American Journal of Food Science and Health
Vol. 4, No. 2, 2018, pp. 7-14
http://www.aiscience.org/journal/ajfsh
ISSN: 2381-7216 (Print); ISSN: 2381-7224 (Online)

Microbial and Biochemical Tests on the Traditional Sorghum Fermentation (*Dabar & Fetirita*)

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Abstract

Two of sorghum varieties flour and starters purchased from Shambat food center while starter was collected from Khartoum, Khartoum north and Omdurman. Themicrobiology and biochemical changes that occurred during the traditional fermentation of Dabar and Fetiritadoughs for Kisra making were studied, the fermentation was found to be mainly a lactic acid fermentation there were no significant differences in numbers and speeches of bacteria between the doughe of the two varieties. Eight species of lactic acid bacteria were isolated from the dough that collected from the different areas in Khartoum states even of them are *lactobaciilus* and one is *streptococcus*. The main metabolic products of the fermentation were lactic acid and acetic acid with end concentration of about 1% and 0.3% respectively the pH dropped from 6.2 to about 3.7. Fermentation with single and mixed culture of the dominant isolates showed that the mixtures were faster in the higher fermentation rates.

Keywords

Fermentation, Flour, Dough, Kisra, *Lacticacid bacteria*, *Yeast*

Received: May 1, 2018 / Accepted: June 19, 2018 / Published online: August 10, 2018

1. Introduction

Fermentation has been known by man as a naturally occurring process for thousands of years. Man has succeeded in utilizing this natural phenomenon for his benefit, particularly in food production. Fermentation is known to affect the nutritive value of food by increasing or decreasing the levels of some of its contents. Sudan is rich in fermented foods, among which Kisra forms the staple food for the majority of Sudanese people [1]. Kisra is a pancake like bread made in a form of thin sheets from sorghum or millet flour [2]. The process of its production falls into three phases: Milling, Fermentation and Baking [3]. In the past limiting factor in Kisra production in the Sudan was the milling capacity. Traditional milling is tedious and time consuming. However, this problem was solved through the introduction of modern mechanical mills which are now common in the Sudan. The development of cities, changes in the food habits, education and movement of women to work outside their houses forced many Sudanese people to shift to the consumption of bread made from wheat. Regardless of the progress made in solving the problem of sorghum milling, fermentation and baking processes still constitute the bottlenecks in Kisra production. A lot of work is needed to improve starter culture production and baking so that people can shift back towards Kisra consumption. [4, 5]. Women in villages have no problem concerning starter culture production because they utilize the remnants of the previous fermented batch, and if this starter is lost for any reason or another, they can get it easily from the neighbors for no charge. However, during the winter season fermentation may

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get delayed. This may necessitate the production of improved starter, capable of carrying the process under low temperature. During summer, the dough may have bitter taste due to over fermentation. Some people say that this bitter Kisra cause some abdominal troubles. In the urban areas Kisra production is not always practiced at home, thus urban women face the problem of lack of starter.

2. Materials and Methods

2.1. Flour and Starter

The flour used in this study was prepared from the sorghum verities locally calledDabar and Fetirita it were perched from the food research center at Shambat and they were kept in a sterilized bottle in the refrigerator. Starters were collected from different households in Omdurman Khartoum North and Khartoum. Fermented dough (Ajien) was prepared according to the traditional methods used in homes. Sorghum flour was mixed with water in ratio of about 1:2 (w/v), then a starter was added to the dough. At a rate of about 10% of the dough. The fermentation was usually completed of in 6-12 hours depending on the temperature and the amount of starter added.

2.2. Microbial Analysis of Flour, Starter and Fermented Dough

Viable Bacterial countand yeast were determined by Standard pour plate count technique according to [3]. Bacterial and yeast counts of fermented dough were carried out at 3 hours intervals using the same methods used in examination of flour and starter.

2.2.1. Lactic Acid Bacteria

Lactic Acid bacteria were enumerated on the modified selective medium of MRS agar medium [6].

2.2.2. Yeast

Yeast extract Agar medium was used for enumeration of yeasts; the plates were incubated at 28°C for 48 hours [3].

2.2.3. Purification of the Bacteria and Yeast Isolates

The different bacteria and yeast isolates were purified by methods described in [6] Identification of Bacteria.

