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Microbiological Quality of a Fermented Dairy Product Containing Brewer's Yeasts

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Abstract

The purpose of this study was to evaluate the microbiological quality of a kefir-like product obtained in a fermentation process of skimmed milk with a complex mixture of microorganisms containing lactobacili, kefir and brewer yeasts. The product was monitorised during the shelf-life (storage at 4 °C from 1 to 21 days) by the cantitative determination (enumeration) of coliforms count, *Escherichia coli*, total staphylococcal count, mold count and the detection of *Sallmonella ssp* and *Listeria monocytogenes*. All the microbiological tests were performed according to Romanian official methods for microbiological investigation of foods. The results showned a good microbiological quality of this type of fermented dairy product; the microbiological parameters, except the molds count, recorded lower values than admissible levels according to the criteria used. The brewer's yeasts addition in the kefir-type product did not affect negatively his microbiological quality and by his presence contribute to the functional properties of the final product.

Keywords: dairy product, brewer's yeast, microbiological parameters

1. Introduction

Many researchers have investigated the benefits of consuming kefir. Over a long period of time, the kefir's consumption had demonstrated that the microorganisms responsable of it's fermentation process are not pathogenic. The regular consumption of kefir can be help to relieve intestinal disorders, promote bowel movement, reduce flatulence and create a healthier digestive system [1]. Kefir exibits antimicrobial activity *in vitro* against Gram-positive and Gram-negative bacteria and some fungi [2]. The exact cause of the inhibition is not known, but may be due to the production of organic acids, hydrogen peroxide, acetaldegyde, diacetyl, carbon dioxide or bacteriocins [3]. Van Wyk (2001) showed that kefir posseses an inhibitory activity against *Staphylococcus aureus, Bacillus cereus, Escherichia coli, Clostridium tyrobutyricum* and *Listeria monocytogenes*. Since kefir has a pH of 4.2-4.6 after fermentation and maturation it may be that the inhibitory activity of the beverage is due to the production of acids by lactic bacteria. Also, a number of researchers have reported that this low pH value is not the only contributor to the antimicrobial activity and it was evaluated the effect of some inhibitory substances on the pathogenic microorganisms [5].

The beneficial properties of strains of some *Saccharomyces spp.* are well known. In addition to their nutritive value (vitamins of the B group, aminoacids, minerals like selenium and chromium), some strains of *Saccharomyces ssp*.are generally resistant to gastrointestinal conditions.

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Yeast preparations have also been successfully applied, in combination with antibiotics, to treat *Clostridium difficile* -related diarrhea commonly known as antibiotic associated diarrhea. Some *Saccharomyces spp* also have a protective effect, and specific activities, against various enteric pathogens. Rodrigues et al. (2000) reported that *Saccharomyces spp.* stimulate sIgA production and the phagocyte system of gnotobiotic mice. Recent works suggests that *Saccharomyces boulardii* is a subspecies of *Saccharomyces cerevisiae* and is regarded as the most prominent representatives of probiotic yeasts within the community of biotherapeutic [7]. *Saccharomyces ssp.* do not appear to alter or adversely affect the normal flora of the gastrointestinal tract and can be consumed with normal probiotic bacteria [8].

Patients treated with *Saccharomyces boulardii* and lactobacilli had a significant faster recovery [9]. Lactobacilli appear to enhance the beneficial effects of *Saccharomyces boulardii* on intestinal mucosa . *Saccharomyces cerevisiae* reduces the growth of *E. coli , Shigella flexnerii, Clostridium difficile and Vibrio cholerae* [10,11].

2. Materials and methods

1.Product manufacturing. In the pilot station of the University of Agricultural Sciences and Veterinary Medicine Cluj-Napoca, Romania, 1.8% skimmed, pasteurized milk was cooled at 30°C and was inoculated with starter cultures. The inoccullum was made up by a mesophilic bacterial culture (10^{10} colony forming units/ mL), FD-DVS-CHN 22, a kefir-yeast culture LAF3 (10^{10} cfu/mL), both provided by Chr. Hansen as freeze-dried powders and Direct Vat Set and a brewer's yeast culture (10^{10} cfu/mL), with a celullar viability of 96%, separated from the secondary beer fermentation (provided by a local brewer). The volumetric ratios (expressed in mL) between milk : bacterial culture: kefir yeast: brewer's yeast were 2000:2:0:4, 2000:2:2:2, 2000:2:4:1, 2000:2:8:0. After inoculation, the product was incubated at 29-30°C for 12 hr, pre-cooled at 18-20°C for 1hr, cooled again at 4-6°C for 10 hr and stored up to 21 days at 0-4°C.

2.Microbiological analysis. The density of microorganisms in inoccullum was determined by direct counting in the Thoma Chamber, while the viability of the brewer's yeast was determined by differential coloration. *Salmonella ssp.* and *Listeria monocytogenes* was detected, while total coliforms count, total mold count, total staphylococcal count was enumerated acccording to Romanian collection of official methods for the microbiological investigation of foods (Table 1). Three replications of all microbiological analysis were carried out for each sample.

