedure according to AOAC (1995).

2.2.2. Amino acids

Amino acid composition was determined by Spackman et al. (1958) method. Samples were hydrolyzed by transferring about 100 mg of sample into 15 ml ampoules, adding 5 ml 6.0 N HCl, sealing the vial under vacuum and digesting at 100 °C for 24 h. Amino acid analysis was performed with a Shimadzu HPLC Model HP 1090.

2.2.3. Antinutritional factors

The trypsin inhibitor activities of dried flour samples were determined according to the method of Kakade et al. (1969), using PAPNA (*N*-benzoyl-DL-arginine *p*-nitroanilide) as synthetic substrate. Amylase inhibitor activity was determined according to Deshpande et al. (1982). The phytic acid content was determined by the ion exchange method of Latta and Eskin (1980). Tannins content were determined using the vanillin – HPLC method of Price et al. (1978).

2.2.4. Statistical analysis

Data, expressed as mean value \pm standard deviation (SD) of three separate determinations. The data were statistically analyzed using one way ANOVA (Steel and Torri, 1980). Duncan's multiple range tests were used to compare means and significance was accepted at $P \leq 0.05$.

3. Results and discussion

3.1. Chemical composition

The changes in proximate composition and soluble sugars of fermented pearl millet flour are presented in Table 1. The obtained data showed no significant ($P \geq 0.05$) change in protein content in pearl millet dough due to fermentation. There was a gradual and insignificant ($P \geq 0.05$) decrease in protein content during the first 20 h of fermentation. At the end of fermentation (24 h) there was a significant ($P \geq 0.05$) increase in protein content compared to 16 and 20 h to initial level. This increase can be attributed to the loss of dry matter mainly carbohydrates. Our results were in agreement with those reported by Abdalla et al. (1998), who reported that the protein content was not significantly ($P \geq 0.05$) changed in pearl millet during 14 h fermentation. Similarly, Ali et al (2003) and Hassan et al (2006) reported a marginal change in protein content of fermented pearl millet. Contradictory reports on protein content in fermented pearl millet are also available. Elyas et al. (2002) and Inyang and Zakari (2008) reported that fermentation significantly increased protein content of pearl millet, while El Hag et al. (2002) observed a decrease in protein in fermented pearl millet. The same trend was observed with lipid, fermentation also was found to cause no change in lipid content of fermented pearl millet (Table 1). Similarly Kazanas and Fields (1981) did not observe any significant change in crude fat content of sorghum after natural lactic acid fermentation for 4 days. Little, have been reported, in literature on change in lipid in cereals during fermentation compared to protein and carbohydrates. This may be due to the fact that cereal grains are in general low in fat content. However, such studies are particularly important in pearl millet due to their high crude fat content compared to other cereal and its effect on flour and its products shelf-life. No definite trend was observed on ash content of fermented pearl millet flour. There was a significant decrease at 4 and 20 h followed by an increase to the initial level. Similar trends were observed in sorghum (Kazanas and Fields, 1981) and sorghum and green gram blend (Chavan et al., 1988). The total carbohydrate significantly decreased from 72.63% to 70.97% after 24 h of fermentation. Early stages of fermentation (4 h) did not change the carbohydrate content.

Table 1 Effect of fermentation on proximate composition and some sugars (on dry weight bases) of pearl millet.