2.2.4. Lactic Acid Bacteria

Before identification the isolates were streaked successively on MRS agar medium plates to produce pure cultures. The isolates were then identified according to [7] Kisra fermented in the laboratory using the isolated microorganism.

Sorghum grain → Dehulling and milling → sterilized of sorghum → addition of sterile distilled water +isolated microorganisms → fermenteddough → baking into thin sheets → kisra

The isolate used for fermentation were as follows

(5 g flour +10 ml H_2O +4 loops inoculum from a fresh slant)

1. Each isolate alone A, B, C, D, E, F, M and N
2. Each two isolate together A+B, A+C, A+D, A+E, B+C, B+D, B+E, C+D, C+E, E+D, F+M, F+N and M+N.
3. Each three isolates together A+B+C, A+B+D, A+B+E, A+C+D, A+C+E, A+D+E and F+M+N.
4. Four isolates together A+B+C+D and A+B+C+E.
5. Five isolate together A+B+C+D+E.

The PH was measured every hour till the pH reached the minimum assessed during the progress of fermentation.

2.3. Analytical Methods

Determination of pH, volatile fatty acids and titratable acidity of the femented dough were carried out at 3 hour intervals.

pH was measured by methods in [8] while the method in [9] described Volatile Fatty Acids (VFA) and

titratable Acidityas determined according to the [10] method.

3. Result and Discussion

Microbial populations of Flour and Starter of Dabar and Fetirita:

The results of microbial population of flour and starter of Dabar and Fetirita were reported in "Table 1". The bacteria showed the higher population in both flour and starter of the two sorghum types. The bacterial count in Dabar was 2×10^6 and 6.3×10^8cfu/g, and that of Fetirita was 1×10^6 and 1.5×10^8 cfu/g for flour and starter respectively. The yeast population was 1.0×10^4 and 3.2×10^5 cfu/g for Dabar flour and starter and 1.2×10^4 and 1.6×10^5cfu/g for Fetirita flour and starter respectively. [11] found that the dominant contaminants of sorghum flour were bacteria with a count of about 10^5 cfu/g. [4] Found that the total counts of bacteria in sorghum flour was about 10^5cfu/g. [12] found that the load of bacteria in Dabar flour was 7.3×10^3 cfu/g.

Table 1. Microbial population of the flour and starter of both Dabar and Fetirita.

Variety	Form	Viable Bacteria	Yeast
Dabar	Flour	2.0×10^6	1.0×10^4
	Starter	6.3×10^8	3.2×10^5
Fetirita	Flour	1.0×10^6	1.2×10^4
	Starter	1.5×10^8	1.6×10^5

3.1. Acid Content and pH of Starter Collected from Khartoum State

"Table 2" presents the pH, lactic acid and volatile fatty acid contents of starters collected from different areas in Khartoum State. The starter of Dabar samples collected from Khartoum Omdurman and Khartoum North had values of pH 3.6, 4.1 and 3.7, respectively. The lactic acid contents of all same samples were about 1%, 1% and 1% respectively, and the volatile fatty acids were about 0.2%, 0.3% and 0.2%, respectively. The Fetirita starters collected from Khartoum, Omdurman and Khartoum North had pH values of 3.7, 3.6, and 3.6 respectively. The lactic acid and volatile fatty acid contents of the same samples were 0.9, 0.8 and 0.8 and the volatile fatty acid 0.35, 0.30 and 0.30 respectively. No reports were found in the literature about the acid contents of household starters [4] found the pH of a starter collected from Khartoum North to be 3.5.

Table 2. Acid content and pH of Dabar and Fetirita starters collected from Khartoum State.

