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Changes in chemical composition and total energy as affected by fermentation and/or cooking of pearl millet flour supplemented with Moringa or fenugreek seeds flour

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Abstract

The effect of fermentation followed by cooking of pearl millet flour (PMF) supplemented with defatted Moringa flour (DMF) or defatted fenugreek flour (DFF) on chemical composition and total energy was investigated. Defatted freeze-dried seeds powder of Moringa or fenugreek was added at different levels (5, 10 and 15%) to PMF using Pearson square.PMF, DMF and DFF had varying chemical compositions and total energy with DMF exhibiting significantly ($p<0.05$) higher crude protein (CP) and total energy contents than PMF and DFF. The dry matter (DM), ash, CP and crude fiber contents were significantly ($p<0.05$) increased after supplementation with DMF but the total energy content was decreased. However, supplementation with DFF significantly ($p<0.05$) increased the chemical compositions and total energy except crude fiber and carbohydrate. The chemical compositions and total energy varied among the different levels of supplementation. Fermentation of both raw and DMF supplemented millet dough significantly ($p<0.05$) increased the CP and oil contents. However the CP and carbohydrate content were significantly ($p<0.05$) increased after fermentation of DFF supplemented millet dough with highest values obtained after 16 h fermentation. Cooking of raw millet dough and DMF and DFF supplemented dough improved the ash, CP and crude fiber contents while other chemical compositions and total energy content fluctuates. Fermentation followed by cooking of PMF supplemented with DFF and DMF improved the chemical compositions while the total energy content was not significantly affected. on the antioxidant compounds and antioxidant capacities of seaweed. Sargassum polycystum portrayed the most antioxidant compounds (37.41 ± 0.01 mg GAE/g DW and 4.54 ± 0.02 mg CE/g DW) and capacities (2.00 ± 0.01 µmol TEAC/g DW and 0.84 ± 0.01 µmol TEAC/g DW) amongst four species of seaweed.

Introduction

Traditional cereal foods are essential in the diet of people in developing world particularly in cereal producing areas like Africa. Flour from various cereals is one of the major raw materials used in the production of popular food products with high acceptability, good storage characteristics and cheap cost (Mbata *et al*., 2009). Pearl millet (*Pennisetum glaucum*) is a main food staple in semi-arid and arid lands of Africa and Asia. Pearl millet is well adapted to drought and sandy acid soil of poor fertility. According to previous researchers (Sawaya *et al*., 1984; Ouattara-Cheik *et al*., 2006; Gassem and Osman 2008), pearl millet is the world's fourth most tropical food cereal. It is used mainly for human food and remains a major source of energy and vital component of food security in the semi-arid areas in the developing world (AbdelRahaman *et al*., 2007). Due to the great importance of millet as a basic staple food for majority of people in developing countries, and its low nutritional value, mainly with respect to protein quality, several methods (irradiation, domestic processing and fortification) have been carried out to improve the biological utilization of millet flour (Hassan *et al*., 2006, Ali *et al*., 2009a, 2009b; Mohamed *et al*., 2010).

Fermentation is one of the methods used in traditional processing of cereal based foods consumed in Africa and it is known to be an effective method of improving the starch and protein digestibility (Ali *et al*., 2003) and bioavailability of minerals (Chauhan *et al*., 1986). It also results in the breakdown of some of anti-nutritional endogenous compounds (Ahmed *et al*., 2006). Other processing method such as cooking has been reported to lower the levels of antinutrients

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in pearl millet thereby increasing the minerals availability (AbdelRahman *et al*., 2005).

Like other cereal, millet is characterized by its poor nutritional quality due to its lower concentration of some essential amino acids such as lysine and methionine. Consequently, there is a growing interest to improve the protein quality in cereal grains by different fortification methods. Therefore, researches have been conducted to fortifying such cereals with legumes to improve their nutritional quality and increase their acceptability (Ali *et al*., 2009a). Legumes are low in methionine and cysteine, but rich in lysine, so combining the two together leveled the protein content of both (Ali *et al*., 2009b). The high lysine content of legumes improves the nutritional quality of cereals by complementing their limiting amino acids (Annan and Plahar, 1995). Legumes are inexpensive and readily available source of high quality protein compared to animal protein (Annan and Plahar, 1995).

Moringa (*Moringa oleifera*) seeds have high protein content, which can be utilized to solving worldwide malnutrition or under nutrition problems by nutritionists and community health cautious persons (Fahey, 2005). According to Anwar and Rashid (2007), the seeds are also rich in essential amino acids, minerals, vitamins, oil, antioxidants. Fenugreek has high protein, fiber, ash, carbohydrate and mineral contents (Patil *et al*., 1997; Nikman *et al*., 2003). Hooda and Jood (2004) reported that the addition of 10% of fenugreek flour to wheat flour increased protein content, fiber, total calcium and total iron of the wheat. Therefore moringa and fenugreek seeds can be incorporated into cereals as supplement for improving the nutritional value of cereals. The aim of this study was to investigate the effect of fermentation followed by cooking of pearl millet flour supplemented with defatted moringa or fenugreek seeds flour on the chemical composition and total energy contents.

