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Jir hur a Fermented Millet (*Penisitum typhoidism*) Product of Sudan

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Abstract: *Jir hur* is a traditional fermented food of the *Fur* tribe of western Sudan prepared from bulrush millet (*Penisitum typhoidism*). The microbiological changes during the fermentation stages were monitored. LAB count increased from 4.48 in raw millet to 7.19 log/cfug in *jir hur* and yeast count increased from 3.77 to 7.55 log/cfug, respectively with significant changes in pH from 6.66 in raw millet to 3.68 in *jir hur*. The fermentation of millet into *jir* was a homo-lactic acid fermentation that involved a complex microbial succession between LAB and yeast. LAB was the predominant microorganisms during the first fermentation with significant increase in yeast counts. *Pediococcus halophilus*, *Pediococcus inopinatus* and *Pediococcus urinaeequi* were the dominant lactic acid bacteria species at all times with the association of *Lactobacillus acidophilus* and *Lactobacillus jensenii* at the second fermentation. The yeast species *Schizosaccharomyces pombe*, *Saccharomycods sinensis* and *Trichosporon adeninovorans* were followed in succession during *jir* fermentation stages.

Key words: Homo-lactic acid fermentation, *Jir hur* preparation, millet fermentation, microbial succession

INTRODUCTION

Jir is a traditional fermented food of the *Fur* tribe of western Sudan prepared from bulrush millet (*Penisitum typhoidism*) their staple cereal. It is considered a prestigious food especially made for celebrities and dignitaries (Dirar, 1993).

Jir produces the finest kind of porridge compared to other cereals indigenous in Sudan. Its preparation is one of the most complicated fermentation processes in Africa. The primary aim behind the steps of *jir* production is to produce a pure white product i.e., pure starch at cottage level which gives *jiriya* (*jir* porridge) an optimum colour and consistency.

The porridge is a translucent, tasteless, jelly-like elastic mass. The pure colourless hue, the smooth touch on the palate and the gel-like setting are the attributes of a quality product (Dirar, 1993).

Jir is not routinely prepared as its preparation takes between 1-2 weeks. This complex process leads to the creation of a great microbial succession during the fermentation steps, besides affecting its nutritional value. The final product is deficient in most of the important nutritional components for a diet.

Traditional procedures and tools used to prepare fermented foods generally and those from cereals in particularly, play a role in the nature of the microbial succession and the microorganisms associated. Furthermore, the variation in the ecological parameters acting on the microbial association such as the nature of the cereal, temperature, size of inoculums, and length of propagation intervals, leads in each case to a characteristic species association. This explains the 46 lactic acid bacteria species and 13 yeast species isolated from the sourdough (Hammes *et al*., 2005). In addition, the specific microflora involved may vary some what from village to village and from family to family within the same village (Mavhungu, 2006).

The microbiology of many of these products is quite complex and not yet exploited. In most of these products the fermentation is natural and involves mixed cultures of yeasts, bacteria and fungi. Some microorganisms may participate in parallel, while others act in a sequential manner with a changing dominant biota during the course of the fermentation (Katongole and Nicholas, 2008).

This study investigated the effects of the complex processes in *jir* preparation, the microbial succession during the fermentation and the ecosystem of microbes associated.

MATERIALS AND METHODS

Materials:

Preparation of jir: Millet was obtained from Omdurman Cereal Market. The traditional method for processing *jir* was carried out at Food Research Center, Sudan (2009-2010) by a woman from *Darfur*. She processed *jir* using traditional tools (wooden pistil and mortar).