Sample	pH	Lactic acid %	Volatile fatty acids
Dabar from Khartoum	3.6	1.1	0.2
Dabar from Omdurman	4.1	0.1	0.3
Dabar from Khartoum North	3.7	1.0	0.2
Fetirita from Khartoum	3.7	0.8	0.4
Fetirita from Omdurman	3.6	0.8	0.3
Fetirita from Khartoum North	3.6	0.8	0.3

3.2. Microbial Population During Kisra Fermentation

The results of lactic acid bacteria and yeasts in fermented dough from Dabar and Fetirita flours were showed in "Figure 1 and 2". The contents of lactic acid bacteria and yeasts increase in similar ways in the two types of dough's with final bacterial concentration of about 10^{10} cfu/g. These results agree with findings reported by many workers. According to [4] the total count of lactic acid bacteria in fermented dough was 9×10^8 cfu/g. [9] found the lactic acid bacteria count to be between 10^8 and 10^{10} cfu/g in different types of dough. [13] found the total bacterial count during Ogi fermentation to be 4.7×10^7, and found the microbial count during the souring stage of sorghum fermentation to be 8×10^8 after 48 hours. [14] found the total count of lactic acid bacteria in spontaneously fermented sorghum dough in samples taken after 24 hours to be 4×10^9 cfu/g and in samples taken after 42 hours of fermentation to be 4.9×10^{10} cfu/g. The yeast population reached a maximum of 10^5 cfu/g and 10^5 cfu/g in Dabar and Fetiritadough's, respectively. [11] reported about the yeast count in Dabar dough of 5×10^4 cfu/g. [13] found yeast counts between 10^4 and 10^5 cfu/g in different types of dough, these counts indicated that yeasts do not play a significant role in Kisrafermentation although they are present in most of these fermentation.

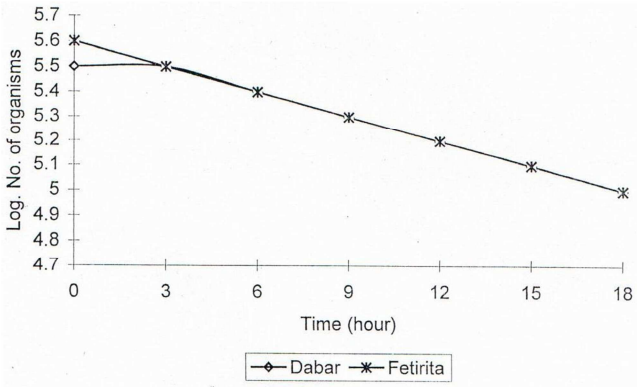

Figure 1. Yeast population during Kisra Fermentation.

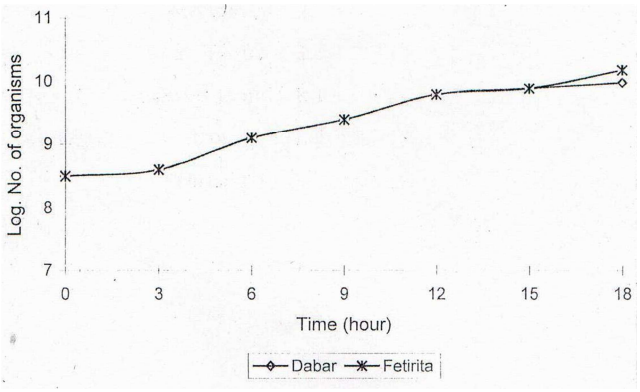

Figure 2. Lactic acid bacteria population during Kisra Fermentation.

3.3. Lactic Acid And Volatile Fatty Acid Contents of Dabar And Fetirita Dough's

The results of lactic acid volatile fatty acids in Dabar and Fetirita fermented dough's are shown in "Figures 3 and 4". The lactic acid content increased during fermentation from 0.2% to 1.1% and from 0.3% to 1.0% during the 18 hours of fermentation in the dough's of Dabarand Fetirita respectively. "Figure 3". The volatile fatty acid content increased during the first 9 hours of fermentation from 0.1% to 0.3% and from 0.1 to 0.3% in the dough's of Dabar and Fetirita, respectively "Figure 4". After that it remained constant till the end of fermentation. The pH dropped from 5.8 to 3.5 and from 5.9 to 3.6 in the Dabar and Fetiritadough's, respectively" Figure 5". These values are comparable to results reported by many workers. [4] found that the most important metabolic product in fermented sorghum dough was lactic acid. This fermentation gave a final concentration of 4-5.5% lactic acid. [10, 15 and 16] found that the pH of fermented dough decreased from 6.7 to 3.8 and the highest lactic acid contents attained in sorghum fermented at 25 ℃ and 35 ℃ were 0.96% and 1.22% respectively. [17] found that the ajeen fermentation had a

pH of 4.0, lactic acid content of 2.2% and a volatile fatty acid content of 0.9% during 35 hours of fermentation. The amount of acid produced in Kisra fermentation is comparable to values reported in many other fermented foods. [14] reported about 0.78% lactic acid in Ogi, 0.6% in Kaffir beer and 1% in Bussa. According to [4] Moroccan traditional dairy products contain between 0.6% to 1% lactic acid. [18] reported about 0.6-1.0%, 2.8%, 0.85% and 1.2% lactic acid in pickled vegetables, Indian Idli, Nigerian Gari and PhilippinianBalaoBalao, respectively. Volatile fatty acids are is not common in fermented foods.