Materials and Methods

Sample preparation

Grain sample of pearl millet seeds (*Pennisetum glaucum L.*) was obtained from Department of Agronomy, Faculty of Agriculture, University of Khartoum. The seeds were cleaned, freed from foreign, broken and shrunken seeds, milled into fine flour using house blender and mortar to pass through a 0.4 mm screen and then stored in polyethylene bags at 4°C for further analysis. Moringa (*Moringa oliefera*) and fenugreek seeds (*Trigonella foenum-graecum L.*) were brought from a local farm, cleaned, freed from extraneous matter. The flour was defatted with cold (4°C) acetone (flour to solvent ratio 1:5 w/v) with constant magnetic stirring provided for 4 h and dried in a freeze dryer (12525, VirTis company, Gardiner, New York), milled into fine powder to pass a 0.4 mm screen and stored in polyethylene bags at 4°C for further analysis.

Supplementation of millet flour

Both defatted Moringa and fenugreek seeds flours were added individually to millet flour using Pearson square to increase nutritive value of millet flour by 5, 10 and 15%. All chemicals used in this study were of reagent grade.

Fermentation of millet flour and supplements

Natural fermentation of millet flour and composite flours was carried out by mixing the flour with distilled water (1:2 w/v). About 250 gm of each sample were mixed with 500 ml distilled water in 750 ml beaker and incubated (Gallenkamp, England) at 37°C for periods 0, 8 and 16 h. After the incubation periods the samples were mixed using a glass rod and transferred to aluminum dishes (30 cm diameter), and dried in a freeze drier (12525, VirTis company, Gardiner, New York). Dried samples were ground to pass through 0.4 mm screen and stored at 4°C for further analysis.

Cooking of millet flour and supplements

Slurry of the fermented dough of each sample was cooked for 10 minutes, cooled and dried in a freeze drier (12525, VirTis company, Gardiner, New York). The dried flakes were milled into fine flour, passed through a 0.4 mm screen and stored at 4°C for further analysis.

Chemical composition of millet flour and supplements

The ash, crude protein, fiber, oil and dry matter contents of moringa, fenugreek, and millet flour with or without supplements were determined according to AOAC methods (AOAC, 1995). Protein was calculated as N% × 6.25 (Methods984.13). Ash content was determined by ashing the samples at 550ºC overnight (Methods 942.05). Crude fiber content was determined according to the acid/alkali digestion method (Methods 962.09). Fat content was determined by ether extraction method using Soxhlet apparatus (Methods 920.39). Dry matter was determined following the removal of moisture content at 105ºC (Methods 934.01). Total carbohydrate of the samples was calculated by subtracting the value of protein, oil, fiber, ash and moisture content from 100. The energy values of samples were calculated

Table 1.Chemical composition (%) and total energy (kcal) of Pearl millet, defatted Moringa and fenugreek seeds flours.

Chemical composition	Dry matter	Ash content	Crude protein	Oil content	Crude fiber	Carbohydrate	Total energy
Millet	92.56(±0.33)[b]	1.54(±0.05)[a]	12.29(±0.10)[c]	5.87(±0.79)[b]	1.29(±0.04)[c]	69.57(±0.04)[a]	388.27(±4.75)[a]
Moringa seeds	93.69(±0.07)[a]	1.33(±0.03)[a]	63.41(±0.40)[a]	1.18(±0.15)[c]	4.68(±0.24)[b]	23.08(±0.31)[c]	356.58(±1.67)[c]
Fenugreek	93.43(±0.27)[a]	0.24(±0.01)[b]	30.33(±0.41)[b]	7.18(±0.25)[a]	7.31(±0.09)[a]	48.86(±0.31)[b]	381.40(±2.51)[b]

Values are means (±SD) of triplicate samples. Means having different superscript letter within a column are significantly different at p< 0.05.

as follows: protein (4kcalg^{-1}), oil (9 kcalg^{-1}) and carbohydrates (4 kcalg^{-1}).

Statistical analysis

All data were subjected to statistical analysis, each determination was carried out and analyzed in triplicate and figures were then averaged. Data was assessed by the analysis of Variance (ANOVA) (Gomez and Gomez, 1984). Duncan Multiple Range Test (DMRT) was used to separate means. Significance was accepted with $p<0.05$.