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Nine kilograms of millet were cleaned using a traditional container (*tabak*) to remove debris and stones. Millet was decorticated by pounding in a wooden mortar. Decorticated millet was transferred to a clean clay container (*zeer*) with about 6 liters of water, then was well covered to start the first fermentation stage for 41 h. The *zeer* was opened and water was removed by a small half-guard (*kass*) out of the *zeer* (millet after the first fermentation had a light white colour and was tender and soft). The decorticated millet was transferred to a plastic container, well washed and spread on a palm mat (*birish*) for exposure to the sun to become semi- dried. It was pounded in the wooden mortar until it became a soft fine paste, was transferred to a plastic container, water added and the contents well mixed. This mixture was strained through a cotton cloth bag (*saffaya*) into the *zeer*. The paste of millet dough in the *saffaya* was returned to the wooden mortar to be pounded again, mixed with water and strained through the *saffaya*. This process was repeated many times in order to exclude the bran in the *saffaya* from the very fine and clearly white mixture of fermented millet. This mixture was transferred into the *zeer* which was then well closed for the second fermentation for 24 h. After the 24 h the *zeer* was opened, the surface water removed by *kass* and the *zeer* closed again for more separation of the water which was repeatedly removed until no water was left on the surface of the dough. The second fermentation lasted for about 39 h; thus total fermentation time to produce *jir hur* was for about 80 h. Three sediment layers of dough were formed. The top layer was a liquid, the second layer was creamy semi-liquid sediment called *Ragabiya* and the third in the bottom; was a white, very hard and stiff sediment called *Jir hur*. The last two sediments were separated by *kass*, placed on aluminium trays and sun dried. *Jir hur* is traditionally used for preparing *jir* porridges.

Methods:

Microorganisms: Microbial populations were isolated and counted using standard methods (Harrigan and McCance, 1976) at all of stages of *jir* fermentation. The bacteria were counted using the pour plate method whereas the spread plate technique was used to count molds and yeast and Lactic Acid Bacteria (LAB). Coliform bacteria were counted by the standard multiple tube fermentation technique two tests: presumptive test and confirmatory test. Colonies appeared were counted as colony forming units (cfu) g^{-1} sample.

Media used: Plate count agar (LAB 149, LAB M™, Bury, Lancashire, and UK.); Malt extract agar (MEA) (LAB37, LAB M™); De man Rogosa and Sharpe, (1960) (MRS) medium agar (LAB94, LAB M™); MacConkey Broth (Oxoid); Brilliant Green 2% Bile Lactose Broth (Oxoid) and Levine EMB Agar (Difco).

Isolation and identification: Colonies of lactic acid bacteria and yeasts based on their distinct morphologies were isolated from the MRS and malt extract agar (MEA) plates randomly. Only gram positive bacteria with catalase negative reactions were observed and the representative isolates were purified by successive streaking onto the same agar substrate. Also the representative yeast colonies on MEA were examined by microscope, purified by successive streaking on MEA and stored at 4ºC for further identification.

Identification of lactic acid bacteria: A total of 63 representative isolates of lactic acid bacteria were identified according to Harrigan and McCance (1976), Barrow and Feltham (1993) and the taxonomic keys laid down in Bergey's Manual of Systematic Bacteriology, volume 2 (Sneath *et al*., 1986).

Identification of yeasts: Sixteen isolates of yeasts were identified to the species level using their morphology and physiological properties according to methods described by Lodder (1970), Barrnett *et al*. (1983) and Barrnett *et al*. (1990).

Determination of pH and titrable acidity: pH was measured using a pH-meter (Dzudie and Hardy, 1996) and titrable acidity as percentage lactic acid (w/v) according to the (AOAC, 1975).

Statistical analysis: Complete Randomized Design (CRD) was used for the statistical analysis of the data according to ANOVA (Duncan, 1955).

RESULTS AND DISCUSSION

Microbiological investigation: The dynamics of the microbial community in *jir* processing was monitored during the various stages of the fermentation. The complicated procedure created variations in the quantity and concentration of the different microorganisms. In the pre-fermentation stage of raw millet (Fig. 1) the pH and titrable acidity were 6.66 and 0.1%, respectively. The total viable bacterial count was 5.60 log/cfug while LAB count was 4.48 log/cfug and yeast count was 3.77 log/cfug (Fig. 2).

Zero time refers to the millet after cleaning, dehulling and mixing with clean water in the *zeer* ready for the first fermentation. There was a slight change in pH and titrable acidity. The total viable bacterial count was 5.36 log/cfug due to the decrease in LAB count to 3.49 log/cfug as a result of the removal of the bran which might have contained LAB. The number of yeast increased to 4.76 log/cfug probably due to the use of traditional tools (*tabak* and wooden mortar) during cleaning and dehulling and yeast contamination of raw millet.

