Journal of Microbiology Research 2013, 3(3): 124-129
DOI: 10.5923/j.microbiology.20130303.04

Impact of Combination of Lactic Acid Bacteria and Yeasts in Fermentation of Jibna-beida

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Abstract Jibna-beida, a semi-traditional cheese of Sudan made from raw cow's milk, goat's milk or combination of both with variable qualities. The present study aimed to investigate the impact of using lactic acid bacteria (LAB) and yeasts initially isolated from traditional cheese in production of laboratory made jiba-beida under controlled conditions and evaluation of the product. The activity of LAB isolates was examined via determination of clotting time, presence of inhibitory activity against pathogenic microorganisms and antibiotic resistance. Two types of jibna-beida were produced: LSKJ cheese prodcued using *Lactobacillus plantrum, Streptococcus thermophillus* and *Klaveromyces lactis* as combined starter and LSSJ, cheese produced using *Lactobacillus lactis, Streptococcus lactis*, *and Saccharomyces rouxii* as combined starter. Significant changes were found in most of the various chemical components of tested cheeses as a result of using different starter cultures. The microbiological analysis revealed absence of colifoms as well as pathogenic bacteria (staphylococci and salmonella) in all tested cheeses. However, $2.85\times10^5\pm 0.421$ cfu/g total bacterial count in were found in LSKJ which were lower than those of LSSJ ($6.55 \times 10^5\pm 2.425$ cfu/g. On the other hand, LAB count of LSKJ ($7.7\times10^4\pm 0.462$ cfu/g) was lower than that LSSJ ($5.6 \times 10^5\pm 4.041$ cfu/g). The yeasts and moulds was $5.0 \times 10^2\pm 1.155$ cfu/g and $4.0 \times 10^3\pm 4.041$ cfu/g LSKJ and LSSJ, respectively. The laboratory made jibna-beida samples were highly acceptable by the panelists. The study demonstrated the potential probiotic ability of the isolated LAB species from jibna-beida prepared at house hold level at El Dueim area.

Keywords Jibna-beida, Lactic Acid Bacteria, Antimicrobial Activity, Antibiotic Resistance

1. Introduction

Jibna-beida (white cheese) is one of the most cheeses available in Sudanese market. Jibna beida invariably contains lactic acid bacteria (LAB), yeasts and coliform bacteria. In the cheese on the market, yeasts have been found to dominate the microflora[1]. On prolonged storage, however, LAB increased to much higher levels than the yeasts[2].

Lactic fermentation has been known for a long time and the expression "Lactic acid bacteria" was used by the early bacteriologists to describe those bacteria that spontaneously soured traditional lactic acid fermented foods.

In developed countries, most of the lactic acid fermentation has been concentrated in dairy and vegetable products while in the developing countries and in particularly Africa, lactic acid fermentation predominates all the indigenous processing of cereals like maize, sorghum, millet and root crops such as cassava[3].

Several previously published reports have indicated the presence of *Lactobacillus* strains in sheep and cow milk[4]. In addition, several studies have shown the inhibitory activities of numbers of LAB such as *Lactobacillus brevis* isolated from Turkish dairy products[5]. and *Lactobacillus acidophilus* isolated from Iranian yoghurt against *Staphylococcus aureus*[4].

Antimicrobial activity of lactic acid bacteria isolated from milk products has been the subject of intensive research due to the potential application of these bacteria as productive cultures in biological preservation[6]. The major groups of inhibitory compounds produced by LAB include: lactic acid and other volatile acids (decrease the pH), other primary metabolites such as hydrogen peroxide, carbon dioxide , diacetyl, and bacteriocins- special antimicrobial compounds.

Each of these groups of compounds, especially a combination of them, can be used to extend the shelf life and safety of food products.

Starter culture is very important for fermentation processes. Growth of fermenting microorganisms can be quite slow for some species under certain conditions when the concentration of cells is too low. Log-phase growth is

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Published online at http://journal.sapub.org/microbiology

powerful, and so one would like to keep cells in this state for the experiment at hand. Different genes are expressed then compared to a stationary phase.

