ISSN: 0378-2700

# PRODUCTION OF VOLATILE COMPOUNDS BY SINGLE STRAINS OF ISOLATED LACTIC ACID BACTERIA IN MODEL FERMENTED MILK

BY

**T.K. Ahmed,*; H.A. Dirar.**and S.H. Hamad**

* Animal Products Department, Food Research Centre
P.O. Box 3231, Khartoum, E.mail:Tag502001@yahoo.com

** Faculty of Agriculture University of Khartoum

**(Accepted 21/8/2005)**

## SUMMARY

***Fifteen*** single strains of lactic acid bacteria isolated from the traditional fermented milk from the three African countries (Sudan, Ethiopia and Uganda) were compared for aroma production in model fermented milk. Seventeen components including esters, aldehydes, ketones and alcohols were identified by gas chromatography – mass spectrometry of volatile compounds produced by lactic acid bacteria. Comparison of the fifteen strains indicated that in the model milk medium all of the strains produced six components including 3- methyl – butanol, acetone, 2-butanone, 2- Heptanone and ethanol .

------------

**Keywords:** aroma, lactic acid bacteria, model milk, strains.

## INTRODUCTION

Dairy products are among the most popular foods and constitute an important item in man's diet in rural areas of the Sudan, where the bulk of the national milk production is produced. Usually most of the milk is preserved through spontaneous fermentation into fermented milk traditionally called "rob". Traditionally, wild lactic acid bacteria represent a natural reservoir for microbial cultures containing diverse genetics information in nomadic communities of Sudan (Dirar, 1993; Ahmed 2000). Some of these strains may be highly resistant to adverse environmental conditions such a high salt concentration and phase inversion. Moreover, they contribute to specific characteristics such as taste, aroma and textural of traditional Sudanese dairy products. Lactic acid bacteria (LAB) have an indispensable technological role in food processing, especially in dairy industry. This unique group of micro-organisms is a part of the dairy diet of virtually people all over the world. They are often added as milk fermentation starter cultures and occur widely as indigenous contaminants in raw milk. They possess a large number of metabolic activities that are responsible for the acceptability of fermented milks as reported by El-soda 1997, (quoted by El-Soda *et al.,* 2003). The isolation and characterization of LAB from traditional dairy products manufactured from raw milk has been considered in the research for new industrially important culture. However, LAB produces various compounds such as organic acids, diacetyl, hydrogen peroxide and bacteriocins or bacterial proteins during lactic fermentation. These organisms and their products give fermented food distinctive flavours, texture and aroma, extending shelf-life and inhibiting pathogenic organisms (Nettle and Barefoot, 1993, Sharaf *et al.*, 1997 and Navarro *et al.*, 2000).

In the present study, the production of aroma components, by pure single strains of lactic acid bacteria was determined in model culture medium. The aromatic potential of strains from various African traditional fermented milks (Ethiopia, Sudan and Uganda) was evaluated to assess the contribution of these single strains to the flavour of model "rob".

## MATERIALS AND METHODS

**Maintenance of Strains :**

The fifteen strains used in this study were isolated from the traditional fermented milks from the three African countries (Sudan, Ethiopia and Uganda). The selection was based on six lactococcus and nine lactobacillus strains (2 and 3 from each country)(Ahmed, 2000).

**Preparation of model fermented milk:**

Pasteurized full cream cow's milk was divided into fifteen equal parts and was inoculated each with one of the isolated strains at the ratio of 5%. The milks were incubated at 30°C (the ambient temperature at which fermentation usually carried – out in rural areas) till the pH of each sample was in the vicinity of 4.5-4.0 (The optimum pH for good quality traditional "rob").

For each strain, the reproducebility of the experiments was evaluated by conducting three replicate model fermented milks.

**Extraction and concentration of the flavour compounds:**

A gas washing bottle containing 200 ml fermented milk and 0.5 ml internal standard (50 mg/L 4.melhyl-1-pentanol in $H_2O$) was placed in a water bath (30°C) over a magnetic stirrer . After a temperature equilibrium time of 10 minute, $N_2$ gas was bubbled through the bottle (gas flow 60 ml/min) and out through a trap consisting of a glass tube (6.8 cmX0.4 cm) containing Porapak Q (50-80 mesh) which had been previously cleaned with pure diethyl ether. After trapping for 60 minutes, the trap was removed and adsorbed volatiles eluted with diethyl ether. The amount of eluent collected was concentrated to about 100 mg with a $N_2$-gas stream.