Time (h)	Protein	Fat	Ash	Carbohydrate	Glucose	Fructose	Maltose
0	15.25 ± 0.21 ^{ab}	5.77 ± 0.25 ^a	1.80 ± 0.03 ^a	72.63 ± 0.56 ^a	6.83 ± 0.35 ^c	1.17 ± 0.06 ^a	1.50 ± 0.10 ^a
4	14.79 ± 0.10 ^{ab}	5.65 ± 0.01 ^a	1.57 ± 0.01 ^b	72.24 ± 0.00 ^{ab}	8.73 ± 0.12 ^d	0.73 ± 0.12 ^b	0.70 ± 0.10 ^b
8	14.99 ± 0.02 ^{ab}	5.75 ± 0.03 ^a	1.82 ± 0.10 ^a	71.65 ± 0.09 ^{bc}	9.40 ± 0.14 ^c	1.11 ± 0.12 ^a	ND
12	14.75 ± 0.36 ^{ab}	5.58 ± 0.06 ^a	1.84 ± 0.00 ^a	71.84 ± 0.01 ^b	10.75 ± 0.17 ^b	1.17 ± 0.06 ^a	ND
16	14.50 ± 0.42 ^b	5.53 ± 0.42 ^a	1.87 ± 0.01 ^a	72.05 ± 0.33 ^{ab}	11.41 ± 0.12 ^a	1.13 ± 0.16 ^a	ND
20	14.55 ± 0.35 ^b	5.60 ± 0.28 ^a	1.65 ± 0.04 ^b	72.66 ± 0.01 ^a	11.35 ± 0.05 ^a	1.20 ± 0.14 ^a	ND
24	15.35 ± 0.35 ^a	5.79 ± 0.03 ^a	1.79 ± 0.10 ^a	70.97 ± 0.37 ^c	7.30 ± 0.58 ^c	0.64 ± 0.17 ^b	ND

Means in the same column with different letters are significantly ($P < 0.05$) different.

Mean ± standard deviation of three determinations.

Between 8 and 12 h, there was a significant ($P \geq 0.05$) decrease in total carbohydrate and a corresponding increase in soluble sugars. As the period of fermentation was further prolonged, there was an unexpected, gradual increase of total carbohydrates to the initial level. However at the end of fermentation (24 h) there was a significant ($P \geq 0.05$) decrease in carbohydrates level probably due to the utilization by microorganisms. The same general trend has been reported for sorghum (El-Tinay et al., 1979; Kazanas and Fields, 1981; Chavan et al., 1988). The initial drop in carbohydrate content was attributed to the action of microbial α - and β -amylase, whereas the increase at 16 and 20 h could be due to the termination of starch degradation by low pH which inhibits amylase activity (El-Tinay et al., 1979), and/or to the presence of tannins that inhibit amylolytic enzymes. The analysis of soluble sugars revealed that glucose is the major soluble sugar in pearl millet. There was a gradual significant ($P \geq 0.05$) increase in glucose content during the first 20 h of fermentation. At the end of fermentation there was a sharp decrease in glucose content. The increase in glucose content during the first 16 h and the corresponding decrease in total carbohydrate may be due to the increase in microbial amylase activity. The sharp decrease in glucose content at the end of fermentation could be due to utilization of glucose by the microorganisms. Similar to glucose, fructose content significantly ($P \geq 0.05$) decreased at end of fermentation. Between 8 and 20 h there was a slight and insignificant increase in fructose content. After 24 h there was significant ($P \geq 0.05$) decrease in fructose content from 1.17 g/100 g to 0.64 g/100 g, (45.3% decrease). Similar results were reported in wheat, oats, rice and sorghum (Hamad and Fields, 1979), finger millet (Sripriya et al., 1996) and pearl millet (Khetarpaul and Chauhan, 1990). The increase and the decrease in reducing sugars during lactic acid fermentation of pearl millet could be attributed to the action of microflora during fermentation (Khetarpaul and Chauhan, 1990). Maltose content decreased very rapidly from 1.5 g/100 g to 0.70 g/100 g at 4 h. At the 8 h fermentation no trace of maltose was evident in fermented pearl millet, indicating that maltase is preferentially metabolized by the microorganisms. Similar findings were reported during the traditional process of converting pearl millet into ben-saalga, a fermented gruel in Burkina Faso (Tou et al., 2007).

3.2. Amino acids

The change in amino acids content during the fermentation of pearl millet is presented in Table 2. A significant ($P \leq 0.05$) decrease in lysine and arginine was observed after 12 h of

fermentation of pearl millet. Fermentation for 24 h significantly ($P \geq 0.05$) decreased glycine, lysine and arginine content. However, the fermentation did not affect the content of other amino acids. The data on the effect of fermentation on amino acids in the present study conflict with the earlier studies by Hamad and Fields (1979), who observed a significant increase in lysine during 6 days fermentation of oats, rice, millet and corn. Similar results were also obtained for sorghum, corn and corn plus soy blends (Wang and Fields, 1978; Kazanas and Fields, 1981), this may be due to short fermentation period used in this study.