In addition, for the culture to out-compete a contaminant if there is one. That is more easily accomplished with a starter culture, which is then used to inoculate a larger culture for scale-up. Inoculating directing into the large-sized flask may allow your bacteria to enter a stationary phase, thus giving an opportunity for other species to out-compete your bacteria. The objective of the present study:

2. Materials and Methods

2.1. Bacterial Strains and Culture Conditions

The LAB strains used in the present study were grown in MRS broth (HiMedia India) at 37ºC for 24-48 h in anaerobic jars. The pathogenic strains used in this study were grown in BHI (HiMedia-India) at 37ºC for 18-24 h under aerobic condition. All strains were maintained at 4˚C (in aerobic condition). The purified isolates were preserved in MRS broth with sterile glycerol (15%) and stored at -70ºC (Badis *et al*., 2004). The isolated LAB were tentatively identified as

Lactobacillus plantrum, Lactobacillus lactis, Streptococc us thermophillus and Streptococcus lactis.

2.2. Activation Test of LAB

To determinee the activity of the LAB isolated from collected cheese samples to be used in production of laboratory made jiban-beida, the following tests were carried out:

2.3. Clotting times and Acidification Rates

To determinee the clotting times and acidification rates of LAB, equal volumes of milk (10 ml) were placed in four test tubes, which were incubated with about (10^8 cfu) of *Lactobacillus plantrum, Lactobacillus lactis, Streptococcus thermophillus and Streptococcus lacti.* The clotting times and acidification rates were recorded after 1- 8 hours.

2.4. Antimicrobial Activity Against Pathogens

Twenty eight LAB isolates demonstrating high antibacterial activity against other selected LAB isolates were further checked against other Gram positive and negative pathogens by agar well diffusion method, the bacterial strains which were used as indicator culture included: *Salmonella spp, Pesudomonas aeuriginosa, Staphylococcus aureus and E. coli* O157:H7 were used as test strains to evaluate the antimicrobial effects of LAB isolated from cheese. *Salmonella sp*., *Pesudomonas aeuriginosa* and *Staphylococcus aureus* isolates were obtained from the Food Research Center and from Faculty of Animal Production, Khartoum University. *E. coli* O157:H7 was isolated from hamburger meat and was obtained from the Food Microbiology Laboratory of Khartoum University.

The antimicrobial activities of the isolates were quantified by modifying the disc diffusion. A well-isolated colony was selected from MRS agar plate culture. The top of the colony was touched with a loop, and the growth was transferred into a tube containing sterile 5ml MRS broth. Then broth culture was incubated at 37˚C for about 24 hrs. To get the culture filtrate a 24hrs cultures were centrifuged (10,000 rpm for 20min, at 4˚C) then was adjusted to pH 7 by 1M NaOH to exclude antimicrobial effect of organic acid[7]. Two control test materials also were prepared using un-inoculated MRS broth.

An actively growing test (indicator) microorganisms in a Tryptone Soya broth (OXOID of 24hrs culture at 35˚C were dipped with a sterile cotton swab which was rotated several times and pressed firmly on the inside wall of the tube above the fluid level to remove excess inoculum from the swab. The dried surface of a Mueller-Hinton agar (HIMIDIA) plates were inoculated by streaking the swab over the entire sterile agar surface. This procedure was repeated by streaking two more times, rotating the plate approximately 60˚C each time to ensure an even distribution of inoculum. A sterile approximately 6 mm in diameter discs (Whatman filter paper No. 1) were delivered with culture filtrate of each isolate by using a wire loop of 20 gauge wire having 2mm of diameter, that can deliver 5µl of the extract to each disc[8].

Four discs (with culture filtrate) were placed on a 100mm plate within 5 to 15 minutes of striking of the test organisms. After 12 and 24 hours of anaerobic incubation, each plate was examined. The diameter of the zones of complete inhibition was measured, including the diameter of the disc. Zones are measured to the nearest whole number in millimeter, using transparent ruler. Method assay procedure of Tadese *et. al.,*[9] was used.

