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Effect of *Leuconostoc mesenteroides* on the visco-elastic properties of sour maize meal

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Abstract: This paper investigates the effect of *Leuconostoc mesenteroides* as starter culture in the fermentation of maize meal for sour maize bread production. The aim was to test and evaluate the possible beneficial role of this organism on the visco-elastic properties of fermented maize meal. 5 ml of inoculum containing 2 x 10^8 log cfu/ml cells of *L. mesenteroides* were inoculated into 50 g moistened maize meal (maize meal : water = 1 : 1 w/v). All viscosity parameters recorded were significantly ($p \leq 0.05$) affected by the use of *L. mesenteroides* as starter culture. Use of *L. mesenteroides* in the fermentation of maize meals increased viscosity of maize meals from 95.22 RVU to 144.00 and 169.25 RVU after 12 h and 24 h of fermentation respectively. Inclusion of *L. mesenteroides* in starter culture development for sour maize dough is identified as important for developing good structural properties of the dough.

Keywords: *Leuconostoc mesenteroides,* viscosity, starter culture, fermentation, sour maize meal

Introduction

Leuconostocs represent a small percentage of the mesophilic micro-flora belonging to the group of lactic acid bacteria that is largely composed of lactobacilli, streptococci, lactococci and pediococci. In spontaneous fermentation processes, the other lactic acid bacteria tend to dominate the growth of *Leuconostoc* species because of their slow growth rates and weak acidifying properties. As a result, Leuconostocs are often not included in mixed starter cultures. This exclusion of Leuconostocs often causes a reduction in flavor and aroma and a general lowering in the typical characteristics of the product (Wood and Holzapfel, 1995). However, in a well-balanced starter culture, Leuconostocs play an important role in the rheological and flavoring quality of the fermented product (Angioloni *et al*., 2006). They are able to perform this role because of the diacetyl (oxidized acetoin) and acetoin produced by certain strains. These compounds contribute to the typical flavor and taste of many foods, particularly dairy products. Previous studies have focused on the antagonistic activity of *Leuconostoc* species and the organism's ability to improve organoleptic properties in fermented vegetables (Lee and Kim, 1988; Valdez *et al*., 1990; Kim *et al*., 1991), wine production (Winbowo *et al*., 1985) and dairy products (Litopolou-Tzenetaki, 1990; Coppola *et al*., 1990; Garrido-Gomez *et al.*, 1991). In carrot and cabbage fermentations, *Leuconostoc mesenteroides* and *Lactobacillus pentosus* are used as starter cultures; *Leuconostoc mesenteroides* produces acetic acid that inhibits the growth of *Listeria monocytogenes* and most other spoilage bacteria and increases the organoleptic quality of the products (Delclos, 1992). The presence of *Leuconostoc* species associated with Lactobacilli is considered very important in the fermentation of sourdough. Azar *et al*. (1977) reported that Leuconostocs produce the desirable flavor compounds and sour taste in Sangak dough, although at that time the correct composition of these bacteria in the complex mixture of microorganisms present in dough fermentation was not fully known. Coppola et al (1998) later investigated the role of *Leuconostoc mesenteroides* in addition to *Saccharomyces cerevisiae* as leavening micro-flora of pizza dough. More recently, research has shown

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that this group of organisms plays a wide variety of roles in the sourdough industry. Leuconostocs produce bacteriocins (Corsetti *et al*., 2004), exo-polysaccharides (Tieking *et al*., 2003) and other metabolic products especially lactic and acetic acids (Wehrle *et al*., 1997) all of which have a great influence on the sensory quality, texture and shelf life of sour dough breads (Goldberg, 1994; Thiele *et al*., 2002). Tieking *et al*. (2003) concluded that the use of leuconostocs in bread production may allow the replacement of additives such as hydrocolloids currently used as texturizing, anti-staling or probiotic agents in bread making. In previous studies, the authors of the present investigation have enumerated the microflora of spontaneously generated sour maize meal (Edema and Sanni, 2006) and evaluated the functional properties of dominant lactic acid bacteria and yeast indigenous to the maize meal for use as starter cultures (Edema and Sanni, 2008). The general objective of the present work was therefore to evaluate and determine the effect of *Leuconostoc mesenteroides* as a mono-culture on the visco-elastic properties of maize meal for sour maize dough.