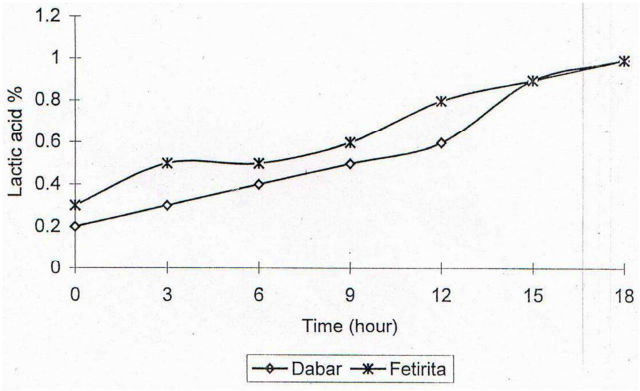

Figure 3. Lactic acid content of Dabar and Fetiritadoughs.

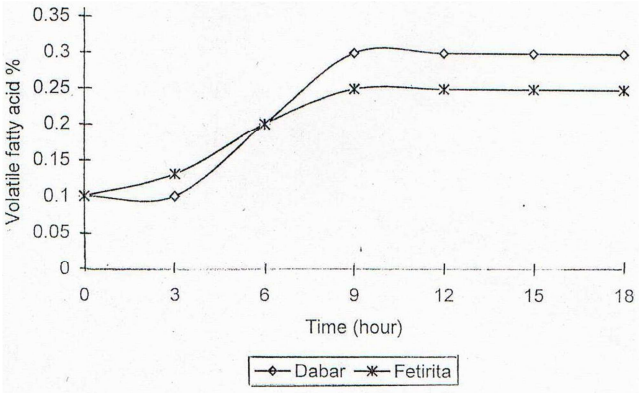

Figure 4. Volatile fatty acid content of Dabar and Fetiritadoughs.

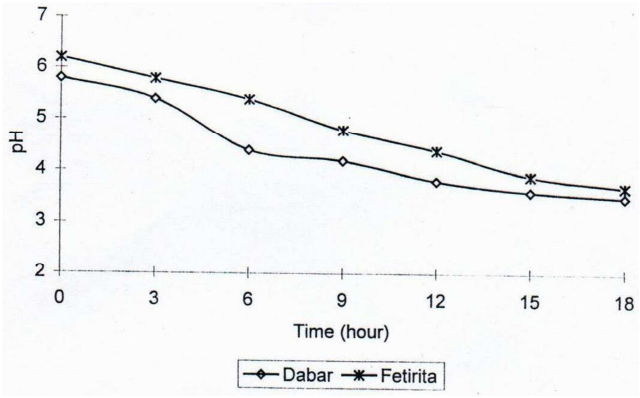

Figure 5. pH.

3.4. Identification of Bacteria Isolated from Dabar and Fetirita Doughs

The results of lactic acid bacteria are presented in "Table 3". Five isolates were made from Dabar dough and 3 from Fetirita dough. They were differentiated according to colony from and color and given the codes shown in "Table 3". All of the isolates were rods except one, which was a *coccus*. According to the results and the tests mentioned above, the seven rod shaped isolates could be related to the genus *lactobacillus* and the *coccus* could be related to the genus *streptococcus*. The eight isolates were grouped into four groups according to their ability to produce gas during glucose fermentation.

The First Group: Consisted of two isolates (D and N), which represented about 50% of the total bacterial population, and they were identified as lactobacilli. Growth was very slow on MRS. A modified MRS containing a vitamin mix promoted better growth. Also growth under anaerobic conditions was faster than under aerobic conditions. Colonies on the modified MRS agar were white and smooth. The cells were big and rod shaped. Both of these isolates produced gas during glucose fermentation. The gas production rate was usually very high since gas bubbles developed quickly in large amounts during the fermentation.

