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Effect of Addition of Different Cereal Flours to Sorghum Flour on Dough Fermentation Pattern and Quality Parameters of Kisra

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Abstract

Kisra is fermented sorghum bread which constitutes the staple diet in Sudan. Traditionally the bread is made by utilizing the natural micro flora in sorghum flour. The fermented dough known as Ajean, is prepared Kisra traditionally by mixing sorghum flour with water in a ratio of 1:2 in a round earthenware container called a khumara. A small amount of the previously fermented dough is then added to the mixture to act as a starter.

Keywords

Lactic Acid Bacteria, Fermentation, Composite Flours, Kisra

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1. Introduction

Sorghum is widely grown in the semi-arid tropical regions of Africa and Asia because of its drought tolerance. It is considered poor people's food and may constitute more than 70% of food intake for people in these regions (Kandler and Weiss, 1986). Kisra, flat bread made of sorghum, constitutes a major part of the staple diet for the people in Sudan. Several studies involving humans, animals, and in vitro digestibility showed that cooked sorghum has low protein digestibility compared with other cereals (Axtell et al., 1982 and MacLean et al., 1981). Fermentation of the sorghum flour has been reported to decrease the levels of the anti-nutritional factors and increase protein availability, in vitro digestibility and nutritive value (Axtell et al., 1982 and Graham et al., 1986, Osman, 2004; Ibrahim et al., 2005; Al-Khalifa et al., 2005; Wedad et al., 2008; Mohammed et al., 2010). Traditionally, the naturally occurring microorganisms in sorghum flour are utilized in these fermentations. Fermentation has been used for several thousand years as an effective and low cost means to preserve the quality and safety of foods. Food fermentations involve mixed cultures of microorganisms that grow simultaneously or in succession. Lactic acid bacteria play an important role in food fermentation as the products obtains with their aid are characterized by hygienic safety, storage stability and attractive sensory properties.

Sorghum protein is of low quality the thing that is reflected on kisra. Traditional cereal foods play an important role in the diet of the people of Africa particularly in cereal producing zones. Flour from various cereals is one of the main raw materials used in the production of popular food products with high acceptability, good storage characteristics and affordable cost (Akinrele, 1970). It is, therefore, thought that addition of higher quality protein flour i.e. wheat flour in small portions might improve Kisra quality. It is also appealing to study the concomitant variation of fermentation patterns. The economic crisis of the country necessitates the search of alternatives of whole-wheat bread. This research project might be considered as an attempt in this respect.

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2. Objectives

1. To study the traditional fermentation pattern of sorghum flour supplemented by wheat flour, whole and decorticated maize, and millet used in Kisra production.
2. To study the quality parameter of sorghum dough Supplemented by wheat flour.
3. To study the quality parameter of traditionally fermented composite flour dough.
4. To study the quality parameters of traditionally fermented composite flour Kisra.

3. Materials and Methods

Flours Cereal grains such as Sorghum and millet were purchased from Khartoum North local market. The experiments were carried out at food microbiology laboratory in Food Research Centre, Sudan.

4. Preparation of Fermented Dough

Fermented dough was prepared in the traditional way used by Sudanese housewives. In the laboratory, sorghum flour was mixed with sterile distilled water in a 1:2 (w/v) ratio. A small amount of the previously fermented dough was then added to the mixture of flour and water to act as a starter (about 5%). This mixture was incubated at 30°C for 24 h in a sterile covered flask (2 kg flour + 4 L water), each fermentation was performed in duplicate during the fermentation period (24 h). Samples of 50 g fermented dough were placed in a sterile stomacher bag and mixed with 450 mL sterile 0.1% peptone water (OxoidCM9) using a stomacher lab-blender 400 (Seward Medical, London, UK) for 2 min. After a further serial dilution in 90 mL 0.1% peptone water, the samples were plated on different selective agar media for enumeration and identification of microorganisms.

Table (1). Proximate composition of raw sorghum, wheat and Millet flour.

Type of flour	Moisture content(%)	Ash content(%)	Crude Protein (%)	Fat content(%)	Crude fiber(%)	CHO(%)
Sorghum	7.37	1.50	13.38	3.32	2.33	70.00
Wheat						
Millet	5.19	1.88	11.61	4.8	6.86	68.50

Table (2). Proximate composition of the composite flour.