Materials and Methods

Sample collection

A commercial flour variety of white maize (*Zea mays*) was obtained from the local market in Ibadan, South-western Nigeria. The grains were milled into maize meal with particle size > 0.2 mm which is particularly valuable as an ingredient for maize bread (Okoruwa, 1995). A knife mill (Fritsch Industriestr. 8 0-55743, Idar-oberstein, Germany) was used for milling. The chemical characteristics of the maize meal were as follows: moisture content 7.15%, fat 4.09%, protein (N x 5.70) 8.96%, fibre 1.48%, ash 1.33% and total carbohydrate 77.06% of dry matter (Edema *et al*., 2005).

Culture conditions for Leuconostoc mesenteroides

Leuconostoc mesenteroides previously isolated from spontaneously generated sour maize meal (Edema and Sanni, 2006) was sub-cultured twice from Hogness' freezing medium by transferring 2 ml of each stock culture into 8 ml of de Mann Rogosa and Sharpe (MRS) broth medium (Oxoid, Hampshire, UK), before use. Incubation was at 30^0C for 48 h under anaerobiosis. The organism was examined for production of antimicrobial compounds and exo-polysaccharides by growing on MRS broth medium. For determination of exo-polysaccharide production, sucrose agar of Garvie (1960) was used according to Harrigan and McCance (1976). Poured dried plates of sucrose agar were inoculated with test cultures by streaking to obtain separate colonies. Incubation was at 37^0C for up to 5 days. Polysaccharide production from sucrose was indicated by the development of mucoid growth.

Fermentation of maize meal

The broth cultures of *Leuconostoc mesenteroides* were inoculated into fresh MRS broth medium and incubated as above. During incubation, 1 ml each of broth culture was plated on MRS agar plates using the pour plate technique to determine broth cultures containing about 2 x 10^8 cfu/ ml. Broth cultures containing the required concentration of viable cells were obtained after about 18 h of incubation. The cultures were centrifuged (Labofuge 200, Kendro Laboratory Products, Germany) at 6000 x g for 10 min, washed in sterile distilled water and re-centrifuged. The washed, harvested cells were then used as inoculum in the fermentation of maize meals. Five milliliters of inoculum was added to 50 grams of moistened maize meal (maize meal:water = 1 : 1) and thoroughly mixed in a glass bowl using glass rod as stirrer. For spontaneous fermentation, 5 ml of sterile water was added to the mixture to give the same volume. Fermentation was carried out at ambient temperature (29±2^{o}C) for 24 hours.

Analyses of fermenting maize meal

The fermenting maize meal was analyzed for:

i. pH with a pH meter (Mettler-Toledo, Essex M3509 Type 340)
ii. Acid equivalent by titration with 1 N NaOH using phenolphthalein as indicator where acid equivalent is the amount of NaOH consumed per gram in milliliters
iii. Cell growth by plating on MRS agar medium and incubating anaerobically at 30^0C for 48 h
iv. Diacetyl production by mixing 10 g fermenting maize meal in 90 ml tap water and titrating 25 ml each of the homogenized mixture to which 7.5 ml of Hydroxylamine solution (1 M) were added in two flasks (one flask was for residual titration. Both flasks were titrated with 0.1 N HCl to a greenish yellow end point using bromophenol blue as indicator (Sanni *et al.,* 1995). The equivalence factor of HCl to diacetyl is 21.52 mg. The concentration of diacetyl produced was calculated thus:

$$Ak = \frac{(R-S)\,(100E)}{W}$$

Ak = Percentage of diacetyl (mg)
R = ml of 0.1 N HCl consumed in residual titration
S = ml of 0.1 N HCl consumed in titration of sample
E = Equivalence factor W = Volume of sample