Results and Discussion

Chemical composition and total energy of PMF, DMF and DFF

The chemical composition and total energy of pearl millet (PMF), defatted Moringa (DMF) and fenugreek (DFF) seeds flour are presented in Table 1. The dry matter (DM) content of PMF (92.56%) was significantly ($p<0.05$) lower than that of DMF (93.69%) and DFF (93.43%) seeds. The DM of PMF in this study was higher than that reported by Elyas *et al*. (2002) for two pearl millet cultivars (93.6%) while the DM of DMF and DFF were lower than that reported by Ijarotimi *et al*. (2013) and Mahmoud *et al*. (2012).

The crude protein (CP) content of the samples ranged between 12.29 and 63.41%, with PMF having significantly ($p<0.05$) lowest value and DFF having highest one. PMF had lower CP than that of pearl millet flour (15.25%) reported by Osman (2011). The CP of DFF and DMF obtained in this study were higher than that reported by Abiodun *et al*. (2012). The higher values were as a result of the displacement of oil from the seed flour thereby increasing other parameters such as protein. The high CP of these defatted flours gives an indication of their usefulness for food fortification of cereals.

The oil contents of PMF, DMF and DFF were 5.87, 1.18 and 7.18%, respectively, ($p<0.05$). DMF had lower oil contents compared to Moringa cake flour (3.06%) reported by Abiodun *et al*. (2012) while the oil contents of DFF was comparable with that reported by Mahmoud *et al*. (2012). The oil content of PMF obtained in this study agrees with that of pearl millet flour reported by Osman (2011). Although cereal grains are generally low in fat content but studies have shown particular interest in pearl millet due to its high crude fat content compared to other cereal and its effect on flour and its products shelf-life (Osman, 2011). PMF (1.29%) had lower crude fiber than DMF (4.68%) and DFF (7.31%). The crude fiber of PMF obtained was lower than that of two pearl millet cultivars studied by Elyas *et al*. (2002). Reports have shown that increase in fiber consumption may help to reduce the incidence of certain diseases such as diabetes, colon cancer and various indigestive disorders (Augustin *et al*., 1978).

The carbohydrate content of the samples ranged from 23.08 to 69.57% with DMF exhibiting the lowest value. However the value obtained for DMF was higher than that of Moringa cake reported by Abiodun (2012). The carbohydrate content of DFF was similar to that reported by Mahmoud *et al*. (2012) for fenugreek seeds (50.06%). On the other hand, PMF (388.27%) had significantly ($p<0.05$) higher total energy content compared to DFF (381.40 kcal) and DMF (356.58 kcal).

Effect of fermentation followed by cooking on chemical composition and total energy of PMF supplemented with DMF

Supplementation of PMF with DMF significantly ($p<0.05$) increased the DM content of the composite flour from 92.56% to 94.72, 94.30 and 93.67% at 5, 10 and 15% supplementation levels, respectively (Table 2). The increment in DM of raw PMF after

Table 2. Effect of fermentation followed by cooking on chemical composition and total energy of millet flour supplemented with different levels of defatted Moringa seeds flour.