**Analysis of volatiles:**

The GC-MS analysis and identification of flavour compounds by GC-MS were as described by Nana, (1998), as follows: Two microlitre extract were injected (split ratio 1:20) using the temperature program: 10 minute at 40°C increased to 24o°C at 6°C/minute and held constant at 240C for 30 minutes. Identification was obtained by probability-based matching with mass spectra in the 5973 NIST – PBH library (Hewlett Packard containing about 75000 reference spectra.

However, quantification was based on relative peak areas which were calculated from total ion chromatogram. (peak area divided by internal standard peak area) and this provided a means of comparing values within compound and not between compounds.

Experimental data were presented as means of triplicate measurements. Data were subjected to ANOVA according to Snedecor and Cochran (1978).

## RESULTS AND DISCUSSION

**Identification of the aromatic substances:**

Chromatographic analysis of aromatic extracts obtained from the fifteen strains in the model milk enabled 23 different peaks to be distinguished on the gas chromatograms (numbered from 1 to 23), but only 20 peaks contained sufficient materials to allow identification. All of these molecules were identified by their mass-spectra and retention time. The concentration of the compounds were expressed in ppb (Table 1).

In model milk medium the strains producing the highest concentration of volatile >2500 ppb were nearly all of the strains except EE 836, E1 875, E1 675, E1 677 and UK 1852 (Table 2).

Ethyl acetate was present only in six cultures SU 99, UK 1560 SU 174, SU 144, SU 171 and UK 1952. Four of these cultures were lactobacillus. Strains SU 99 and UK 1560 were *lactoccocus lactis*.. The highest concentration was present in UK 1560 culture rather than the, other cultures (Table 1).

**Aldehydes:**

All aldehydes produced in the model milk by these cultures, were derived from non-lipid compounds, 2-methyl- propanal, 2-methyl-butanal and 3-methyl-butanal were present in minor quantities. In spite of their minute quantities they are important contributors to the aroma of fermented milks. 3-Methyl-butanal which is most probably derived form leucine via a Streker degradation was found in minute amounts almost in all model milk media expect that model milk cultured by UK1394 and EI 675, while 2-methyl-butanal was present in model milk culture by SU 189 only . Butanal was detected in model milk media cultured by EI 677. 3- methyl-1-Pentanal was present in model milk culture by SU 189, EE 836 and UK 186, while hexanal was detected in very minute amount in samples SU 189, SU 99, UK 1560, SU 171, EI 675, EI 474 and UK 1952 (Table 1). Similar findings were reported by Moio *et al.* (1993).

**Ketones:**

All 15 strains studied produced acetone, 2-butanone, 2,3-butanedione and 2-heptanone in minute quantities. These compounds occupy a special position in the flavour formation of the fermented milk and in spite of their minute quantities, their effect on aroma is very prominent. (Margalith, 1981). 3-hydroxy-2-butanone was present only in sample UK 1560 in very low concentration. Similar amounts were reported for yoghurt by Gaafar (1992). Among the 20 components identified, ketones contribute >70% for the majority of the strains studies (Table 2), almost ten times more than ketones reported for raw milk by Moio *et al.* (1993).