3.3. Trypsin inhibitory activities

The effect of traditional fermentation process on trypsin inhibitory activity (TIA), amylase inhibitor activity (AIA) phytic acid, and tannins is presented in Table 3. During 24 h fermentation trypsin inhibitor activity was significantly ($P \leq 0.05$) reduced. Generally, it decreased from 7.33 to 6.65 (9%). A reduction in TIA during natural lactic acid fermentation of cereals has been reported by several investigators. Ejigui et al. (2005) reported 41.7% reduction in TIA in yellow maize during 4 days fermentation in controlled environmental chamber. Similarly, Osman (2004) observed 37–58% decrease in

Table 2 Effect of fermentation on amino acid profile of pearl millet (g/16 g N).

Amino acids	0 h	12 h	24 h
Aspartic acid	7.94 ± 0.00 ^a	7.87 ± 0.21 ^a	7.57 ± 0.37 ^a
Threonine	3.68 ± 0.02 ^a	3.58 ± 0.11 ^{ab}	3.34 ± 0.15 ^b
Serine	4.56 ± 0.09 ^a	4.47 ± 0.02 ^a	4.32 ± 0.23 ^a
Glutamic acid	23.73 ± 0.01 ^a	23.21 ± 0.92 ^a	22.54 ± 1.31 ^a
Praline	4.82 ± 0.12 ^a	4.59 ± 0.09 ^a	4.51 ± 0.08 ^a
Glycine	2.72 ± 0.03 ^a	2.60 ± 0.08 ^{ab}	2.48 ± 0.04 ^b
Alanine	8.53 ± 0.01 ^a	8.48 ± 0.37 ^a	8.31 ± 0.39 ^a
Valine	5.41 ± 0.02 ^a	5.39 ± 0.28 ^a	5.05 ± 0.08 ^a
Methionine	1.43 ± 0.30 ^a	1.53 ± 0.10 ^a	1.47 ± 0.02 ^a
Isoleucine	4.34 ± 0.08 ^a	4.28 ± 0.21 ^a	4.11 ± 0.24 ^a
Leucine	11.34 ± 0.09 ^a	11.15 ± 0.49 ^a	10.76 ± 0.55 ^a
Tyrosine	2.52 ± 0.15 ^a	2.54 ± 0.08 ^a	2.58 ± 0.04 ^a
Phenylalanine	5.41 ± 0.01 ^a	5.31 ± 0.18 ^a	5.44 ± 0.42 ^a
Histidine	2.25 ± 0.01 ^a	2.16 ± 0.11 ^a	2.00 ± 0.10 ^a
Lysine	2.06 ± 0.03 ^a	1.85 ± 0.04 ^b	1.78 ± 0.04 ^b
Arginine	4.28 ± 0.01 ^a	3.58 ± 0.13 ^b	3.28 ± 0.09 ^c

Means in the same row with different letters are significantly ($P < 0.05$) different.

Mean ± standard deviation of two determinations.

Table 3 Changes on antinutritional factors of pearl millet during fermentation.

Time (h)	Trypsin inhibitor (TIA)	Amylase inhibitor (AIA)	Phytic acid (mg/100 g)	Tannins % as catechin equivalent
0	7.33 ± 0.01 ^a	80.16 ± 0.46 ^a	647.00 ± 27.01 ^a	0.010 ± 0.004 ^c
4	7.03 ± 0.00 ^b	56.82 ± 0.45 ^b	535.85 ± 10.54 ^b	0.027 ± 0.002 ^d
8	6.95 ± 0.01 ^{cb}	56.48 ± 0.92 ^b	456.95 ± 2.33 ^c	0.036 ± 0.002 ^{abc}
12	6.95 ± 0.03 ^{cb}	46.42 ± 0.00 ^c	348.25 ± 5.87 ^d	0.041 ± 0.001 ^a
16	6.90 ± 0.03 ^c	47.72 ± 0.00 ^c	342.45 ± 14.07 ^{cd}	0.041 ± 0.002 ^a
20	6.76 ± 0.11 ^d	45.29 ± 3.44 ^c	319.22 ± 7.00 ^{ed}	0.030 ± 0.001 ^{cd}
24	6.65 ± 0.04 ^d	39.45 ± 2.52 ^d	310.95 ± 7.00 ^e	0.035 ± 0.004 ^{cb}