The Second Group: Consisted of isolates A and C. they made up 30% of the total bacterial population of the dough. The members of this group produced gas from glucose but in a far lower rate than the members of group one. The cells were short rods. Because of their ability to produce gas, group one and two are considered hereto-fermentative *lactic acid bacteria*.

The Third Group: Consisted of isolates B, E and M, which made up about 18% of the total bacterial dough population. The members of this group were also identified as *lactobacilli.* This group differed from the first two groups in that the members did not produce gas from glucose. They are therefore homo-fermentative *lactobacilli.*

The Fourth Group: Consisted of one isolate; namely the *Coccus*. It produced no gas from glucose, it is a homo-fermentative lactic acid bacterium. Many workers reported about the micro flora of Kisra fermentation [19-22, 13, 4, 23, 11]. These workers agree on the fact that this fermentation is mainly a *lactic acid* one a variety of *lactic acidbacteria*were found to dominate the fermentation at differing conditions. [21] found *Loctobacillus, Streptococcus and Pediococcus*, [22] reported about *Lactobacillus,* and [13] found *Pediococcuspetosaceus to* dominate together with *Lactobacillus confusus, L. brevis* and *L. intermedia*, [24] found that 50% of the isolates made from fermented sorghum dough were *lactovacilli*, [4] found that the micro flora of

fermented sorghum dough contained greater than 99% *lactobacilli,* dominated by the species *Lactobacillus*fermentum, *L. reuteri* and *L. amylovorus*. [11] isolated three groups of lactic acid bacteria from fermented Kisra dough, which consisted of seven isolates identified as *lactobacilli.* Five strains were classified as *L. vaginalis* and the other two strains were assigned to *L. helveticus*.

Table 3. Identification tests of lactic acid bacteria isolated from Dabar and Fetirita samples.

	Tests	A	B	C	D	E	F	M	N
Colony	Color	White	White	White	White	White	White	White	White
	Surface	Smooth	Smooth	Smooth	Smooth	Smooth	Smooth	Smooth	Smooth
Cell	Shape	Rod	Rod	Rod	Rod	Rod	Coccus	Rod	Rod
	Oxidase	-ve	-ve	-ve	-ve	-ve	-ve	-ve	-ve
	Catalase	-ve	-ve	-ve	-ve	-ve	-ve	-ve	-ve
	Gram stain	+ve	+ve	+ve	+ve	+ve	+ve	+ve	+ve
	Spores test	-ve	-ve	-ve	-ve	-ve	-ve	-ve	-ve
	Motility	-ve	-ve	-ve	-ve	-ve	-ve	-ve	-ve
	Optimum temp. for growth	37℃	37℃	37℃	37℃	37℃	37℃	37℃	37℃
	Gas production from glucose	++	-	++	++++	-	-	-	++++
	Aerobic growth	Weak	Weak	Weak	Weak	Weak	Weak	Weak	Weak
	Anaerobic growth	+ve	+ve	+ve	+ve	+ve	+ve	+ve	+ve