Visco-elastic properties of fermented maize meal

Visco-elastic properties of the fermented maize meals were determined with a series 3 RVA rapid visco-analyser, which runs with the Thermocline for windows software (Newport Scientific Pty. Ltd, Warriewood, Australia). The rapid visco-analyser is an automatic heating and cooling viscometer configured especially for testing starch-based and related products requiring precise control of temperature and shear. The visco-elastic properties of the samples were characterized using the parameters recorded on the viscosity trace: peak, trough, breakdown, final viscosity, setback, pasting temperature and pasting time. Viscosity was recorded in RVU.

Analysis of data

All determinations were made in triplicate trials and the data generated were subjected to one-way analysis of variance (ANOVA) at 5% level of significance using SPSS11.0 for windows. Means were separated by Duncan's multiple range tests.

Results and Discussion

Leuconostoc mesenteroides previously isolated from spontaneously fermented maize meal was used in this study. It was found to produce an exo-polysaccharide on sucrose agar. Table 1 shows the pH changes, acid and diacetyl production as well as cell growth taken on MRS agar during fermentation of maize meals, with and without inoculation of *Leuconostoc mesenteroides*. Significant changes were observed in all parameters examined at 5% level of significance. *L. mesenteroides* was able to lower pH from 5.66 in the unfermented maize meal which served as the control, to 3.37 after 12 h with a resultant significant increase in the amount of acid produced from 0.38 ml of NaOH consumed per gram in unfermented maize meal to 2.5 ml in maize meal fermented with *L. mesenteroides* for 12 h. The spontaneously fermented maize meal also significantly lowered pH and produced more acid than the unfermented maize meal with values of 5.28 and 0.80 respectively for pH and acid equivalent after 12 h of fermentation. The visco-elastic properties of the fermented maize meals are presented in Table 2. Fermentation of maize meals with *L. mesenteroides* increased final viscosity from 95.22

Table 1. Acidity and cell growth during fermentation of maize meal

Sample/property	pH	Acid equivalent (ml)	Diacetyl production (mg)	Cell growth on MRS (cfu/g)
Unfermented maize meal	5.66_{e}	0.38_{a}	25.08_{a}	$4.21 \times 10^{4}{}_{a}$
Spontaneously fermented meal (12 h)	5.28_{d}	0.80_{b}	84.47_{b}	$5.20 \times 10^{5}{}_{b}$
Spontaneously fermented meal (24 h)	4.54_{c}	1.26_{c}	96.25_{c}	$6.35 \times 10^{5}{}_{c}$
L. mesenteroides fermented meal (12 h)	3.37_{b}	2.50_{d}	129.12_{d}	$5.46 \times 10^{8}{}_{d}$
L. mesenteroides fermented meal (24 h)	3.35_{a}	2.71_{e}	134.70_{e}	$6.22 \times 10^{8}{}_{e}$

Key: Values are means of three replicates; Mean values followed by different subscripts within columns are significantly different by Duncan's multiple range tests ($P \leq 0.05$)

Table 2. Visco-elastic properties of fermented maize meals

Sample/property	Peak	Trough	Breakdown	Final viscosity	Setback	Pasting time	Pasting temperature
Unfermented maize meal	39.32_{a}	34.90_{a}	4.42_{b}	95.22_{a}	60.32_{c}	4.57_{a}	88.70_{e}
Spontaneously fermented meal (12 h)	52.08_{b}	50.67_{b}	1.41_{a}	117.28_{b}	66.61_{d}	4.83_{b}	84.35_{d}
Spontaneously fermented meal (24 h)	58.15_{c}	53.46_{c}	4.69_{c}	120.31_{c}	66.85_{d}	4.87_{c}	80.62_{c}
L. mesenteroides fermented meal (12 h)	162.40_{d}	105.26_{d}	57.14_{e}	144.00_{d}	38.74_{a}	5.15_{d}	79.31_{b}
L. mesenteroides fermented meal (24 h)	169.00_{e}	128.25_{e}	40.75_{d}	169.25_{e}	41.00_{b}	5.27_{e}	78.05_{a}