Means in the same column with different letters are significantly ($P < 0.05$) different.

Mean ± standard deviation of three determinations.

TIA in three sorghum cultivars after 24 h fermentation. A decrease in TIA level has also been reported for corn and corn soybean blend (Chompreeda and Fields, 1981) and in millet based-gruel (Yassmin and Pattabiraman, 1988). The lower than expected reduction in TIA (9.0%) indicate the need for longer fermentation period or may due to increase of tannin content during fermentation. Trypsin inhibitor have been implied to be one of the factors responsible for reducing protein digestibility, pancreatic hypertrophy and poor growth performance in rats, mice and chicks (Gertler et al., 1967; Gumbmann et al., 1989; Osman et al., 2003). Therefore, reduction of TIA may be useful in improving nutritional quality of pearl millet with respect to protein digestibility.

3.4. Amylase inhibitory activity

Data on the effect of fermentation on amylase inhibitor activity (AIA) of pearl millet (Table 3) indicated that as the period of fermentation increases a significant ($P \leq 0.05$) decrease in AIA occurred. After 24 h, the amylase inhibitor level reduced from 80.16 to 39.45 (50.8%). Sharma and Kapoor (1996) observed almost complete removal of AIA during 48 h fermentation of pearl millet. Such pattern were also reported on sorghum (Osman, 2004), and yellow corn (Ejigui et al., 2005). The reduction in amylase inhibitor could be attributed to the microbial degradation during fermentation. The fermenting organisms have found to process metabolic pathway that degrade and utilize these compounds, that is often lacking in human (Chavan and Kadam, 1989). Other processes such as heat treatment (Mulimani and Supriya, 1993), germination (Sathe et al., 1983), extrusion (Alonso et al., 2000) and radiation (Sidduraju et al., 2002) were also found to reduce amylase inhibitor level. The reduction or elimination of AIA may be useful for improving carbohydrate utilization of pearl millet.

3.5. Phytic acid content

During 24 h fermentation of pearl millet, phytic acid content significantly ($P \leq 0.05$) decreases from 647.0 to 310.95 mg/100 g (51.9% reduction) (Table 2). These results were similar to those observed for pearl millet (Eltayeb et al., 2007; Abdel-Rahaman et al., 2005; El Hag et al., 2002), sorghum (Abdel-Haleem et al., 2008; Kayode et al., 2007; Towo et al., 2006; Osman, 2004), rice (Liang et al., 2008), corn (Lopez et al., 1983), and maize (Abedel-Hady et al., 2005; Ejigui et al., 2005). The decrease in phytic acid content may be attributed to microbial phytase and endogenous pearl millet phytic.

3.6. Tannins content

Data on the effect fermentation on tannin content of pearl millet is shown in Table 3. In contrast to enzymes inhibitors and phytic acid, tannin content significantly ($P \leq 0.05$) increased with increase in fermentation period. Significant ($P \geq 0.05$) increase in tannin was first observed after 8 h of fermentation. Between 8 and 16 h, there was a slight and insignificant ($P \leq 0.05$) increase in tannin content. After 20 h significant decrease in tannin was observed compared to 16 h, but significantly higher than initial content Overall the tannin content increased from 0.010 to 0.035 (350%). Similar trend was observed during the brewing process of opaque sorghum beer (Kayode et al., 2007). Abdel-Rahaman et al. (2005) observed a significant decrease in tannin content of pearl millet during 8 h of natural fermentation. However, when the fermentation was continued up to 12 h there was a gradual and significant increase in tannin content. Contrary to our study, tannin content was reported to decrease during natural lactic acid fermentation in pearl millet, sorghum, and maize (El Hag et al., 2002; Osman, 2004; Abdel-Haleem et al., 2008; Abedel-Hady et al., 2005). The increase in tannin content could be attributed to hydrolysis of condensed tannins such as proanthocyanidin. While the decrease may be due to their binding with cotyledon endosperm that are usually undetected by routine method due to their insolubility in solvent (Emambux and Taylor, 2003) or may be due to microbial phenyl oxidase action.