3.5. Fermentation Experiments with the Isolated Bacteria

The eight bacterial isolates were used for Kisra fermentation as described in material and methods. The experiments were done to test the potentialities of single isolates and of combinations of these isolates. This can help in the choice of bacterial strains suitable for starter culture production. The results of these experiments are shown in. “Tables 4, 5, 6, 7, 8, 9 and 10”. “Table 4” contains the results of the individual isolates. “Tables 5, 6, 7 and 8” contain results of combinations of the five isolates made from Dabar dough and “Tables 9 and 10” contain results of the combinations of the three isolates obtained from Fetirita. As can be seen from table 4, isolate M lowered the pH from 6.1 to 3.8 in 12 hours. This was the fastest rate of fermentation. Isolates A, C and D lowered the pH to 3.8 in 13 hours, whereas isolate N did that in 15 hours. Isolates E, B and F gave the same results in 16, 18 and 20 hours, respectively. The pH value for all isolates remained constant at 3.8 except for isolates F and M where the PH dropped to 3.7. These results indicate that isolates A, C, D and M are fast fermenters. Isolate N can also be put in this group, whereas isolates B, E and F have slower rates of fermentation. Isolates F and M are the most acid tolerant bacteria because they dropped the pH to 3.7. When the Dabar isolate were put in pairs, the combinations A+B, A+C, and B+C gave the best performance where the PH dropped to 3.8 in 10 hours. The second best combinations were A+D, A+E and C+D, which gave the same result in eleven hours “Table 5”. it is worth mentioning here that isolates A, C and D gave relatively high fermentation rates as individuals, while isolates B and E were slow fermenters when grown singly. The combination with the fast fermenters improved their performance in the caseof A+E and B+C. The combinations B+D and C+E had a fermentation time of 12 hours. The combination of the fast fermenter B with the slow fermenter E and the fast fermenter C with the slow fermenter E was not so effective. The slowest rate of fermentation was given by the combination of the slow fermenters B+E and the combination of the slow fermenter E and the fast fermenter When the Dabar isolates were put in a combination of three, the performance of all combinations was about the same“Table 6”. The pH dropped to 3.8 in seven hours in four of the combinations, and in eight hours in the other two combinations. The combinations of three isolates are much faster than single strains and also faster in their fermentation rate than the pairs. Combinations of four isolates reduced the pH to 3.8 in about 4-5 hours “Table 7”. ThepH dropped further to 3.5 in 6 to 7 hours and remained constant. This combination was faster in its rate of fermentation and was more acid tolerant than the individual isolates and then combinations of two or three. Finally the combination of five isolates reduced the pH to 3.8 in less than four hours and a final pH of 3.4 was produced in 6 hours “Table 8”. With regard to isolates made from Fetirita, the combinations of two strains reduced the pH to 3.8 in about 11 to 14 hours “Table 9 ”. This performance is comparable to that of the group isolated from Dabar. The combination of three of these strains (F + M + N) reduced the pH to 3.8 in 10 hours “Table 10” which means that this combination has a higher fermentation rate than the combinations of two members. However, the Fetirita isolates in a combination of three is still slower than the Dabar isolates in a similar combination. This point to differences between the two groups of flora, which constitutes a subject for further studies.

Table 4. Changes in pH values during dough fermentation of five Bacterial isolates (A, B, C, D and E) obtained from Dabar and three Bacterial isolates (F, M and N) obtained from Fetirita.

Time (hours)	A	B	C	D	E	F	M	N
0	5.8	5.8	5.8	5.8	5.8	6.1	6.1	6.1
1	5.8	5.8	5.8	5.8	5.8	6.1	6.1	6.1
2	5.7	5.8	5.6	5.6	5.7	6.1	5.8	5.9
3	5.6	5.7	5.5	5.5	5.6	6.1	5.6	5.8
4	5.4	5.6	5.4	5.4	5.4	6	5.4	5.5
5	5.2	5.5	5.3	5.1	5.2	5.9	5.2	5.5
6	5.0	5.4	5.1	4.9	5.1	5.8	5.0	5.2
7	4.9	5.2	4.9	4.6	5.0	5.7	4.7	5.0
8	4.8	5.1	4.8	4.4	4.9	5.6	4.5	4.8
9	4.6	5.0	4.7	4.3	4.8	5.5	4.3	4.6
10	4.4	4.9	4.3	4.2	4.7	5.4	4.0	4.4
11	4.2	4.8	4.2	4.1	4.6	5.3	3.9	4.3
12	4.0	4.7	4.1	4.0	4.5	5.2	3.8	4.2
13	3.8	4.6	3.8	3.8	4.4	5.1	3.7	4.0
14		4.5			4.3	5		3.9
15		4.4			4.2	4.8		3.8
16		4.2			3.8	4.6		3.7
17		4.0				4.4		
18		3.8				4.1		
19						4.0		
20						3.8		
21						3.7		

Table 5. Changes in pH values during dough fermentation of two different bacterial isolate combinations isolated from Dabar.