Supplementation level (%)	Fermentation time (hrs)	Dry matter (%)	Ash content (%)	Crude protein (%)	Oil content (%)	Crude fiber (%)	Carbohydrate (%)	Total energy (kcal)
0	Fermented dough							
	0	92.56(±0.33)^{ab}	1.54(±0.05)^{de}	14.29(±0.10)^{hi}	5.87(±0.79)^{b}	1.29(±0.04)^{g}	69.57(±0.69)^{b}	388.27(±4.75)^{b}
	8	93.99(±0.29)^{d}	2.57(±0.37)^{bc}	14.83(±0.05)^{h}	6.02(±0.35)^{a}	1.94(±0.07)^{cd}	68.63(±0.10)^{bcd}	388.03(±2.57)^{b}
	16	94.76(±0.14)^{g}	1.37(±0.05)^{def}	15.32(±0.33)^{fg}	6.03(±0.18)^{a}	2.05(±0.05)^{c}	68.98(±0.28)^{bc}	391.47(±1.51)^{a}
	Fermented/cooked dough							
	8	94.76(±0.36)^{g}	1.94(±0.03)^{cd}	15.38(±0.06)^{gh}	5.71(±0.34)^{b}	2.22(±0.52)^{bc}	69.51(±0.40)^{b}	390.94(±1.89)^{ab}
	16	92.11(±0.49)^{a}	1.67(±0.03)^{d}	15.67(±0.29)^{g}	2.03(±0.14)^{j}	1.90(±0.08)^{cd}	70.84(±0.54)^{ab}	364.29(±2.05)^{gh}
5	Fermented dough							
	0	94.72(±0.23)^{g}	2.07(±0.14)^{c}	16.91(±0.16)^{i}	4.24(±0.81)^{c}	2.39(±0.26)^{b}	69.11(±0.26)^{b}	382.25(±2.98)^{bc}
	8	94.60(±0.18)^{ef}	1.63(±0.18)^{de}	17.50(±0.11)^{ef}	2.77(±0.26)^{g}	1.16(±0.21)^{gh}	71.54(±0.31)^{a}	381.07(±1.50)^{c}
	16	94.25(±0.09)^{e}	3.01(±0.18)^{ab}	18.18(±0.24)^{e}	3.69(±0.15)^{de}	0.63(±0.53)^{hi}	68.10(±0.52)^{bcde}	380.86(±1.69)^{c}
	Fermented/cooked dough							
	8	92.65(±0.15)^{ab}	1.94(±0.19)^{cd}	19.25(±0.25)^{de}	3.59(±0.29)^{e}	1.51(±0.25)^{e}	66.72(±0.46)^{cd}	376.22(±0.18)^{d}
	16	93.52(±0.22)^{bc}	2.08(±0.17)^{c}	19.39(±0.09)^{de}	3.84(±0.12)^{d}	1.61(±0.41)^{de}	66.60(±0.36)^{cd}	378.50(±0.79)^{cd}
10	Fermented dough							
	0	94.30(±0.16)^{e}	1.92(±0.20)^{cd}	19.40(±0.25)^{de}	2.47(±0.39)^{h}	2.55(±0.47)^{ab}	67.57(±0.46)^{c}	371.41(±0.51)^{e}
	8	93.72(±0.18)^{cd}	1.78(±0.023^{d}	19.89(±0.12)^{cd}	3.58(±0.41)^{e}	1.23(±0.39)^{g}	67.74(±0.42)^{c}	380.73(±0.81)^{c}
	16	92.65(±0.54)^{c}	2.19(±0.18)^{c}	20.75(±0.06)^{c}	3.59(±0.24)^{e}	1.37(±0.12)^{ef}	65.75(±0.26)^{d}	378.36(±2.76)^{d}
	Fermented/cooked dough							
	8	94.18(±0.23)^{e}	3.45(±0.19)^{a}	20.85(±0.11)^{c}	2.31(±0.45)^{i}	1.25(±0.40)^{g}	66.32(±0.41)^{cd}	369.47(±0.97)^{ef}
	16	93.52(±0.17)^{d}	3.13(±0.10)^{ab}	22.02(±0.15)^{bcd}	2.54(±0.23)^{h}	2.29(±0.18)^{bc}	63.99(±0.19)^{ef}	366.90(±1.03)^{g}
15	Fermented dough							
	0	93.67(±0.13)^{c}	1.72(±0.07)^{d}	22.25(±0.21)^{bc}	3.18(±0.19)^{i}	3.06(±0.11)^{a}	63.96(±0.11)^{ef}	371.45(±0.49)^{e}
	8	94.64(±0.08)^{ef}	2.59(±0.12)^{bc}	22.46(±0.13)^{b}	3.63(±0.33)^{de}	1.35(±0.06)^{ef}	64.61(±0.13)^{e}	380.94(±1.07)^{c}
	16	92.15(±0.16)^{a}	1.91(±0.13)^{cd}	23.61(±0.18)^{ab}	3.65(±0.41)^{de}	1.81(±0.17)^{d}	61.17(±0.10)^{fg}	372.07(±1.42)^{de}
	Fermented/cooked dough							
	8	93.42(±0.11)^{bc}	3.05(±0.14)^{ab}	23.63(±0.16)^{ab}	3.67(±0.29)^{de}	0.92(±0.25)^{h}	62.15(±0.05)^{f}	376.17(±1.10)^{d}
	16	93.14(±0.24)^{b}	2.87(±0.14)^{b}	24.93(±0.01)^{a}	3.54(±0.22)^{e}	1.74(±0.23)^{de}	60.06(±0.39)^{g}	372.54(±2.91)^{de}
$Lsd_{0.05}$		0.4409*	0.0261*	0.5352**	0.6521*	0.0141*	0.5236**	3.0863**

Values are means (±SD) of triplicate samples. Means having different superscript letter within a column are significantly different at p< 0.05.

supplementation with DMF may be due to high value of DM in Moringa seeds flour. Fermentation of raw PMF significantly ($p<0.05$) increased the DM to 93.99% but it was decreased after supplementation with 5, 10 and 15% DMF. The increment could be due to the increase in crude fiber content after fermentation of raw PMF while the decrease in the carbohydrate content of PMF supplemented with moringa after fermentation may be responsible for the decrease in DM. The DM of all flour samples were above 91% thereby giving the flours a better shelf-life (Aryee *et al*., 2006). The DM of the supplemented millet dough varied with fermentation time. The results were higher than those reported for whole millet seeds flour of Ashana cultivar (Mohamed *et al*., 2010). Cooking followed by fermentation significantly decreased the DM of both raw PMF and DMF supplemented composite flour compared to fermentation alone.