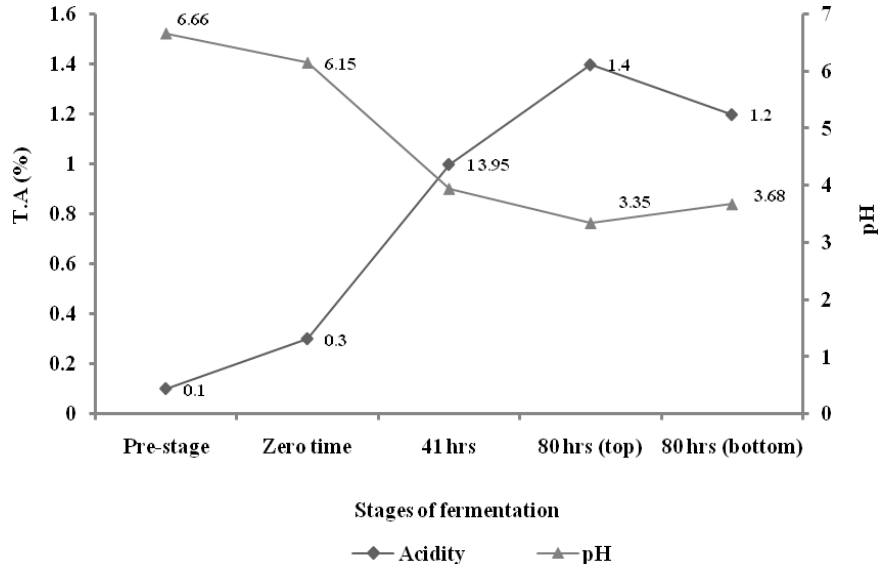

Fig. 1: Relationship between pH and titrable acidity at 5 stages of *jir* fermentation

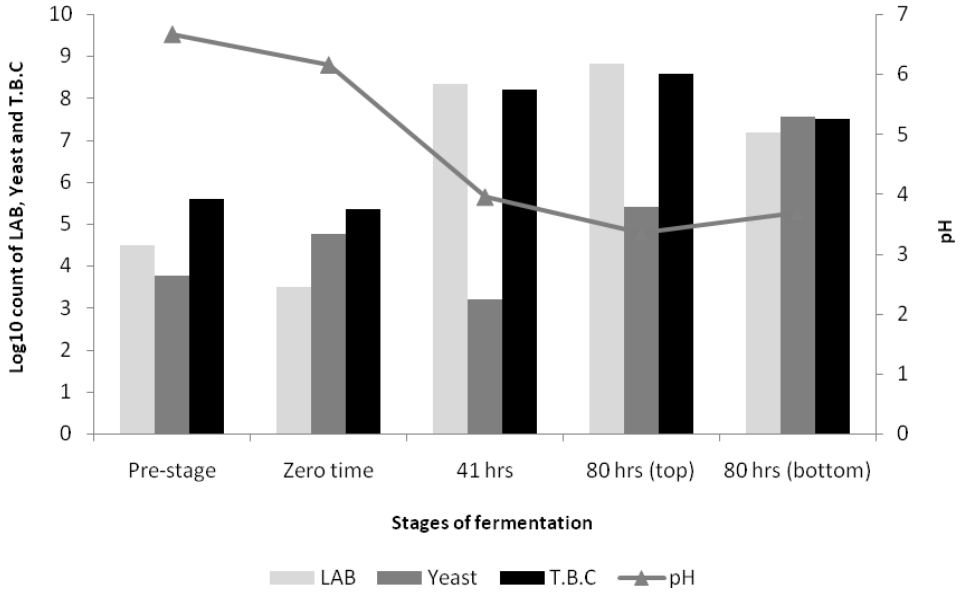

Fig. 2: Relationship between LAB, Yeast, T.B.C and pH at 5 stages of *jir* fermentation

pH was 6.15 and titrable acidity 0.3%. That change might be due to the time spent in pounding, dehulling and transferring the millet to *zeer* to initiate the first fermentation.

The first fermentation (41 h) is considered a soaking step in other traditional fermented African foods i.e. *ben-saalga* (Tou *et al*., 2006). During the fermentation there was significant changes ($p \leq 0.05$) in the microorganisms counts; the total viable bacterial count was 8.20 log/cfug and LAB count 8.32 log/cfug. The pH decreased to 3.95 and titrable acidity increased to 1%. The drop in pH may be attributed to the fermentation by lactic acid bacteria and non-lactic acid bacteria (Abdelrahim, 2003). This result is similar to that of Lei and Jakobsen (2004) in *koko* fermentation and Tou *et al.* (2006) in *ben-saalga* fermentation.