Type of flour	Lactic acid content (%)	Ash content (%)	Protein content (%)
Sorghum + Wheat	1.2	0.23	5.76
Sorghum + Millet	1.6	0.21	5.65

5. Microbiological Methods

5.1. Preparation of Serial Dilutions

Representative 10 ml were aseptically mixed with 90 ml distilled water and homogenized by shaking. Subsequent decimal dilutions were prepared with the same diluents and in all cases duplicate-counting plates were prepared of appropriate dilutions (Harrigan and MacCance, 1976).

5.2. Total Viable Count

Total viable count was carried out using the pour plate method described by Harrigan (1998).

5.3. Total Yeast and Moulds

Yeasts were enumerated by surface plating on malt extract agar (Oxoid) with 0.01% chloramphenicol as bacterial inhibitor and incubated aerobically at 25oC for 2-3 days (Harrigan and MacCance, 1976).

5.4. Lactic Acid Bacteria

Numbers of *LAB* were determined on selective media MRS agar. Appropriate dilutions were plated on MRS agar and incubated an aerobically using the anaerobic jars and the BBL, Gas Pak, anaerobic system envelopes (Becton, Dickinson, Cockeysville, USA) at 37°C for 3 days (Harrigan and MacCance, 1976).

5.5. Staphylococcus Aureus

*Staphylococcus aureus*was performed on Baird-Parker Agar (Oxoid). The plates were incubated at 37oC for 48 h (Harrigan and MacCance, 1976).

5.6. Coliform Bacteria and *E. coli*

The coliform test was done according to Harrigan (1998) by plating one ml sample onto MacConkey broth media. The plates were incubated at 37ºC for 48 hours and the counts were presented as colony forming unites per gram (cfu/g). Plates showing positive coliform were subjected to the confirmed test using Brilliant green bile lactose broth in test tubes with Durham tubes. The test tubes were then incubated at 44ºC for 48 hours. Each confirmed positive tube was subcultured into E.C. broth medium and then incubated at 44.5ºC for 24 hours. Tubes showing any amount of gas

production were considered to be positive *E. coli* presence.

5.7. Detection of *Salmonella*

The stage of the pre-enrichment of *Salmonella* medium was done by mixing 25g of sample with 225 ml of buffer peptone water in a sterile microbiological bag.The pre-enrichment culture was incubated for 24 hours at 37°C. The stage of theselective enrichment of *Salmonella* was done by pepetting one ml of the preenrichment culture to 10 ml of selective Selenite broth and incubating at 37°C for24 hours. The stage of plating on selective agar media was done by transferring

a loop full of the selective enrichment media to the surface on selective agar media (bismuth sulphate agar) and spreading to obtain isolated colonies.

5.8. Physico-Chemical Analysis

Samples of dough was taken during fermentation and analyzed for, titratable acidity and pH.

5.9. PH Determination

Ten grams of sample were shaken into 90 ml distilled water, left to stand for twenty minutes and then the pH of the suspension was measured using a pH meter model 7020.AACC (1983).

5.10. Total Titratable Acidity

Titrable acidity was determined according to the AOAC (1975) method. 10 ml aliquots (triplicates) were pipetted and titrated against O.1 MNaOH to phenolphthalein end-point and the acidity was calculated as g lactic acid/100.

6. Proximate Analysis

6.1. Protein Content

This was done according to the method described by AACC (2006).

6.2. Ash Content

Total ash content was determined by the AACC (2006) method. Constant weight was obtained after igniting the sample in an electric muffle furnace.

7. Results and Discussion

Table 3and 4 shows that, there was a significant increase ($P<0.05$) in total bacterial count of (sorghum+ wheat) flour $5.60x10^6$cfu/g at zero time when compared with that of (sorghum+ millet) flour which contained $6.25x10^5$cfu/g. Also it shows that, there was a significant increase ($P< 0.05$) in total bacterial count of (sorghum+ wheat) flour $2.04x10^8$cfu/g after 19 hours. On the other hand, after 24 hours, the total bacterial count was 5.83 x 109cfu/g. These results were in agreement with Ahmed (1994) who found that the total bacterial count in dough fermentation was 5.20x106cfu/g at zero time fermentation. Mohammed et al. (1991) and Hamad et al. (1992, 1997) studied changes in the population of microorganisms during Kisra fermentation. They found that the bacterial population increased with fermentation time and reach platue after 9 h and lactic acid bacteria were dominant micro-organism. In order to commercialize the Kisra production, it is necessary to understand the fermentation process and identify the micro-organism involved.