2.5. Antibiotic Resistance

Four strain identified *L. lactis, L. thermophillus, S. Lactis and S. plantarum,* were selected on the basis of their desirable technological characteristics[10]. They were tested for resistance to 8 antibiotics produced by Biodiscs were; Chloramphenicol (25mcg), Erythromycin (5mcg), Fusidic acid (10mcg), Methicllin (10mcg), Novobiocin (5mcg), Penicillin-G (1unit), Streptomycin (10mcg), Tetracycline (25mcg). This testing was performed using the standard disc diffusion method .

2.6. Manufacture of Jibna-beida

Jibna-beida was made as described by Osman[11] using two types of starter cultures, (1) starter culture consisting of *Lactobacillus plantrum, Streptococcus thermophillus* and *Klaveromyces lactis* as combined starter (LSK), and (2) starter culture consisting of *Lactobacillus lactis, Streptococcus lactis*, *and Saccharomyces rouxii* as combined starter (LSS). Those LAB and yeasts strains (2%) which were previously isolated from collected cheese samples,

were added to the pasteurized milk at 40 ºC, then rennet (0.07 % g/L) was dissolved in milk. Salt (7 g/L) and the inoculated milk were stirred for 5 minutes, and then the mixture was left to develope a curd. The curd after coagulation was cut by stainless kitchen knife and left for 5 minutes to separate the whey. This was collected, and kept in room temperature. The curd was collected and transferred into clean wooden moulds lined with clean clothes then pressed with 1 kg weight overnight. In the next day the curd was cut into cubes and weighted, then transferred into plastic containers and the whey was added. Cheese was then stored for 1 day first, and then transferred to refrigerator at 5 ºC.

2.7. Chemical Analysis of Laboratory-made Jibna-beida

The various chemical analysis which included mositure, protein, fat, ash, total soluble solid,, lactose and pH were determined in laborotary- made jibna-beida according to AOAC[12].

2.8. Microbiological Analysis of Laboratory-made Jibna-beida

The various microbiological analysis which included total bacterial count, coliform, *Staphylococcus aureus*, yeast and mould, detection of Salmonella and lactic acid bacteria were estimated in laborotary- made jibna-beida using standard methods.

2.9. Sensory Evalution

Jibna-beida sample were subjected to sensory evalution using (15-20) panelists, the panelists were asked to assess each sample for colour, appearance, flavour, texture and overall acceptability A 9 point hedonic scale with 1 as the extremely bad and 9 the excellent. All analysis took place in a room free from disturbing noises, and in which fresh air was circulation conditions were equalized for all the tests. The order of presentation for samples was randomized and the samples were given codes before being tested.

2.10. Statistical Analysis

Statistical analysis was done using Statistical Package for Social Studies Software[13]. Complete randomized design was used to estimate chemical, microbiological and sensory characteristics of the jibna-beida.

3. Results and Discussion

3.1. Activation test of LAB using for Manufacture of Jibna-beida

Table (1) shows the time/hour of clotting and acidification rates of *Lactobacillus plantrum, Lactobacillus. lactis, Streptococcus. thermophillus and Streptococcus. lactis,* the result shows that the time of clotting rates of these microorganisms were after 6, 8, 7 and 8 hours, respectively. These results indicate that the *Lactobacillus plantrum* was faster to clot (after 6 hours). On the other hand the acidification rates according to clotting time were 3.8, 3.8, 3.9 and 3.8, respectively.

Table 1. The clotting time and acidification rate of lactic acid bacteria

Time(hr)	L. p	L. l	S.t	S.l
0	6.28	6.24	6.30	6.37
1	6.00	6.10	6.20	6.30
2	5.80	5.71	5.82	5.75
3	5.00	5.40	5.20	5.50
4	4.80	5.00	4.90	5.50
5	4.00	4.80	4.50	5.10
6	3.80 *	4.30	4.10	4.70
7	3.70	4.00	3.90 *	4.40
8	-	3.80 *	-	3.80 *

*≡Determine the time of clotting
L. p ≡ Lactobacillus plantrum; L. l ≡ Lactobacillus lactis;
S. t ≡ Streptococcus thermophillus; S. l ≡ Streptococcus lactis

3.2. Antimicrobial Activity

Table 2. The diameter (mm) of inhibition zones determined by LAB against tested organisms