Although conditions were also probably suitable for yeast growth, the count decreased to 3.19 log/cfug due to the competition with lactic acid bacteria and much lower rates of natural specific growth than that of LAB as was

reported by Olstorpe *et al*. (2008) in the fermentation of wild forest *noni*.

The second fermentation (80 h) continued until settlement and separation of the different sediments. There were three sediments: a top paste layer in which the total viable bacteria and LAB counts reached their maximum level of 8.58 and 8.81 log/cfug, respectively, with a significant change ($p \leq 0.05$) in pH to 3.35 and reaching the maximum concentration of 1.4% lactic acid. Yeast count increased to 5.40 log/cfug probably due to the availability of essential nutrients for lactic acid bacteria growth and a favorable environment for yeast activity (nutrients and pH). The nutrients such as proteins and vitamins from pearl millet were made more easily available to lactic acid bacteria through reduction in size during pounding of the grains and also by the autolysis of yeast or other biological factors as was reported by Tou *et al*. (2006) in the *ben-saalga* fermentation. Furthermore, lactic acid bacteria secretion of organic acids lowered the pH and allowed the yeast population to become competitive in the immediate environment (Kosikowski, 1977; Vedamuthu, 1982; Steinkrauss, 1982; Bankole and Okagbue, 1992).

The co-metabolism between yeast and lactic acid bacteria illustrated their count changes during *Jir* fermentation stages, whereby the bacteria provided the acid environment, which selected the substrate for the growth of yeast and the yeast provided the vitamins and other growth factors to the bacteria (Gobbetti *et al*., 1994; Steinkrauss, 1996).

The second sediment layer the *Ragabia*, had a creamy colour. Total viable bacteria and LAB count declined to 7.82 and 8.04 log/cfug respectively, while yeast count increased to 6.93 log/cfug.

Jir hur was the third sediment at the bottom of the *zeer* with a hard texture and pure white colour. Total viable bacteria and LAB counts decreased to 7.49 log/cfug and LAB count to 7.19 log/cfug with an increase in pH to 3.68 and a drop in titrable acidity to 1.2%. The drop in LAB count might be due to its growth inhibition by the high concentration of lactic acid (1.4%) and the alcohol produced by yeast. Yeast count increased significantly to the maximum level 7.55 log/cfug.

The change in lactic acid bacteria and yeast counts in the last two sediments was due to the high competition between them, the shortage in the essential nutrients to lactic acid bacteria for growth, the low pH which inhibited growth of LAB and possibly the suppression by the alcohol as reported by Sherfi (1997). In the second fermentation the environment was suitable for yeast growth (low pH) and not that of LAB which lowered the competition between them. This agrees with Tou *et al.* (2006) in *ben-saalga* fermentation.

Coliform bacteria count was initially high >1100/mL in raw millet and continued untill the end of the first fermentation. This agreed with Mbugua (1986) who reported that *Enterobacteiaceae* were usually active in the early stages of fermentation of cereal–based slurries and their activity was eliminated when an enriched culture was used. After the second fermentation the coliform bacteria count decreased to <3/mL due to the decrease in pH from 3.95 in the first fermentation to 3.35 in the second fermentation. Thus the lowering of pH inhibited the growth of spoilage and pathogenic microorganisms as was reported earlier (Murrali *et al*., 1984; Narasimha, 1995). Result is also in accordance with Nout *et al*. (1989) and Masha *et al*. (1998) that an initial increase is followed by a decrease in the number of *Enterobacteiaceae* from their death kinetics studies in similar natural fermented plant materials.

Coliform count increased in *jir hur* after drying in the aluminum trays to 9 mL; hand contamination was one of the causes.

Microorganisms: The local millet variety, traditional tools, procedure for preparing *jir* and the environment during preparation was responsible for the microorganisms involved in *jir* fermentation.

The predominant representative isolates from different stages of *jir* fermentation were fully identified till species level. The LAB were *Pediococcus halophilus*, *Pediococcus inopinatus*, *Pediococcus urinaeequi*, *Lactobacillus acidophilus*, *Lactobacillus jensenii* and the yeast *Schizosaccharomyces pombe*, *Saccharomycods sinensis* and *Trichosporon adeninovorans*. These genuses of these dominant microorganisms were identified in other indigenous fermented foods, the genus but not the species. Generally, the few microbiological studies on spontaneously fermented millet products identified *Lactobacillus salivarius*, *Lactobacillus casei*, *Lactobacillus acidophilus*, *Lactobacillus jensenii*, *Lactobacillus cellobiosus*, *Lactobacillus plantarum*, *Pediococcus* spp. and *Leuconostoc* spp. (Oyeyiola, 1991; Antony and Chandra, 1997; Olasupo *et al*., 1997).