Addition of 5, 10 and 15% DMF to PMF significantly ($p<0.05$) increased the ash content of PMF from 1.54% to 2.07%, 1.92% and 1.72%, respectively. This result agreed with that reported by Awadalkareem *et al*. (2008) and Serrem *et al*. (2011) where increase in ash content was observed after supplementation of sorghum flour with soy protein concentrate. The ash content varied after fermentation but significantly ($p<0.05$) higher values were observed after 16 h fermentation for 5 and 10% DMF supplemented millet dough compared to unsupplemented dough and this could be due to utilization of ash during the growth of microorganisms. Further significant ($p<0.05$) increase in ash content was observed after cooking of fermented dough supplemented with 5 and 10% DMF, 1.94 and 3.45%, respectively or 10 and 15% DMF, 3.45 and 3.05% respectively, and the values were not significantly different with increase in fermentation time followed by cooking. The values obtained were higher than the range (1.69-2.44%) reported by Nour *et al*. (2014) for cooked pearl millet flour supplemented with defatted Moringa seed flour. Similarly, increase in ash content after cooking of raw and supplemented pearl millet flour has been reported by Nour *et al*. (2014).

The CP content of raw PMF (14.29%) was significantly ($p<0.05$) increased to 16.91, 19.40 and 22.25% after supplementation with 5, 10 and 15% DMF, respectively and this could be due to high CP content of DMF. The values obtained were higher than that of 10 millet cultivars (8.5-15.1%) reported by Abdalla *et al*. (1998). A similar observation on the increase in CP of millet flour supplemented with whey protein isolate has been reported by Mallasy *et al*. (2010) and Ibrahim *et al*. (2005). Fermentation slightly ($p<0.05$) increased the CP of both raw PMF and DMF supplemented composite flours with significant ($p<0.05$) increase observed after 16 h fermentation and this could be due to the synthesis of amino acid needed for growth by micro-organisms during fermentation. Similarly, Inyang and Zakari (2008) reported that fermentation significantly ($p<0.05$) increased the protein content of pearl millet flour and Nour *et al*. (2014) reported an increase in composite flour. Cooking of fermented raw and supplemented flours further increased their CP contents with 5, 10 and 15% DMF supplemented composite flours having the values of 19.39, 22.02 and 24.93%, respectively, after 16 h fermentation followed by cooking. This report contradicts previous findings that cooking decreased the protein content of millet flour supplemented with defatted Moringa seed (Nour *et al*., 2014) and soybean (Ali *et al*., 2009a; Mohamed *et al*., 2010) and this may be due to combine effect of fermentation and cooking.

The crude fiber (CF) content of raw PMF (1.29%) was significantly ($p<0.05$) increased to 2.39, 2.55 and 3.06% after supplementation with 5, 10 and 15% DMF, respectively. The increase in fiber content of supplemented flour could be ascribed to the high content of fiber in DMF (4.68%). Fermentation for 8 h decreased the CF of both raw PMF and DMF supplemented composite flours but slight increase was observed after 16 h fermentation, though the values were still lower than that of unfermented samples. The reduction in crude fiber content of fermented dough may be due to enzymatic degradation of the fiber during fermentation (Ikenebomeh *et al*., 1986). Cooking of fermented dough slightly increased the CF except that of 15% DMF supplemented millet dough but the values were significantly ($p<0.05$) lower than that of unfermented dough. The increase in fiber content obtained after cooking could be due to a shift from insoluble to soluble dietary fiber as well as the formation of resistant starch and enzyme-resistant indigestible glucans (Vasanthan *et al*., 2002). This finding is important because crude fiber has useful role in providing roughage that aids digestion and reduces the risks of cardiovascular diseases (Verma and Banerjee, 2010; Dhingra *et al*., 2011; Sharma *et al*., 2012).

Supplementation with 5, 10 and 15% DMF significantly ($p<0.05$) lowered the oil content of raw PMF (5.87%) to 4.24, 2.47 and 3.18%, respectively. Fortification of sorghum or bread wheat flours with defatted soy flour significantly affected the oil content of the flours (Serrem *et al*., 2011). Fermentation of raw PMF, 10 and 15% DMF supplemented dough increased the oil content to 6.03, 3.59% and 3.65% after 16 h fermentation. Previous studies reported no significant effect of fermentation on oil of sorghum and green gram blend (Chavan *et al*., 1988) and pearl millet flour (Osman, 2011). Cooking of fermented raw and 10% supplemented millet dough significantly ($p<0.05$) lowered their oil contents but no significant effect of cooking was observed in 5 and 15% DMF supplemented dough compared to fermentation alone. The decrease in oil content after cooking of fermented dough may be due to evaporation of volatile oils and this may increase the shelf-life of the raw flour and composite flours. Nour *et al*. (2014) reported that cooking lowered the oil content of millet flour supplemented with defatted Moringa seed.