Table 1. Official methods used for microbiological analysis

Microbiological indicators	**Methods**
Coliforms	SR ISO 5541/1-94 SR ISO 5541/2-94
Escherichia coli	SR ISO 7251/1996
Salmonella ssp.	SR ISO 6579/2003
Listeria monocytogenes	SR ISO 11290/2/2000
Staphylococcus ssp. (coagulase-positive)	SR ISO 6888/1/2/2002
Molds	SR ISO 7954/2001

The data obtained were compared to the criteria contained in Regulament 1441/2007 on the acceptable levels of microbiological contamination in foods.

3.Biochemical tests. Identification of *E. coli* and *Staphylococcus aureus* were realised using some biochemical tests. To identify *E. coli,* we used the following tests: Phenylalanin-deaminase, urease, indol and H_2S production, lisine decarboxilase, citrate utilisation and motility at 37°C. To identify *Staphylococcus aureus* the tests used were: anaerobe fermentation of glucose, H_2O_2 production, plasmo-coagulase and motility. This tests were performed according the official methods (protocols on biochemical tests) presented in Table1.

4.The pH determination. The pH of samples was determinated using an electronic pH-mater (Hanna Instruments Inc.)

5.Statistical Analysis. The data were analyzed by using the variance analysis (ANOVA, software Graph Pad Prism 5.00), considering a confidence interval of 95% ($p< 0.05$) as threshold of significance.

3. Results and Discussion

Results shown in Table 2 revealed that pH was ranged between 4.31 and 4.77 for all the tested samples. Also we calculated the means (±SEM) for the four product types, manufactured with different ratios between milk: bacterial culture: kefir yeast : brewer yeast (table 2).

This low pH value prevents the growth of most spoilage and pathogenic organisms but also is

considered promising to growth of yeasts.[12]. As we reported in our previous work [13], the brewer's yeast cell density increased from 10^4 cfu/ml in the 1th day, to 10^5 cfu/ml in the 7th and 14th days of shelf- life and to 10^6 cfu/ml in the 21th days of storage. This final value was founded in the samples with 4 ml, respectively 2 ml of brewer yeast added in 2L of milk.

Fermented foods are normally considered to be safe again food-borne diseases because of their low pH [14]. Nearly similar results were founded in the literature however, smaller values were recorded for other types of fermented dairy products [13,15].

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Table 2. The pH values of examined product samples during the shelf-life

Product Samples with different ratios M:C:K:B	**pH values during shelf-life**				Mean ±SEM
	1 day	7 days	14 days	21 days	
2000:2:0:4	4.32	4.54	4.51	4.58	4.4875±0.043
2000:2:2:2	4.31	4.52	4.59	4.64	4.515±0.039
2000:2:4:1	4.31	4.56	4.68	4.63	4.545±0.041
2000:2:8:0	4.31	4.58	4.77	4.72	4.595±0.0399

**M:C:K:B- The volumetric ratios (expressed in mL) between milk : bacterial culture: kefir yeast: brewer's yeast used for inoculation*

Table 3. The incidense of different microorganisms in the examined samples during the shelf-life period (1-21 days)

	Coliforms/g	E. coli/g	Staphylococcus ssp. *(coagulase-positive)*/g	Sallmonela/ 25 g	L. monocytogenes/ 25 g	Molds/g
No. of positive	9	6	1	ND	ND	7
%	18.75	12.5	2.08	ND	ND	14.58

ND-not detected

Table 4. Microbial counts in the examined samples during the shelf-life (1-21 days)

	Coliforms	E. coli	Total staphylococci	Sallmonela	Listeria monocytogenes	Molds
Minimum	0.1x10	0.3x10	0	ND	ND	1.1 x10
Maximum	1.5 x 10	1.5x10	0.7 x 10	ND	ND	$2.5x10^3$
Mean	0.7x10	0.65x10	0.2x10	ND	ND	2.8 x10
± SEM	0.12	0.245	0.051	ND	ND	0.482

ND-not detected

Table 5. Biochemical characteristics of microorganisms (*E. Coli* and *Staphylococcus aureus*)

Biochemical characteristics of microorganisms	***Escherichia coli***
Phenylalanin-deaminase	-
Urease	-
Indol Production	+
H_2S Production	-
Lisine decarboxilase	+
Citrate utilisation	-
Motility at 37°C	+
Biochemical characteristics of microorganisms	***Staphylococcus aureus***
Anaerobe fermentation of glucose	+
H_2O_2 Production	+
Motility	-
Plasmo-coagulase test	+

+Positive, -Negative

Table 6. Effect of brewer's yeasts addition on the product's microbiological profile