The microbial succession depended on the nature of these microorganisms themselves. Lei and Jakobsen (2004) explained the differences of dominating microorganisms at the different production stages as due to the changes in the microbiological environment, such as, pH, presence of different microorganisms in different production vessels, changes in the viscosity of the product and competition amongst the microorganisms.

Microbial succession: *Pediococcus halophilus* and *Schizosaccharomyces pombe* were the dominating microorganisms during the pre-stage for preparing millet till the zero time of fermentation (Table 1). During the first fermentation and after 41 h *Schizosaccharomyces pombe* was absent which may be due to the lowering of pH, with the appearance of *Saccharomycods sinensis* and

Table1: The microbial succession during *jir* fermentation

Stage of fermentation	Species of microorganisms (lactic acid bacteria and yeast)
Pre-stage (raw millet)	*Pediococcus halophilus* and *Schizosaccharomyces pombe*
Zero time	*Pediococcus halophilus* and *Schizosaccharomyces pombe*
First fermentation (41 h)	*Pediococcus halophilus, P. urinaeequi, P. inopinatus, Saccharomycods sinensis* and *Trichosporon adeninovorans*
Second fermentation (80 h) top layer	*Pediococcus halophilus, P. urinaeequi, P. inopinatus, Lactobacillus acidophilus L. jensenii* and *Saccharomycods sinensis*
Jir hur (80 h) bottom layer	*Pediococcus halophilus, P. urinaeequi, P. inopinatus, L. acidophilus, L. jensenii* and *Trichosporon adeninovorans*

Trichosporon adeninovorans. On the other hand, *Pediococcus halophilus* continued appearing with the two other species *P. urinaeequi* and *P. inopinatus* during all the fermentation process. That exchange in the *Pediococcus* spp. was attributed to their differences in a favoured selective environment, mainly on the basis of tolerance to temperature, pH and sodium chloride (Sneath *et al*., 1986; Dykes, 1991). After 80 h and during the second fermentation to the final product (*jir hur*), *Lactobacillus acidophilus and Lactobacillus jensenii* was the dominating LAB with *Pediococcus* species.

In the second fermentation and specifically in the top layer, the dominating yeast was *Saccharomycods sinensis* with absence of the other yeast species. *Trichosporon adeninovorans* was the dominant one in the *jir hur* bottom layer.

In this fermentation, differences in pH (6.66 to 3.68) and nutritional factors were the suitable explanation for the rapid appearance of *Pediococcus* spp. before *Lactobacillus* sp. which appeared later (second fermentation). Lei and Jakobsen (2004) explained the differences in the domination of *pediococcus* and *lactobacillus* at different stages in *koko* preparation as an indication of the ability of *Pediococcus* spp. to survive throughout the *koko* production. Ayres *et al*. (1980) found earlier that fermentation is usually initiated by the spherical bacteria and that the rod-shaped *lactobacilli* normally succeed the spherical bacteria at the desired lower pH (3.6). Thus, the pH in the second fermentation dropped from 3.95 to 3.35 when *Lactobacillus acidophilus* and *Lactobacillus jensenii* appeared. Also, *Lactobacillus spp.* appeared in the second fermentation due to the suitable nutritional requirements (amino acids and vitamins) available at that stage.

CONCLUSION

Jir is a unique product compared to other African millet fermented foods. The process of *jir* fermentation is similar to other African fermented millet products in a step or two in the first fermentation. In African fermented millet products the genus *Lactobacillus* is dominant while in *jir hur* it is *Pediococcus*.

LAB was predominant during the first fermentation with significant increase in yeast counts. *Pediococcus halophilus*, *Pediococcus inopinatus* and *Pediococcus urinaeequi* were the dominant lactic acid bacteria species at all times with the association of *Lactobacillus acidophilus* and *Lactobacillus jensenii* at the second fermentation. *Schizosaccharomyces pombe* was the only yeast species present at the zero time but *Saccharomycods sinensis* and *Trichosporon adeninovorans* were present in the first fermentation but separated after the second fermentation to the three layers of sediments.

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