Key: Values are means of three replicates; Mean values followed by different subscripts within columns are significantly different by Duncan's multiple range tests ($P \leq 0.05$)

in the unfermented to 144.00 and 169.25 after 12 h and 24 h of fermentation respectively compared with values obtained for spontaneously fermented samples (117.28 and 120.31 after 12 and 24 h of fermentation respectively). Pasting temperatures were significantly lower for samples fermented with *L. mesenteroides* (78.05 – 79.31) although pasting time was found to be higher (5.15 – 5.27) compared with the values obtained for the spontaneously fermented maize meals Figure 1a-e).

The influence of *Leuconostoc mesenteroides* as a mono-culture in fermentation of maize meal for sour maize bread was investigated in this study. *L. mesenteroides* previously isolated from the indigenous microflora of the maize meal was used (Edema and Sanni, 2006). In sour dough research, spontaneous fermentation is sometimes used in order to obtain starters from particular flours. This is because many sour dough starters are usually developed from indigenous flora and they are often found to be more efficient in the sour dough system (Youngman, 2002). Most of the sour dough starters used in Swedish bakeries today originated from spontaneously generated sour doughs. It is also well known that organisms develop niches where they thrive and to transplant an organism from one natural environment to another is not a good formula for success, particularly in sour dough fermentations. Sour dough is a very complex biological system. Its properties depend on many different concurrent factors besides the composition of the micro-flora. Studies in sour doughs have emphasized the importance of functional properties such as pH, acidity as well as production of other antimicrobial compounds such as diacetyl in the development of starter cultures for sour doughs (Lonner and Preve-Akesson, 1988; Lonner and Preve-Akesson, 1989; Torner *et al.*, 1992; Gül *et. al*., 2005; Arendt *et. al*., 2007). *L. mesenteroides* used in this study was found to have a significant positive effect on the visco-elastic properties of maize meal for sour maize dough. This could be as a result of the exo-polysaccharide produced by the organism. Lactic acid bacteria, especially species of *Leuconostoc* are known to produce dextran, an extracellular exo-polysaccharide which has been reported to improve baking properties such as volume and texture. The use of dextran in baking is not wide-spread, but potential applications have been described (Wehrle and Arendt, 1998). Recent research in the development of novel sour doughs has identified the potential benefits of organisms such as the leuconostocs on properties of the sour dough system. Prominent among these