LAB isolate	E. coli	Pseud.	Sal.	S. aureus
Streptococcus thermophillus	1.4	1.0	1.3	1.0
Streptococcus laclis	1.5	1.1	1.0	1.2
Lactobacillus plantrum	1.0	1.2	1.0	1.3
Lactobacillus laclis	1.2	1.0	1.1	1.4

E.coli = Escherischia coli; Pseud.= Pesudomonas aeuriginosa
Sal. = Salmonella spp ; S. aureus ≡ Staphylococcus aureus

Table (2) shows the antimicrobial activity/hour of the tested LAB. Inhibition zone diameters of Streptococcus. thermophillus was <1.0 mm for Salmonella spp, E. coli O157:H7, whereas it was only 1.0 mm for Pesudomonas aeuriginosa and Staphylococcus aureus, these indicate that Streptococcus.thermophillus more effective against E. coli O157:H7 followed by Salmonella spp, Pesudomonas aeuriginosa and Staphylococcus aureus. Inhibition zone diameters of Streptococcus. Lactis was <1.0 mm for E. coli O157:H7, Pesudomonas aeuriginosa and Staphylococcus aureus, where it was only 1.0 mm for Salmonella spp. On the other hand inhibition zone diameters of Lactobacillus. plantrum were 1.0, 1.2, 1.0 and 1.3 for E. coli O157:H7, Pesudomonas aeuriginosa, Salmonella spp and Staphylococcus aureus, respectively, these indicate that the Lactobacillus. Plantrum more effective against Staphylococcus aureus followed by Pesudomonas aeuriginosa, E.coli O157:H7 and Salmonella spp.While Lactobacillus. Lactis more effective against Staphylococcus aureus followed by E. coli O157:H7, Salmonella spp and Pesudomonas aeuriginosa, which inhibitor zone diameters were 1.4, 1.2, 1.1 and 1.0, respectively.

3.3. Antibiotic Resistance

Table (3) shows the antibiotic resistance/hour of LAB. The result shows that *Streptococcus lactis* was moderately sensitive to Chloramphenicol and Novobiocin antibiotic; resistant to erythromycin, penicillin-G, streptomycin and tetracycline antibiotic; sensitive to Methicillin antibiotic. On the other hand *Streptococcus thermophillus* was resistant to

Novobiocin, Penicillin-G, and Streptomycin antibiotic; sensitive to Chloramphenicol, erythromycin, Methicllin and Tetracycline antibiotic. *Lactobacillus. Lactis* resistant to Novobiocin and Penicillin-G antibiotic; sensitive to Chloramphenicol, erythromycin, Methicillin, Streptomycine and Tetracycline antibiotics. Generally most of the LAB isolated from collected cheese samples are resistant, that indicating their high activity during fermentation.

Table 3. The antibiotic resistance of lactic acid bacterial strain

Antibi -otic	Streptococcus lactis	Streptococcus thermophillus	Lactobacillus lactis	Lactobacillus plantrum
C	1.5^{I}	2.2^{S}	2.3^{S}	2.6^{S}
E	1.3^{R}	2.7^{S}	2.6^{S}	2.7^{S}
Fl	1.5	1.0	1.3	1.0
M	2.0^{S}	3.8^{S}	2.7^{S}	2.8^{S}
Nv	2.0^{I}	1.5^{R}	1.5^{R}	1.6^{R}
P	2.5^{R}	2.5^{R}	2.5^{R}	2.7^{R}
S	1.0^{R}	1.0^{R}	1.6^{S}	1.5^{S}
T	1.3^{R}	2.8^{S}	2.7^{S}	2.7^{S}

S=Sensetive; I= Intermediate; R= Resistance
C = Chloramphenicol; E = Erythromycin; F l = Fusidic acid; M = Methicllin
Nv = Novobiocin; P = Penicillin-G; S = Streptomycin; T = Tetracycline

3.4. Chemical Composition of Laboratory Made Jibna-beida

The chemical composition of laboratory – made jibna-beida is presented in Table (4). The moisture content of jibna-beida produced by using combined starter LSK (LSKJ) (50.27±1.25%) was higher than that produced by using combined starter LSS (LSSJ) (49.82±1.33%). However, these findings were higher than those reported by Aly and Galal[14], Sulieman[15] and Ibrahim[1] who found that the moisture contents in pasteurized milk jibna-beida were 47.28, 43.0, and 44%, respectively.