Time (hours)	A+B	A+C	A+D	A+E	B+C	B+D	B+E	C+D	C+E	E+D
0	5.8	5.8	5.8	5.8	5.8	5.8	5.8	5.8	5.8	5.8
1	5.6	5.6	5.6	5.6	5.6	5.6	5.6	5.6	5.6	5.6
2	5.4	5.4	5.5	5.4	5.4	5.4	5.4	5.4	5.4	5.4
3	5.2	5.2	5.4	5.3	5.2	5.3	5.2	5.2	5.2	5.2
4	5.1	5.1	5	5.3	5.1	5.2	5.1	5.1	5.2	5.1
5	4.8	5	4.5	5	5	5.1	5	4.8	5.1	5
6	4.6	4.7	4.9	4.8	4.9	5	4.8	4.6	5.1	5
7	4.4	4.5	4.6	4.6	4.8	4.9	4.8	4.4	4.9	4.9
8	4.3	4.1	4.4	4.4	4.4	4.8	4.8	4.3	4.8	4.8
9	4.1	3.9	4.1	4.1	4	4.6	4.4	4.1	4.6	4.6
10	3.8	3.8	4	3.9	3.8	4.4	4.2	4	4.2	4.3
11			3.9	3.8		4.1	4.1	3.8	4.1	4.2
12			3.8			3.8	4		3.8	4
13							3.8			3.8

Table 6. Changes in pH values during dough fermentation of three Different bacterial Isolate combinations isolated from Dabar.

Time (hours)	A+B+C	A+B+D	A+B+E	A+C+D	A+C+E	A+D+E
0	5.8	5.8	5.8	5.8	5.8	5.8
1	5.6	5.6	5.6	5.6	5.6	5.6
2	5.2	5.4	5.2	5.2	5.2	5.4
3	5.0	5.2	5.0	5.0	5.0	5.0
4	4.9	4.6	4.9	4.9	4.9	4.4
5	4.8	4.4	4.6	4.6	4.8	4.2
6	3.9	3.9	3.9	3.9	4.6	4.0
7	3.8	3.8	3.8	3.8	3.9	3.9
8	3.7	3.7	3.7	3.7	3.8	3.8
9					3.7	3.7

Table 7. Changes in pH values during dough fermentation of four Different bacterial isolate combinations isolated from Dabar.

Time (hours)	A+B+C+D	A+B+C+E
0	5.8	5.8
1	5.6	5.6
2	4.2	5.2
3	4.0	4.1
4	3.9	4.0
5	3.6	3.9
6	3.5	3.8
7		3.7
8		3.5

Table 8. Changes in pH values during dough fermentation of five different bacterial isolate combinations isolated from Dabar.

Time (hours)	A+B+C+D+E
0	5.8
1	5.4
2	4.6
3	4.1
4	3.6
5	3.5
6	3.4

Table 9. Changes in pH values during dough fermentation of two different bacterial isolate combinations isolated from Fetirita.

Time (hours)	F+M	F+N	M+N
0	6.1	6.1	6.1
1	6.1	6.1	6.1
2	6.0	6.1	6.0
3	5.9	6	5.8
4	5.7	5.9	5.5
5	5.5	5.7	5.2
6	5.3	5.3	5.0
7	5.0	5.1	4.8
8	4.7	5.0	4.6
9	4.3	4.9	4.0
10	4.0	4.4	3.9
11	3.9	4.0	3.7
12	3.7	3.9	
13		3.8	
14		3.7	
15			

Table 10. Changes in pH values during dough fermentation of three different bacterial isolate combinations isolated from Fetirita.

Time (hours)	F+M+N
0	6.1
1	6.1
2	5.9
3	5.7
4	5.5
5	5.2
6	5.0
7	4.7
8	4.4
9	3.9
10	3.8

4. Conclusion

In conclusion, fermented sorghum in form of (kisra) is one of the important staple foods in most Sudanese States. This study showed the microbiology and biochemical changes that occurred during the traditional fermentation oftwo sorghum varieties (Dabar and Fetirita) doughs for Kisra making, the fermentation was found to be mainly a lactic acid fermentation. Sevenspecies of lactic acid bacteria were isolated from the dough are *lactobaciilus* and one is *streptococcus*. It is interesting to note that with increase in the number of isolates the rate of fermentation and acid production and acid tolerance increased. The bacterial combinations were more efficient than individual strains. This can explain why this fermentation is always a mixed fermentation. Starters from individual strains will not be expected to be as effective as mixed populations. This can help in the choice of bacterial strains suitable for starter culture production.

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