4. Conclusion

The fermentation process was found to cause no changes in protein, lipid and ash contents, but it significantly reduced carbohydrate content. The change in amino acids was varied, glycine, lysine and arginine were significantly decreased during fermentation. Fermentation process significantly reduced the enzymes inhibitors and phytic acid whereas tannin content increased. This increase in tannins content may adversely affect the nutritional quality of lohoh bread.

Acknowledgements

This study was funded by the College of Food and Agricultural Sciences Research Center at King Saud University. The authors wish to thank Mr. Abu Bakar ALhady for his technical assistance.

References

- Abdalla, A.A., El Tinay, A.H., Mohamed, B.E., Abdalla, A.H., 1998. Effect of traditional processes on phytate and mineral content of pearl millet. *Food Chem.* 63, 79–840.
- Abdel-Haleem, W.H., El Tinay, A.H., Mustafa, A.I., Babiker, E.E., 2008. Effect of fermentation, malt-pretreatment and cooking on antinutritional factors and protein digestibility of sorghum cultivars. *Pakistan J. Nutr.* 7, 335–341.
- Abdel-Rahaman, S.M., Babiker, E.E., El-Tinay, A.H., 2005. Effect of fermentation on antinutritional factors and HCl extraction of minerals of pearl millet cultivars. *J. Food Technol.* 3 (4), 516–522.
- Abdel-Hady, A.S., Hassan, A.B., Ali, I.M., Babiker, E., 2005. Antinutritional factors content and availability of protein, starch and mineral of maize (*Zeamays linmanus*) and lentil (*Lens culinaris*) as influenced by domestic processing. *J. Food Technol.* 3, 523–528.
- Ali, A.M., El-Tinay, H.A., Abdalla, H.A., 2003. Effect of fermentation on the *in vitro* digestibility of pear millet. *Food Chem.* 80, 51–54.
- Alonso, R., Aguirre, E.A., Marzo, F., 2000. Effect of extrusion and traditional processing methods, on antinutritional and *in vitro* digestibility of protein and starch in faba and kidney beans. *Food Chem.* 68, 159–165.
- AOAC, 1995. Official Methods for Analysis, 16th ed. Association of Official Analytical Chemists, Arlington VA, USA.
- Burton, G.W., Wallace, A.T., Rachile, K.O., 1972. Chemical composition and nutritive value of pearl millet. *Crop Sci.* 12, 187–188.
- Chavan, J.K., Kadam, S.S., 1989. Nutritional improvement of cereals by fermentation. *Crit. Rev. Food Sci. Nutr.* 28, 349–400.
- Chavan, V.D., Chavan, J.K., Kadam, S.S., 1988. Effect of fermentation on soluble protein and *in-vitro* protein digestibility of sorghum, green gram and sorghum green gram blends. *J. Food Sci.* 53, 1573–1574.
- Chompreeda, P.T., Fields, M.L., 1981. Effect of heat and fermentation on extractability of minerals from soy bean meal and corn meal blends. *J. Food Sci.* 49, 566–570.
- Deshpande, S.S., Sathe, S.K., Salunkhe, D.K., Cornforth, D.P., 1982. Effect of dehulling on phytic acid, polyphenol and enzymes inhibitors of dry beans (*Phaseolus vulgaris* L.). *J. Food Sci.* 47, 1846–1850.
- Ejigui, J., Savoie, L., Marin, J., Desrosiers, T., 2005. Beneficial changes and drawbacks of a traditional fermentation process on chemical composition and antinutritional factors of yellow Maize (*Zea mays*). *J. Biol. Sci.* 5 (5), 590–596.
- EL Hag, M.E., El Tinay, A.H., Yousif, N.E., 2002. Effect of fermentation and dehulling on starch, total polyphenols, phytic acid and *in vitro* digestibility of pearl millet. *Food Chem.* 77, 193–196.
- Eltayeb, M.M., Hassan, A.B., Sulieman, M.A., Babiker, E.E., 2007. Effect of processing followed by fermentation on antinutritional factors content of pearl millet (*Pennisetum glaucum* L.) cultivars. *Pakistan. J. Nutr.* 6, 463–467.
- El-Tinay, A.H., Abdel Gadir, A.M., El-Hidi, M., 1979. Sorghum fermented Kisra bread. 1. Nutritional value of Kisra. *J. Sci. Food Agric.* 30, 859–862.
- Elyas, S.H., El-Tinay, A.H., Yosif, N.E., Elsheikh, E.A., 2002. Effect of fermentation on nutritive value and *in vitro* protein digestibility of pearl millet. *Food Chem.* 78, 75–79.
- Emambux, M.N., Taylor, J.N., 2003. Sorghum kafirin interaction with various phenolic compounds. *J. Sci. Food Agric.* 83, 402–407.
- Fancher, B.I., Jensen, L.S., Smith, R.L., Hana, W.W., 1987. Metabolize energy content of pearl millet. *Poult. Sci.* 66, 1693–1696.