Table (3) indicates that the coliform bacterial (E. coli) in fermented dough of (sorghum+ wheat) decreased from >2,400cfu/g to 34 cfu/g at 19 hours, while after 24 hours, these microbial groups decreased to 15 cfu/g. These results were in agreement with Hamad et al., (1992) who found that, the coliform bacteria (E. coli) in fermented dough decreased to11cfu/g at 19 hours.

There was significant decrease ($P<0.05$) in yeast and moulds count of (sorghum+wheat) flour, which contained $2.56x10^4$cfu/g, $7.30x10^7$cfu/g and $9.11x10^7$cfu/g compared to that of (sorghum+ millet) flour, which contained $6.15x10^5$cfu/g, $6.86x10^6$cfu/g and $7.93x10^7$cfu/g at zero time, 19 hours and 24 hours fermentation, respectively (Table3 and 4).

Table (5) indicates that the chemical analysis of the (sorghum+ wheat) and (sorghum+ millet) showed that there were some differences in their chemical components. Also there were significant changes occurred in pH of sorghum flour dough due to fermentation process, the initial pH of the dough at start of fermentation was 6.24 for (sorghum + wheat), with the progress of fermentation pH reached 4.00. At the end of fermentation the pH decreased to 3.86 for (Sorghum + wheat) and 3.88 for (Sorghum + millet).After 24 h fermentation, the maximum titrable acidity was 1.2 for (sorghum + wheat) and 1.6% for (sorghum + millet).

Table (3). Changes in microbiological characteristics during different fermentation periods of sorghum and wheat flour.

Sorghum + Wheat	0 time	19hrs	24hrs
Lactic acid bacteria	Nil	2.08b × 108	5.53ab x 109
Total viable count	5.60ax106	2.04b x 108	5.83ab x 109
Yeasts and Moulds	2.50b x 104	7.30ax107	9.11ab x 107
Staphylococcus sp.	2.04bx103	6.87a x 102	2.81a x 102
E.coli	>2.400	34	15
Salmonella sp.	Nil	Nil	Nil

Means having different superscript letter in each row differ significantly ($p < 0.05$).

Table (4). Changes in microbiological characteristics during different fermentation periods of sorghum and millet flour.

Sorghum + Millet	0 time	19 hrs	24 hrs
Lactic acid bacteria	Nil	6.40ax106	7.73ax107
Total viable count	6.25ax105	6.60ax106	5.53bx107
Yeasts and Moulds	6.15ax 105	6.86bx106	7.93ax107
Staphylococcus sp.	Nil	Nil	Nil
E.coli	Nil	Nil	Nil
Salmonella sp.	Nil	Nil	Nil

Table (5). The pH-values of fermented dough.

Type of flour	0 time	19 hrs	24 hrs
Sorghum + Wheat	6.24a	4.00b	3.86c
Sorghum + Millet	6.34a	4.05b	3.88c

Means having different superscript letter in columns and rows differ significantly ($P \leq 0.05$).

Lactic acid bacteria were the dominant microflora and their number increased during fermentation of the three sorghum varieties, in the fermentation of the three varieties and at each interval the number of lactic acid bacteria was greater than the number of yeasts and molds and they dominated till the end of fermentation. This fact implied that Kisra fermentation is mainly lactic acid fermentation. This was in agreement with Sanni et al. (2002), who reported that lactic acid bacteria species were the predominant microorganisms during fermentation of Ghanaian maize. The increase in acid producing bacteria resulted in increased the amount of lactic acid produced with concomitant drop in pH with an increase in fermentation time. similar sequence of changes in lactic acid bacteria with drop in pH an increase in acidity during fermentation were reported by El-Hidai (1978), Mohammed et al. (1991) and Hamad et al. (1992, 1997) in kisra, Gassem (1999) in khamir Nout (1991) in sorghum based infant food formula, Ijabadeniyi (2007) in ogi produced traditionally. The counts of the Enterobacteriaceae increased in the first stages of fermentation then not detected after 19h. The inhibition of Enterobacteriaceae could be due to the growth of lactic acid bacteria, which outnumber the Enterobacteriaceae and result in fast acid production.

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