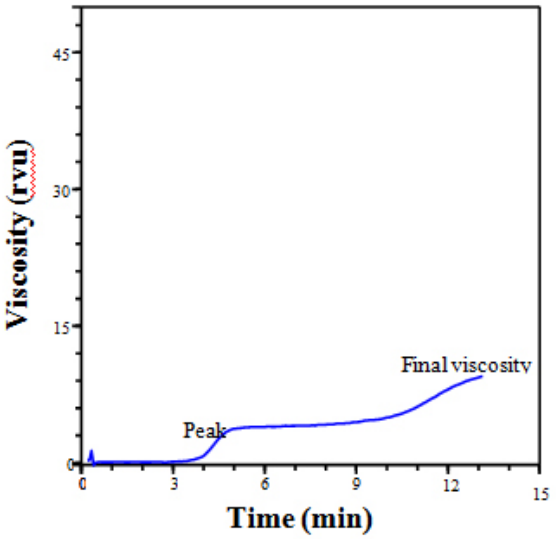

Figure 1a. Viscosity graph of unfermented maize meal

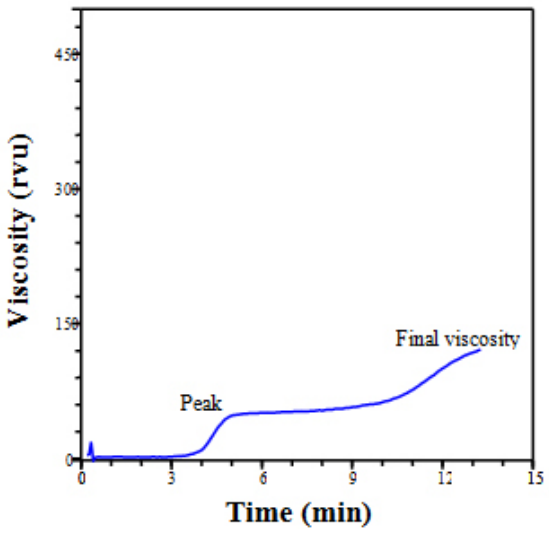

Figure 1b. Viscosity of spontaneously fermented maize meal (fermentation time = 12 h)

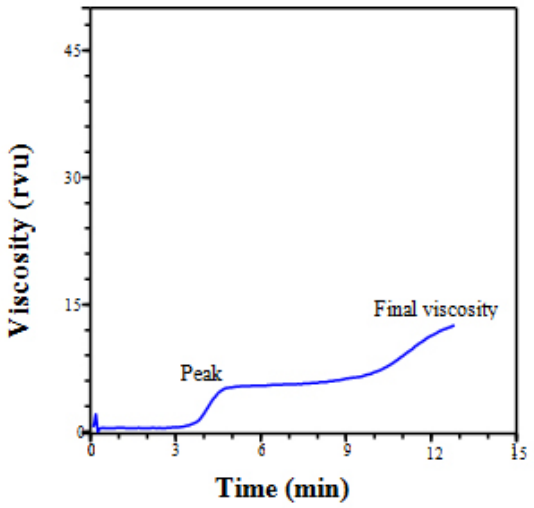

Figure 1c. Viscosity of spontaneously fermented maize meal (fermentation time = 24 h)

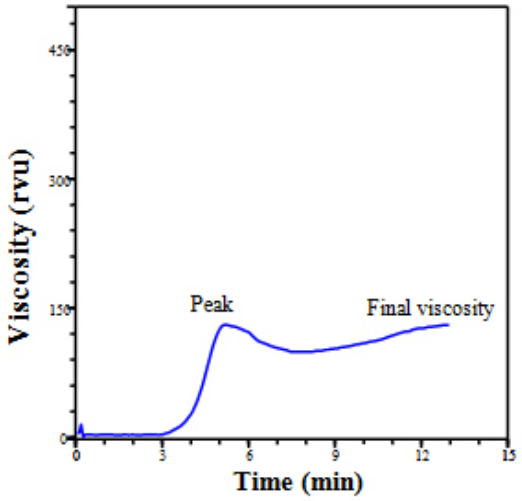

Figure 1d. Viscosity of *Leuconostoc mesenteroides* fermented maize meal (fermentation time = 12h)

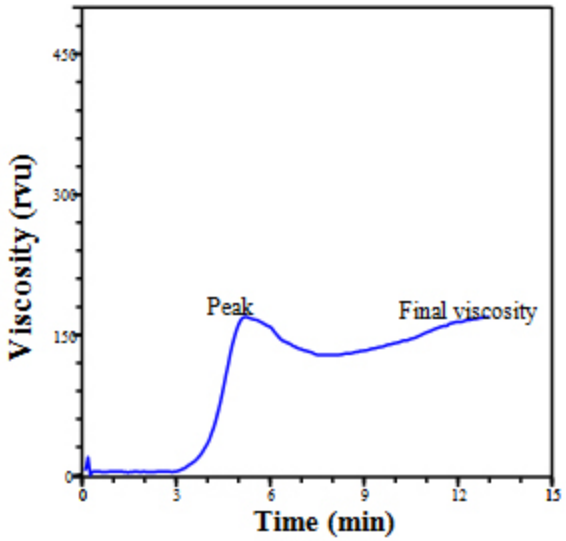

Figure 1e. Viscosity of *Leuconostoc mesenteroides* fermented maize meal (fermentation time = 24h)