The carbohydrate content of raw PMF was significantly ($p<0.05$) decreased to 69.11, 67.57 and 63.96% after supplementing with 5, 10 and 15% DMF, respectively and this could be due to lower carbohydrate content of DMF. Similarly, Kayitesi *et al*. (2010) reported that fortification of sorghum flour with marama bean flour significantly ($p<0.05$) lowered the carbohydrate content. Fermentation of both raw and DMF supplemented flour showed no significant effect on their carbohydrate contents and the values were slightly lowered after 16 h fermentation probably due to the utilization of some sugars by micro-organisms. Similar observation was reported for pearl millet flour during fermentation (Osman, 2011). Cooking of fermented raw millet dough has no significant effect on the carbohydrate contents but significantly ($p<0.05$) decreased that of DMF supplemented millet dough compared to unfermented dough. This agrees with the findings of Nour *et al*. (2014) who reported that cooking of millet flour supplemented with 5 and 10% defatted Moringa seed had no significant effect on the carbohydrate content.

The total energy content of raw PMF (388.27 kcal) was significantly ($p<0.05$) lowered to 382.25, 371.41 and 371.45 kcal after supplementation with 5, 10 and 15% DMF, respectively. In agreement with our results, a reduction in the total energy content due to supplementation of sorghum and/or wheat flours with defatted soy flour has been reported (Serem *et al*., 2011). Significant ($p<0.05$) increase in total energy content was observed after fermentation of raw, 10 and 15% DMF supplemented millet dough. Cooking of 16 h fermented dough significantly reduced the total energy content of raw and DMF supplemented composite millet flours. This contradicts the report of Nour *et al*. (2014) where cooking alone was observed to increase total energy of both raw millet flour and millet flour supplemented with defatted fenugreek seeds.

Table 3. Effect of fermentation followed by cooking on chemical composition and total energy of millet flour supplemented with different ratios of defatted fenugreek seed flour.

Supplementation level (%)	Fermentation time (hrs)	Dry matter (%)	Ash content (%)	Crude protein (%)	Oil content (%)	Crude fiber (%)	Carbohydrate (%)	Total energy (kcal)
0	Fermented dough							
	0	92.56(±0.33)^{a}	1.54(±0.05)^{c}	14.29(±0.10)^{cd}	5.87(±0.79)^{bc}	1.29(±0.04)^{d}	69.57(±0.04)^{a}	388.27(±4.75)^{j}
	8	93.99(±0.29)^{c}	2.57(±0.37)^{ab}	14.83(±0.05)^{cd}	6.02(±0.35)^{b}	1.94(±0.07)^{c}	68.63(±0.10)^{ab}	388.03(±2.57)^{j}
	16	94.76(±0.14)^{ef}	1.37(±0.05)^{cde}	15.32(±0.33)^{c}	6.03(±0.18)^{b}	2.05(±0.05)^{bc}	68.98(±0.28)^{a}	391.47(±1.51)^{g}
	Fermented/cooked dough							
	8	94.76(±0.36)^{ef}	1.94(±0.03)^{bc}	15.38(±0.06)^{c}	5.71(±0.34)^{bc}	2.22(±0.52)^{b}	69.51(±0.40)^{a}	390.94(±1.89)^{h}
	16	92.11(±0.49)^{a}	1.67(±0.03)^{cd}	15.67(±0.29)^{c}	2.03(±0.14)^{de}	1.90(±0.08)^{c}	70.84(±0.54)^{a}	364.32(±2.05)^{n}
5	Fermented dough							
	0	92.79(±0.67)^{ab}	1.62(±0.04)^{c}	15.35(±0.04)^{c}	7.80(±0.19)^{a}	1.33(±0.03)^{d}	67.36(±0.54)^{b}	401.06(±2.98)^{a}
	8	93.59(±0.36)^{bc}	1.85(±0.15)^{c}	15.36(±0.16)^{c}	5.83(±0.78)^{bc}	1.80(±0.11)^{c}	68.74(±0.70)^{ab}	388.88(±4.59)^{j}
	16	95.10(±0.48)^{f}	2.25(±0.27)^{b}	17.06(±0.18)^{c}	4.94(±0.11)^{c}	1.56(±0.38)^{cd}	69.29(±0.70)^{a}	389.83(±2.38)^{i}
	Fermented/cooked dough							
	8	94.41(±0.40)^{de}	2.13(±0.08)^{b}	18.27(±0.48)^{b}	5.70(±0.67)^{bc}	1.64(±0.41)^{cd}	67.67(±0.95)^{b}	392.64(±1.48)^{f}
	16	94.60(±0.43)^{e}	2.23(±0.07)^{b}	17.50(±0.00)^{bc}	2.99(±0.20)^{d}	2.61(±0.36)^{a}	69.27(±0.13)^{a}	373.98(±1.78)^{l}
10	Fermented dough							
	0	94.33(±0.28)^{d}	1.78(±0.03)^{c}	15.76(±0.08)^{c}	6.27(±0.32)^{b}	1.47(±0.15)^{cd}	68.95(±0.55)^{a}	398.24(±0.93)^{b}
	8	92.91(±0.35)^{ab}	0.94(±0.03)^{d}	15.77(±0.31)^{c}	5.11(±0.20)^{c}	1.87(±0.09)^{c}	69.22(±0.21)^{a}	385.45(±2.16)^{k}
	16	94.26(±0.47)^{d}	1.94(±0.07)^{bc}	17.08(±0.07)^{bc}	4.81(±0.38)^{c}	2.04(±0.34)^{bc}	68.39(±0.44)^{ab}	385.19(±4.96)^{k}
	Fermented/cooked dough							
	8	94.59(±0.38)^{e}	1.99(±0.06)^{bc}	18.15(±0.36)^{b}	6.22(±0.50)^{b}	1.63(±0.29)^{cd}	66.6(±0.81)^{c}	396.65(±2.53)^{c}
	16	93.59(±0.44)^{bc}	1.99(±0.02)^{bc}	20.17(±0.24)^{a}	2.27(±0.56)^{de}	2.51(±0.26)^{ab}	66.65(±0.17)^{c}	367.69(±4.39)^{m}
15	Fermented dough							
	0	92.61(±0.05)^{ab}	2.25(±0.08)^{b}	15.78(±0.11)^{c}	8.07(±0.29)^{a}	1.59(±0.18)^{cd}	63.08(±0.67)^{cd}	394.93(±0.13)^{ef}
	8	93.31(±0.48)^{b}	0.95(±0.04)^{d}	17.49(±0.28)^{bcd}	6.48(±0.32)^{b}	2.67(±0.17)^{a}	67.43(±0.41)^{b}	392.47(±2.95)^{f}
	16	95.54(±0.41)^{g}	1.17(±0.16)^{cd}	19.83(±0.14)^{ab}	4.75(±0.67)^{cd}	2.17(±0.15)^{b}	67.62(±0.76)^{b}	388.56(±3.79)^{j}
	Fermented/cooked dough							
	8	93.97(±0.14)^{c}	2.18(±0.11)^{b}	19.33(±0.37)^{ab}	6.85(±0.85)^{ab}	1.93(±0.10)^{c}	63.68(±0.52)^{cd}	393.67(±4.48)^{e}
	16	95.53(±0.51)^{e}	2.89(±0.10)^{a}	20.89(±0.08)^{a}	1.81(±0.95)^{e}	2.09(±0.25)^{bc}	67.53(±0.48)^{b}	367.28(±6.39)^{m}
$Lsd_{0.05}$		0.2087**	0.0869*	0.3426**	0.3084**	0.0497*	0.4211*	2.6325**