The proteins content of LSKJ (22.40±1.905%) was higher than that LSSJ which was 21.33±1.241%. These values were higher than that determined by Salih[16], who found average of (19.84%) in white cheese made from pasteurized milk.

Fat content of LSKJ (18.61±1.443) was slightly lower when compared with that of LSSJ which was 19.72±1.22%. These results were slightly lower than that reported by Salih[16] in jibna-beida produced from pasteurized milk and Elowni and Hamid[17] in Sudanese white cheese, who found a value of 20.30 and 22.8%, respectively .

Table (4) also shows that the range of ash content was 5.65±0.664% and 5.78±0.739% for cheese produced from combined starter culture LSK and LSS, respectively. While the lactose content was 3.07±0.098% and 3.35±0.185% in LSKJ and LSSJ, respectively. The change in lactose and protein of jibna beida produced by using different starter culture might be due to microbial enzymatic action, normally the chemical changes of carbohydrate appear in conversion of lactose to lactic acid and small amount of acetic acid and pyruvic acid in addition to CO_2. As for fats, they are hydrolyzed to glycerol and fatty acids as a result of microbial action[18].

Table 4. Chemical composition (%) of laboratory–made Jibna-Beida (LSK and LSS)

Parameter	LSK	LSS
Moisture	50.27±1.247	49.82±1.328
Protein	22.40±1.905	21.33±1.241
Fat	18.61±1.443	19.72±1.224
Ash	5.65± 0.664	5.78± 0.739
Lactose	3.o7± 0.098	3.35± 0.185
Total solid (T.S)	49.73±1.328	50.18±1.212
pH	5.90± 0.589	6.50± 0.346

LSK: Contained combined starter :
Lactobacillus planturm + Streptococcus thermophills + Klaveromyces lactis
LSS: Contained combined starter :
Lactobacillus lactis + Streptococcus lactis+ Saccharomyces rouxii

The total solids content were lower in LSKJ (49.73±1.328) when compared with that produced of LSSJ (50.18±1.212%). These results were higher if compared with those reported by El-Zubeir[19] for jibna beida, Sulieman *et. al.*,[20] for Maddafra cheese, Ohaj (2009) for Gouda cheese. Whereas, it was lower than those reported by, Sulieman (2007) and Salih[16], who found 50.48% and a range between (50.40 to 52.20%), respectively. On the other hand, pH value of jibn-beida made from LSK (5.90±0.589) was lower than that of jibn-beida from LSS (6.50±0.346). These results were higher than those reported by Salih[16], who found a range 5.45 to 5.58 for jibna-beida produced in the Sudan.

3.5. Microbiological Characteristics of Laboratory Made Jibna-beida

The microbiological analysis of laboratory made jibna–beida (Table 5) revealed presence of $2.85\times10^{5}\pm 0.421$ cfu/g of total bacterial count in jibna-beida made by using LSK starter culture (LSKJ) which were lower than those of laboratory made jibna-beida from LSS starter culture (LSSJ) ($6.55 \times 10^{5}\pm 2.425$ cfu/g), and these results were in close agreement to those reported by Ahmed, (2010), who obtained a value of 2.8×10^{6} cfu/g in cheese manufactured from raw milk and 1.3×10^{5} cfu/g in cheese manufactured from pasteurized milk. The lower level of total bacterial count in laboratory made jibna- beida were probably due to the effect of starter culture (LAB) and heat treatment which suppress the growth of microorganisms, and also could be attributed to the proper hygienic condition of cheese production.