**Table (1) Flavour components of robe produced by single strains (ppb)**

| Peak No. | Compound | SU 189 | SU 99 | EE 861 | EE 836 | UK 1394 | UK 1560 | SU 174 | SU 144 | SU 171 | EI 675 | EI 474 | EI 677 | UK 1952 | UK 3.338 | UK 186 |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | **1. Ester** | | | | | | | | | | | | | | | |
| 4 | Ethylacetate | - | 5 | - | - | - | 115 | 35 | 40 | 70 | - | - | - | 5 | - | - |
| | **2. Aldehydes** | | | | | | | | | | | | | | | |
| 4 | Butanal | - | - | - | - | - | - | - | - | - | - | - | 0.009 | - | - | - |
| 3 | 2- Myethyl Propanal | - | - | 0.022 | - | 1.530 | 0.009 | 0.088 | - | - | - | - | - | - | - | - |
| 16 | 2- Methyl butanal | 0.009 | - | - | - | - | - | - | - | - | - | | | | | |
| 4 | 3- Methyl butanal | 0.172 | 0.008 | 5.272 | 0.030 | - | 3.092 | 0.024 | 0.772 | 0.024 | - | 0.005 | 0.012 | 0.011 | 0.007 | 0.012 |
| 10 | 3-Methyl pentanal | 0.208 | - | - | 0.106 | - | - | - | - | - | - | - | - | - | - | 0.008 |
| 13 | Heptanone | 0.013 | 0.008 | - | - | - | 0.008 | - | - | 0.007 | 0.001 | 0.009 | - | 0.007 | - | - |
| | **3. Ketones** | | | | | | | | | | | | | | | |
| 2 | Acetone | 730 | 625 | 1855 | 1075 | 825 | 1940 | 40 | 815 | 200 | 518 | 600 | 805 | 895 | 760 | 745 |
| 5 | 2- butanone | 605 | 540 | 680 | 630 | 620 | 605 | 20 | 385 | 15 | 535 | 490 | 520 | 515 | 500 | 485 |
| 8 | 2,3- Butanedione | | 690 | 725 | - | 495 | 1360 | 255 | 300 | - | - | 1535 | 280 | 585 | 2755 | 1715 |
| 23 | 3- Hyderoxy -2- butanone | - | - | - | - | - | 20 | - | - | - | - | - | - | - | - | - |
| 17 | 2- Heptanone | 65 | 80 | 175 | 150 | 160 | 65 | 60 | 55 | 405 | 85 | 225 | 70 | 170 | 120 | 150 |
| | **4. Alcohols** | | | | | | | | | | | | | | | |
| 7 | Ethanol | 15 | 605 | 90 | 50 | 770 | 1680 | 2370 | 1210 | 8305 | 125 | 100 | 200 | 155 | 90 | 65 |
| 9 | Propanol | - | - | - | - | - | 300 | 50 | - | - | - | - | - | - | - | - |
| 10 | 2- butanol | 5 | 10 | - | 10 | 5 | 5 | 345 | 145 | 380 | 10 | 5 | 10 | 10 | 5 | 5 |
| 15 | 3- methyl Propanol | - | 10 | 20 | 10 | 5 | 270 | 10 | 15 | 30 | - | - | 15 | - | - | 5 |
| 20 | 3- methyl butanol | 3670 | 10 | 780 | 40 | 40 | 740 | 410 | 375 | 150 | 10 | 5 | 25 | 20 | 10 | 50 |

SU 189 = *Streptococcus salivarius*; SU 99; EE 861; EE 836; UK 1394; UK 1560 = *Lactococcus lactis*, SU 174; SU 144 = *Lactobacill Fermentum*, SU171 = *Lactobacillus heleviticus*, E147 4, 675, 677; UK 1952 = *Lactobacillus plantarum;* UK 1560, 3.338 = *Lactobacill casei* (in this and in subsequent tables)

**Table (2): Level of volatile components grouped according to chemical classes in "rob" produced by single strains (in ppb)**

| Chemical group | SU 189 | SU 99 | EE 861 | EE 836 | UK 1394 | UK 1560 | SU 174 | SU 144 | SU 171 | EI 675 | EI 474 | EI 677 | UK 1952 | UK 3.338 | UK 186 |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| Esters | - | 5 | - | - | - | 115 | 35 | 40 | 70 | - | - | - | 5 | - | - |
| Aldehydes | 402 | 16 | 5294 | 136 | - | 4630 | 33 | 810 | 31 | 11 | 14 | 21 | 18 | 7 | 20 |
| Ketones | 1400 | 1935 | 3435 | 1855 | 2090 | 3990 | 3370 | 1555 | 620 | 1435 | 2850 | 1675 | 2165 | 4135 | 3095 |
| Alkohols | 3690 | 635 | 890 | 110 | 820 | 3005 | 3165 | 1745 | 8865 | 145 | 110 | 250 | 185 | 105 | 125 |
| Total | 5492 | 2591 | 9619 | 2101 | 2910 | 11740 | 3603 | 4150 | 9586 | 1591 | 2974 | 1946 | 2374 | 4247 | 3240 |

**Alcohols:**

Data in table 1, clearly indicated that all of the strains used in this study produced primary alcohols in different quantities ranging in the vicinity of 5-8305 ppb. While propanol was present only in two strains UK 1560 and SU 174. However, most of the strains produced primary alcohols such as ethanol, Propanol, 2-butanol, 3-methyl – Propanol and 2-methyl – butanol, by the catabolism of amino acids. These results are in accord with observation of Degoree-Dumas *et al.* (1984); Latrasse *et al.* (1987) and Jolivet *et al.* (1994).