Values are means (±SD) of triplicate samples. Means having different superscript letter within a column are significantly different at $p < 0.05$.

Effect of fermentation followed by cooking on chemical composition and total energy of PMF supplemented with DFF

The DM content of raw PMF was increased to 92.79, 94.33 and 92.61% after supplementation with 5, 10 and 15% DFF, respectively (Table 3). The DM content significantly ($p<0.05$) increased after fermentation of both raw and DFF supplemented composite flours. This increase could be attributed to the high crude fiber and carbohydrate contents of the fermented samples. High DM content in food samples increased the storage periods of the food products as reported by Alozie *et al*. (2009). Fermentation followed by cooking showed no effect on the DM content of the samples but significant reduction in DM content was observed after cooking of 16 h fermented raw PMF.

The ash content of raw PMF (1.54%) was increased to 1.62, 1.78 and 2.25% after supplementation with 5, 10 and 15% DFF, respectively. The ash content fluctuates after fermentation of both raw and supplemented composite flours but significant ($p<0.05$) increase was observed after 16 h fermentation of millet flour supplemented with 5 and 10% DFF. The values obtained were higher than those reported by Nour *et al*. (2014) for cooked pearl millet flour supplemented with defatted fenugreek seed flour (1.69-2.33%). Cooking of fermented dough was more effective in increasing the ash content with significantly ($p<0.05$) higher value of 2.89% obtained after cooking of 16 h fermented millet flour supplemented with 15% DFF. Our findings supported that of Mohamed *et al*. (2010) who reported that cooking enhanced the ash content of two pearl millet cultivars.