Legend:
Viscosity in rvu – rapid viscosity units
Time in min – minutes

benefits are improvement of dough stability, texture and acceptability (Vandamme *et al*., 1997, Lacaze *et al.*, 2007). The findings of this study have shown that the inclusion of *L. mesenteroides* in the development of starter cultures for sour maize bread will significantly improve the visco-elastic properties of the sour dough, among other known positive effects attributed to the growth of the leuconostocs in sour doughs started on cereals other than maize. However, it is yet to be determined the appropriate fermentation time for the use of *L. mesenteroides* as mono-culture in sour maize meal fermentation. The ecology, kinetics and optimal fermentation conditions for the application of *L. mesenteroides* in maize sour dough are the focus of on-going research by the authors.

References

Angioloni, A., Romani, S., Pinnavaia, G. G. and Marco, D. R. 2006. Characteristics of bread making doughs: influence of sourdough fermentation on the fundamental rheological properties. European Food Research Technology 222: 54–57.

Arendt, E. K., Liam, A. M. R. and Dal Bello, F. 2007. Impact of sourdough on the texture of bread. Food Microbiology 24 (2): 165-174.

Azar, M., Ter-Sarkissian, N., Ghavifek, H., Ferguson, T. and Ghasseni, H. 1977. Microbiological aspects of Sangak bread. Journal of Food Science and Technology (India) 14: 251-254.

Coppola, S., Vilani, F., Coppola, R. and Parente, E. 1990. Comparison of different starter systems of water buffalo Mozzarella cheese manufacture. Le lait 70: 411- 423.

Coppola, S., Pepe, O. and Mauriello, G. 1998. Effect of leavening micro-flora on pizza dough properties. Journal of Applied Microbiology 85 (5): 891-897.

Corsetti, A., Settanni, L. and Van Sinderen, D. 2004. Characterization of bacteriocin-like inhibitory substances (BLIS) from sourdough lactic acid bacteria and evaluation of their in vitro and in situ activity. Journal of Applied Microbiology 96: 521–534.

Delclos, M. 1992. Vegetables preservation by a mixed organic acid Fermentation. Ph.D. Thesis. University of Surrey, Guildford, U.K.

Edema, M. O., Sanni, L. O. and Sanni, A. I. 2005. Evaluation of maize-soybean flour blends for sour maize bread production in Nigeria. African Journal of Biotechnology 4 (9): 911-918.

Edema, M. O. and Sanni, A. I. 2006. Micro-population of fermenting maize meal for sour maize bread production in Nigeria. Nigerian Journal of Microbiology 20 (2): 937 – 946.

Edema, M. O. and Sanni, A. I. 2008. Functional properties of selected starter cultures for sour maize bread. Food Microbiology 25: 616 – 625.

Garido- Gomez, M. P., Barneto, R. and Quintana, M. A. 1991. Microbiological and physico – chemical study of Aracena cheese. Chemie Microbiologie Technologie der Lebensmitel 13: 173-177.

Garvie, E. I. 1960. The genus *Leuconostoc* and its nomenclature. Journal of Dairy Research 27: 283-285.

Goldberg, I. 1994. Functional Foods, Chapman and Hall Inc., U.S.A 571pp

Gül, H., Ozcelik, S., Sagdic, O. and Certel, M. 2005. Sourdough bread production with lactobacilli and *S. cerevisiae* isolated from sourdoughs. Process Biochemistry 40: 691–697.

Harrigan, W. F. and McCance, M. E. 1976. Laboratory methods in food and diary microbiology. Academic Press, London, U.K. 452 pp.

Kim, S. Y., Souane, M., Kim, G. E. and Lee, C. H. 1991. Microbiological characterization of *Jangsu*. Korean Journal of Food Science and Technology 23: 689-694.