- Gassem, M.A., Osman, M.A., 2008. Chemical composition and nutritional potential of pearl millet grown in Jazan region of Saudi Arabia. *J. Saudi Soc. Agric. Sci.* 7, 1–22.
- Gertler, A., Birk, Y., Bondi, A., 1967. A comparative study of the nutritional and physiological significance of pure soybean trypsin inhibitor and ethanol extract soybean meals in chicks and rats. *J. Nutr.* 91, 358–370.
- Gumbmann, M.R., Dugan, G.M., Spangler, W.L., Baker, E.C., Rackis, J.J., 1989. Pancreatic response in rats and mice to trypsin inhibitor from soybean and potato after short and long term dietary expose. *J. Nutr.* 119, 1598–1609.
- Hamad, M.A., Fields, M.L., 1979. Evaluation of protein quality and available lysine of germinated and fermented cereals. *J. Food Sci.* 44, 456–460.
- Hassan, A.B., Mohamed Ahmed, I.A., Osman, N.M., Eltayeb, M.M., Osman, G.A., Babiker, E.E., 2006. Effect of processing treatment followed by fermentation on protein content and digestibility of pearl millet (*Pennisetum typhoideum*) cultivars. *Pakistan J. Nutr.* 5 (1), 86–89.
- Inyang, C.U., Zakari, U.M., 2008. Effect of germination and fermentation of pearl millet on proximate, chemical and sensory properties of instant “Fura–Nigerian cereal food. *Pakistan J. Nutr.* 7, 9–12.
- Kakade, M.L., Simons, N., Liener, I.E., 1969. An evaluation of natural vs synthetic substrate for measuring the antitryptic activity of soy bean samples. *Cereal Chem.* 49, 518–526.
- Kayode, A.P., Hounhougigan, J.D., Nout, M.J., 2007. Impact of brewing process operation on phytate, phenolic compounds and *in vitro* solubility of iron and zinc in opaque sorghum beer. *LWT* 40, 834–841.
- Kazanas, N., Fields, M.L., 1981. Nutritional improvement of sorghum by fermentation. *J. Food Sci.* 6, 819–821.
- Khetarpaul, N., Chauhan, B.M., 1990. Effect of germination and fermentation on available carbohydrates content of pearl millet. *Food Chem.* 38, 21–26.
- Latta, M., Eskin, M., 1980. A colorimetric method, for phytate determination. *J. Agric. Food Chem.* 28, 1313–1315.
- Liang, J., Han, B., Nout, M.J., Hamer, R.J., 2008. Effect of soaking, germination and fermentation on phytic acid, total and *in vitro* soluble zinc in brown rice. *Food Chem.* 110, 821–828.
- Lopez, Y., Gordon, D.T., Fields, M.L., 1983. Release of phosphorus from phytate by natural lactic fermentation. *J. Food Sci.* 48, 953–954.
- Mulimani, V.H., Supriya, D., 1993. Alpha amylase inhibitor activity in sorghum grain. Effect of cooking and UV radiation. *J. Food Sci. Technol.* 30, 321–323.
- Osman, M.A., 2004. Changes, in sorghum enzyme inhibitors, phytic acid, tannins, and *in vitro* protein digestibility occurring during Khamir (local bread) fermentation. *Food Chem.* 88, 129–134.
- Osman, M.A., Reid, M.R., Weber, C.W., 2003. The effect of feeding tepary bean (*Phaseolus acutifolius*) proteinase inhibitors on the growth and pancreas of young mice. *Pakistan J. Nutr.* 2, 111–115.
- Ouattara Cheik, A.T., Aly, S., Yaya, B., Alfred, T.S., 2006. A comparative study on nutritional and technological quality of fourteen cultivars of pearl millets (*Pennisetum glaucum* (L) Leek} in Burkino Faso. *Pakistan J. Nutr.* 5 (6), 512–521.
- Price, M.L., Socoyoc, S.V., Butler, L.G., 1978. A critical evaluation of the vanillin reaction as an assay for tannin in sorghum grain. *J. Agric. Food Chem.* 26, 1214–1218.
- Sathe, S.K., Deshpande, S.S., Reddy, N.R., Goll, D.E., Slunkhe, D.K., 1983. Effect of germination on protein, raffinose, oligosaccharides and antinutritional factor in the great northern beans (*Phaseolus vulgaris* L.). *J. Food Sci.* 48, 1796–1800.
- Sawaya, N.W., Khalil, K.J., Safi, J.W., 1984. Nutritional quality of pearl millet flour and bread. *Qual. Plant Food Hum. Nutr.* 34, 117–125.
- Sharma, A., Kapoor, A.C., 1996. Levels of antinutritional factors in pearl millet as affected by processing treatment and various types of fermentation. *Plant Food Hum. Nutr.* 49, 241–252.
- Sidduraju, P., Osoniyi, O., Makkar, H.P.S., Becker, K., 2002. Effect of soaking and ionizing on various antinutritional factors of seeds from different species of an unconventional legumes, Sesbana and common legume, green gram (*Vigna radiate*). *Food Chem.* 79, 273–281.