Supplementation of millet flour with 5, 10 and 15% DFF increased the CP content of raw millet flour to 15.35, 15.76 and 15.78%, respectively. The high CP of DFF may have resulted in increase in CP of the composite flours. Fermentation of both raw and DFF supplemented composite dough slightly increased the CP content and this increased with increase in fermentation time. This was similar to the findings of Osman (2011) who reported an increase in protein content of pearl millet flour after 24 h fermentation compared to 16 and 20 h. This incrementin protein content of fermented raw and composite flour may be due to protein synthesis and solublization of insoluble proteins. Cooking of fermented dough further increased the CP content with a significant ($p<0.05$) increase obtained for millet flour supplemented with 10 and 15% DFF compared to fermentation process alone. This disagreed with a previous report of Nour *et al*. (2014) who observed a decrease in CP of pearl millet flour supplemented with defatted fenugreek seeds after cooking

The oil content of raw PMF was increased to 7.80, 6.27 and 8.07% after 5, 10 and 15% DFF supplementation, respectively and these values were higher than that of millet cultivars (2.7-7.1%) reported by Abdalla *et al*. (1998). The increase in oil may be due to high oil content exhibited by DFF (7.18%) used as a supplement. Fermentation slightly increased the oil content of raw PMF while reduction in oil content was observed in fermented DFF supplemented composite dough which decreased with increase in fermentation time. Our results contradict those reported by Osman (2011) who observed no significant change in oil content of pearl millet flour after fermentation for 4 days. Cooking of fermented raw PMF further lowered the oil content. However, cooking of 8h-fermented DFF supplemented dough increased the oil content compared to fermentation alone but the values were lower than that of unfermented dough. Increasing fermentation period to 16 h followed by cooking significantly ($p<0.05$) reduced the oil contents of the samples. A similar observation on significant reduction of oil content due to cooking of two millet flour cultivars has recently been reported (Mohamed *et al*., 2010). The decrease in oil may extend the shelf-life of the raw and composite flours by decreasing the chance of rancidity and will also contribute to low energy value of samples.

Supplementation with 5, 10 and 15% DFF increased the crude fiber content of raw PMF to 1.33, 1.47 and 1.59%, respectively. Significant ($p<0.05$) increase in crude fiber was observed after fermentation of both raw and DFF supplemented composite flour and this varied with fermentation time, with 15% DFF supplemented millet dough having the highest value. Cooking of 8 h fermented DFF supplemented dough significantly ($p<0.05$) lowered crude fiber content but increased that of raw PMF to 2.22%. Previously a significant decrease in fiber content of both whole and dehulled millet seed flour after cooking has been reported by Mohamed *et al*. (2010). However cooking of 16 h fermented millet dough supplemented with 5 and 10% significantly ($p<0.05$) increased the crude fiber to 2.61 and 2.51%, respectively, and this could be due to synergistic effects of longer fermentation time and heat treatment that probably resulted in formation of resistant starch and enzyme resistant indigestible glucans (Vasantha *et al*., 2002). The high crude fiber can help to soften stools and lowers plasma cholesterol level in the body (Dhingra *et al*., 2011).

The carbohydrate content of raw PMF decreased to 67.36, 68.95 and 63.08% after supplementation with 5, 10 and 15% DFF, respectively. The decrease may due to low carbohydrate content of DFF (48.86%) which had a diluting effect on carbohydrate content of the composite flour. A similar reduction in carbohydrate content of sorghum fortified with defatted soy flour has been reported (Serrem *et al*., 2011). Fermentation showed no significant effect on carbohydrate content of the flour but a significant increase was observed after supplementation with 15% DFF (67.62%) which could be due to termination of starch degradation by low pH that inhibits amylase activity (El-Tinay *et al*., 1979). Fermentation followed by cooking has no significant effect on carbohydrate content of the dough but a significant ($p<0.05$) reduction was observed after supplementation with 10% DFF. This contradicts previous report on increase in carbohydrate contents after cooking of defatted fenugreek supplemented millet dough (Nour *et al*., 2014).

Supplementation with 5, 10 and 15% DFF increased the total energy content of raw PMF to 401.06, 398.24 and 394.93%, respectively. The high total energy could be as a result of high total energy content of DFF which added to that of PMF. Fermentation slightly decreased the total energy content of the supplemented dough while that of raw millet dough fluctuates. Cooking of 8 h-fermented 5, 10 and 15% DFF supplemented millet dough increased the total energy content to 392.64, 396.65 and 393.67 kcal, respectively, however, the values were higher than unfermented dough. However, fermentation for long period of time (16 h) followed by cooking significantly ($p<0.05$) lowered the total energy content of both raw and DFF supplemented composite dough. Similarly, a reduction in total energy of supplemented dough due to cooking has been reported (Nour *et al*., 2014).

Conclusion

The result showed variations in chemical compositions of PMF, DMF and DFF with DMF and DFF having higher dry matter, CP and crude fiber than PMF. Supplementation with DMF and DFF improved the CP and ash content of millet dough while other chemical compositions and total energy content were not affected. However, supplementing the dough with DMF lowered the oil, carbohydrate and total energy while DFF lowered only carbohydrate content. The chemical compositions and total energy varied among the different levels of supplementation. Fermentation followed by cooking improved some of the chemical compositions of PMF while the total energy content was not significantly affected.

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