Lacaze, G., Wicka, M. and Cappelle, S. 2007. Emerging fermentation technologies: Development of novel sourdoughs. Food Microbiology 24 (2): 155-160.

Lee, S. H. and Kim, S. D. 1988. Effect of starters on the fermentation of *Kimchi*. Journal of the Korean Society for Food Nutrition 17: 342-347.

Litopolou-Tzenetaki, E. 1990. Changes in numbers and kinds of lactic acid bacteria during ripening of *kefalotyri* cheese. Journal of Food Science 55: 111-113.

Lonner, C. and Preve-Akesson, K. 1988. Acidification properties of lactic acid bacteria in rye sour doughs. Food Microbiology 5: 43-58.

Lonner, C. and Preve-Akesson, K. 1989. Effect of LAB on the properties of sour dough bread. Food Microbiology 6 (1): 19-28.

Okoruwa, E.A. 1995. Utilization and processing of maize. IITA research guide No. 35, p.29.

Sanni, A.I., Fapounda, E. M. and Onilude, A. A., 1995. Characteristic properties of lactic acid bacteria isolated from the rumen of Maradi goats. Chemie Microbiologie Technologie der Lebensm 17 (314): 99- 104.

Sozzi, T. and Pirovano, F. 1993. Bacteriophages of *Leuconostoc* spp. In: Proceedings, Symposium on Biotechnology and Molecular biology of lactic acid bacteria for the improvement of foods and feeds quality (eds. Zamorani, A., Manachini, P.L., Bottazzi, V. and Coppola, S.) Institute Poligrafico e zeeca dello statok pp 252-263. Rome.

Thiele, C., Ganzle, M. G. and Vogel, R. F. 2002. Contribution of sourdough lactobacilli, yeast and cereal enzymes to the generation of amino acids in dough relevant for bread flavour, Cereal Chemistry 79: 45–51.

Tieking, M., Korakli, M., Ehrmann, M. A., Ganzle, M. G. and Vogel, R. F. 2003. *In situ* production of exo-polysaccharides during sour dough fermentation by cereal and intestinal isolates of lactic acid bacteria. Applied and Environmental Microbiology 69 (2): 945- 952.

Torner, M. J., Martinez – Anaya, M. A., Antuna, B. and Benedito de Barber, C. 1992. Headspace flavor compounds produced by yeasts and Lactobacilli during fermentation of preferments and bread doughs. International Journal of Food Microbiology 15 (1-2): 145-152.

Valdez-de, G. F., Gori, G. S., Garro, M., Mozzi, F. and Oliver, G. 1990. Lactic acid bacteria from naturally fermented vegetables ,Microbiologie Aliments Nutrition 8: 175-179.

Vandamme, E. J., Renard, C. E. F. G., Arnaut, F. R. J., Vekemans, N. M. F. and Tossut, P. P. A. 1997. Process for obtaining improved structure build-up of baked products, EP 0 790 003 B1.

Wehrle, K. and Arendt, E. K. 1998. Rheological changes in wheat sourdough during controlled and spontaneous fermentation, Cereal Chemistry 75: 882–886.

Wehrle, K., Grau, H. and Arendt, E. K. 1997. Effects of lactic acid, acetic acid, and table salt on fundamental rheological properties of wheat dough. Cereal Chemistry 74: 739–744.

Wibowo, D., Eschenbruch, R., Davis, C. R., Fleet, G. H. and Lee, T. H. 1985. Occurrence and growth of lactic acid bacteria in wine. American Journal of Ecology and Viticulture 36: 302-313.

Wood, B. J. B. and Holzapfel, W. H. 1995. The Genera of the lactic acid bacteria: The genus *Leuconostoc*. Blackie academic and professional, London. PP 235-269.

Youngman, J. 2002. rec. food. sourdough FAQ Questions and answers. Greenwood D. (Edt) http://www.nyx.net/~dgreenw/ Accessed January 2003).