Generally, in the model milk medium all the strains produced common five components such as (3-methyl – butanol, acetone, 2- butanone, 2- Heptanone and ethanol. (Table 1). While the concentration of all these components in the extracts were low and generally equal to or below the sensory threshold level (24000 ppb for 3- methyl –butanol). In this medium all strains produced very high level of concentration of methyl ketone (70 ppb in milk., Bandings 1984).

## REFERENCES

Ahmed, T.K. (2000): Standardization of traditional "Rob" production procedure. Ph.D thesis, University of Khartoum, Sudan.

Badings, H-T. (1984): Flavour and off-flavour. In Dairy Chemistry and physics. pp. 336-357, (Eds) P.Walstra and R. Jenness, New York, Wiley.

Degorcc-Dumas, J.R; More, J.G. and leveau, J.Y (1984):. Aroma production by microorganisms. The potentialities. Industries Agricolc et Alimentaries, 101:11-15

Demirci, Y. and Hemme, D.(1995): Acidification test based on the use of biomas for screening of leuconostoc. Application to Lu. mesenteroids strains isolated from French raw milk cheese. J. Dairy Res., 62:521-527.

Dirar, A.H (1993): The Indigenous Fermented Foods of the Sudan (A study in African Foods and Nutrition) Book, published by CAB International, Walling Ford, U.K.

El-Soda, M.; El – Ziny; M; Awad, S., Osman, G.; Omran, N.; Gamal, G.; Ezzat, N. and El-Shafei, H (2003): A culture collection of lactic acid bacteria isolated from raw milk and traditional Egyptian dairy products. Egyptian J. Dairy sci, 31:23-41

Gaafar, A.M. (1992): volatile flavour compounds of yoghurt. Inter. J. Food Sci. & Tech., 27:87-91

Jellivet, N; Chatand,J., Vaysier, Y.; Bensousan, M. and Belin, J.M. (1994): production- volatile compounds in model milk and cheese media by eight strains of Geotrichum Candidum Lunk. J. Dairy Res., 61:241-248.

Latrasse, A.; Dameron, P.; Hassoni, M and Staron, T.(1987): Production of a fruity aroma by Geotrichum Candidum (strains), Science de aliments,7:637-645.

Margalith, P.Z. (1981): Flavour Microbiology, Pub. Charles, C. Thomas, Illinois, U.S.A., pp 33-43

Moio, L.; Dekimple, J.; Etievant, P. and Addeo, F. (1993): Neutral volatile compounds in raw milks from different species. Journal of Dairy Research 60:199-213.

Navarro,L., zarazaga, M.; Saenz, J;Ruiz-Larrea, F. and Tarres, C. (2000): Bacteriocin production by lactic acid bacteria isolated from Rioja red wins. J. Appl. Micro. 88:44

Nettles, C.G. and Barefoot, S.F. (1993): Biochemical and genetic characteristics of bacteriocins of food associated lactic acid bacteria . J. Food Port. 56:338

Sharaf, O.M.; Ibrahim, G.A.;El-Shafei, K. and Twafik, N.F. (1997): Comparison of bacteriocins antimicrobial activity of bacteriocins from some lactic acid bacteria, propionibacteria and enterococci against food bacterial pathogens. Annals of Agric. Sc. Moshthor. 35: 1413.

Snedecor, G.W. and Cochran, W.G. (1978): statistical methods, sixth edition. The Iowa StateUniversity press. Ames, Iowa, U.S.A

## إنتاج مركبات طياره بواسطة سلالات منفردة معزولة من بكتيريا حامض اللاكتيك في نموذج لبن متخمر

تم عزل سلالات لبكتريا حمض اللاكتيك من الألبان المتخمرة التقليدية لثلاث دول إفريقية (السودان ـ أثيوبيا ـ أوغندا). إستخدم ١٥ سلالة منفردة من السلالات المعزولة في تخمر لبن متخمر نموذجي ودرست الإختلافات في مكونات النكهة الطيارة في كل منها. وقد أمكن تميز ١٧ مكون ضمت أسترات وألدهيدات وكيتونات وكحولات بإستخدام الفصل الكروماتوجرافي الغازي ـ المقترن بطيف الكتلة. وقد وجد أن السلالات الخمسة عشر المختبرة تشترك في إنتاج ٦ مكونات يشمل ٣- ميثيل بيوثانول، الأسيتون، ٢- بيوثانون ، هيتانون، الإيثانول.