Table 5. Microbiological characteristics of laboratory made jibna– beida

Cheesetype	Total bacterial count	Coliforms	Lactic acid bacteria (cfu/g)	*Salmonella*	*Staphylococcus aureus* (cfu/g)	Yeast and Mould (cfu/g)
LSKJ	$2.85\times10^5\pm0.421$	Nil	$7.7\times10^4\pm0.462$	-ve	-ve	$7.7\times10^4\pm0.462$
LSSJ	$6.55\times0^6\pm2.425$	Nil	$5.6\times10^5\pm4.041$	-ve	-ve	$4.0\times10^3\pm4.041$

LSKJ= Jibna-beida prepared using LSK starter culture
LSSJ= Jibna-beida prepared using LSS starter culture

LAB count of LSKJ ($7.7\times10^4\pm$ 0.462 cfu/g) was lower than that LSSJ ($5.6\times10^5\pm$ 4.041 cfu/g). The LAB count in this study was higher than that reported by Hamid and Elowni[21] who reported (5.06 log cfu/g) in Sudanese white cheese.

Table (5) also, shows that yeasts and moulds of laboratory LSKJ was lower ($5.0\times10^2\pm$ 1.155 cfu/g) than that of LSSJ ($4.0\times10^3\pm$ 4.041 cfu/g). The result was in close agreement to those reported by Kameni *et.al.,* (2006) and Ahmed, (2010), who report the values 7.6×10^3 cfu/g and 7.0×10^3 cfu/g, respectively. However, the reduction of yeast and mould count could be attributed to the proper hygienic condition employed in cheese. Fortunately, colifoms and the pathogenic microorganism *Salmonella* and *staphylococcus aureus* cells were not found in all laboratory made jibna-beida.

3.6. Sensory Characteristics of Laboratory-Made Jibna-Beida

Data presented in Table (6) show the mean scores of the sensory characteristics of lab-made jibna-beida made from pasteurized milk and defined starter cultures (LSK and LSS). Comments given by the panelists showed preference for the product which has good texture , colour and appearance, little salty and mild acidity flavour.

Table 6. Panelists mean score of sensory characteristics of laboratory-made jibna-Beida

Cheese samples	Colour and appearance	Flavour	Texture	Overall acceptability
LSKJ	7.9	7.5	7.5	7.8
LSSJ	6.9	7.5	7.4	7.3

LSKJ = Cheese prepared using combined starter:
Lactobacillus planturm + Streptococcus thermophills + Klaveromyces lactis)
LSSJ = Cheese prepared using combined starter
Lactobacillus lactis + Streptococcus lactis+ Saccharomyces rouxii

Jibna-beida produced by LSK as combined starter culture had higher scores for colour and appearance compared to that produced by LSS starter culture, with no significant differences ($p\geq0.05$) between the two types of cheeses. There were no significant differences between two types of cheese in flavor which was high in all samples. However the high scores for flavor may be due to high values acetaldehyde and diacetyle produced as results of microbial action (LAB) on lactose, breakdown of protein to flavor compounds and breakdown of fat to volatile fatty acids[22]; [23]. Statistically, significant variation ($p\leq0.05$) were observed in overall acceptability scores between cheese samples. Generally, colour and appearance, flavour, texture and overall acceptability properties of cheese produced from pasteurized milk under control condition were highly acceptable by the panelists.

4. Conclusions

Jibna-Beida can be prepared in the laboratory under controlled conditions using combinations of dominating LAB (with resistance to antibiotics) and yeasts as starter culture and pasteurized milk. Most of the chemical components of laboratory made-jibna- beida samples were in close agreement with data in the literature. The microbiological analysis revealed absence of colifom bacterial and pathogens (salmonella and staphylococci). The sensory analysis indicated high acceptance of the cheese samples by panelists. The inhibitory activity possessed by LAB isolates might be used for the control of pathogens and spoilage bacteria in jibna-beida and other dairy products especially those produced traditionally. So, the findings of the present study suggest that the selected LAB strains isolated might be appear to possess probiotic potential against pathogens and spoilage microorganisms in traditionally fermented dairy products. Since the selected LAB strains isolated from traditionally made jiban-beida possess probiotic potential, so, they could be exploited further for their use in other fermented dairy products. It is recommended that these LAB species can be further studied according to selection criteria like stimulation of immunological system and adhesion to epithelium tissue.

ACKNOWLEDGEMENTS

The authors express their gratitude for all who assisted in the implementation of this work, with special thanks to the microbiological laboratory technicians of the University of Gezira.

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