- Spackman, D.H., Stein, W.H., Moore, S., 1958. Automatic recording apparatus for use in chromatography of amino acids. *Anal. Chem.* 30, 1190–1199.
- Sripriya, G., Antony, U., Chandra, T.S., 1996. Changes in carbohydrate, free amino acids, organic acids, phytate and HCL extractability of mineral during germination and fermentation of finger millet (*Eleusine coracana*). *Food Chem.* 58, 345–350.
- Steel, R.G., Torri, J.H., 1980. Principles and procedures of statistics. McGraw-Hill Book, Co., Inc., New York, USA.
- Tou, E.H., Mauquet-Rivier, C., Picq, C., Traore, A.S., Treche, S., Guyot, J.P., 2007. Improving the nutritional quality of ben-saalga, a traditional fermented millet-based gruel, by co-fermenting with groundnut and modifying the process method. *LWT* 40, 1561–1569.
- Towo, E., Matuschek, E., Svanberg, U., 2006. Fermentation and enzyme treatment of tannin sorghum gruels: effect on phenolic compounds, phytate and in vitro accessible iron. *Food Chem.* 94, 369–376.
- Wang, Y.D., Fields, M.L., 1978. Feasibility of home fermentation to improve the amino acid balance of corn meal. *J. Food Sci.* 43, 1104–1109.
- Yassmin, M., Pattabiraman, T.N., 1988. Changes in protease inhibitory activity in plant seeds on heat processing. *J. Sci. Food Technol.* 25, 59–62.