Samples with different ratios M:C:K:B	Microorganisms	Shelf-life (days)			
		1	7	14	21
2000:2:0:4	Coliforms (ufc/g)	0.3x10	0.1x10	ND	ND
	E. coli (ufc/g)	ND	ND	ND	ND
	Staphylococcus ssp. *(coagulase-positive)* (ufc)/g	ND	ND	ND	ND
	Molds (ufc/g)	ND	ND	ND	1.1x 10
2000:2:2:2	Coliforms (ufc/g)	0.7x10	0.3x10	ND	ND
	E. coli (ufc/g)	0.3x10	0.3x10	ND	ND
	Staphylococcus ssp. *(coagulase-positive)* (ufc)/g	ND	ND	ND	ND
	Molds (ufc/g)	ND	ND	1.1x10	1.4x10
2000:2:4:1	Coliforms, (ufc/g)	1.1x10	1.1x10	0.3x10	ND
	E. coli, (ufc/g)	1.1x10	1.1x10	ND	ND
	Staphylococcus ssp. *(coagulase-positive)* (ufc/g)	ND	ND	ND	ND
	Molds, (ufc/g)	ND	ND	9x10	$1.6x10^2$
2000:2:8:0	Coliforms, (ufc/g)	1.5 x10	1.5x10	ND	ND
	E. coli, (ufc/g)	1.5x10	1.1x10	ND	ND
	Staphylococcus ssp. *(coagulase-positive)*,(ufc/g)	0.7x10	ND	ND	ND
	Molds, ufc/g	ND	ND	$3.2 x10^2$	$2.5x10^3$

Data represent the means of three independent experiments

Listeria monocytogenes and *Salmonella ssp.* were not detected in any of the tested samples. As seen in Table 3, 18.75% of the samples tested during the shelf- life period had coliforms but any of them did not exceed regulatory standard. In all samples, coliforms were present in the first stage of the shelf-life period. Only in the samples with the inoculation ratio 2000: 2:4:1 we founded coliforms in the 14^{th} day of storage.

E. coli was present in 12.5% of the tested samples but at a low level. As seen in the table 6, samples with inoculation ratios 2000:2:8:0 and 2000:2:4:1 have revealed a greater level than the samples with higher density in brewery yeasts cells (2000:2:2:2). It must be mention that in the samples with the highest concentration in brewery yeasts (2000:2:0:4), E.coli was absent.Even if the coliforms and *E. coli* are present in small count, this may be a consequence of a low level of hygiene in manufacturing process, including the handlers, quality of water used and the ustensils.

Staphylococcus ssp. (coagulase-positive) were founded only in the blank, so in the samples without added brewer's yeast (2000:2:8:0). In this sample the staphylococcal count exceeded the limits. *Staphylococcus aureus,* confirmated by the biochemical tests (Table 5), was found only in one sample of the 48 tested samples. We consider that the *S. aureus* presence is only accidental and due to human handling during the manufacturing process.

Molds were present in 14.58% of tested samples. Molds count exceeded regulatory limits in all tested samples. Molds growth was observed in the second half of shelf-life, probably because of the substrate transformation and consumption of nutritive substances by the lactobacili and brewer yeasts. The lower level in molds was found in the samples with the highest concentration in brewery yeasts.These results highlights a good microbiological quality of the product and must be correlate with strict observance of hygiene rules in the manufacturing process.

However, the presence of coliforms bacteria, further more, the presence of *Escherichia coli* and *Staphylococcus aureus* in some of the products emphasized the importance of production hygiene during manufacturing of dairy products. Other researchers also reported a good microbiological quality for cultured dairy products during the shelf-life [14,16].

4. Conclusion

The fermented dairy product containing brewer's yeasts demonstrated a good microbiological quality during the shelf-life. The presence of the brewer's yeasts did not affect negatively the product's quality. The increase of the proportion of brewer's yeast added in the product, can be the reason of a lower level of coliforms, E.coli, staphylococcus and molds count.

References

1. Otles, S.; Cagindi, O., Kefir- a probiotic dairy-composition,nutritional and therapeutical aspects, *Pakistan Journal of Nutrition,* **2003**, *2*(2), 54-59
2. Garrote,G.L., Abraham, A.G., De Antoni, G.L., Inhibitory power of kefir: the role of organic acids, *Journal of Food Protection,* **2000***, 63,* 364-369
3. Farnworth, E.R., (editor), Handbook of fermented functional foods, *CRC Press, Boca Raton, USA*, 2003
4. Van Wyk, J.,*The inhibitory activity and sensory properties of kefir targeting the low-income African consumer market*, M.Sc., Thesis, University of Stellenbosch, Stellenbosch, South Africa, 2001
5. Powell, J., *Bacteriocins and bacteriocin producers present in kefir and kefir grains*, MSc. Thesis University of Stellenbosch, Stellenbosch,South Africa, 2006
6. Rodrigues,A.C.P., Cara,D.C., Fretez,S.H.G.G., Cunha,F.Q., Vieria,E.C., Nicoli,J.R., Vieira,L.Q. *Saccharomyces boulardii* stimulates sIgA production and the phagocytic system of gnotobiotic mice, *J. of Applied Microbiology*, **2000**, *89*: 404-414.
7. Dixit, Kalpana and Gandhi, D.N., *Biotherapeutic properties of probiotic yeast Saccharomyces species in fermented dairy foods*, 2006, http://www.dairyscience.info/probiotics/105-biotherapeutic-probiotic-yeast.html?showall=1 .
8. Elmer, G.W., McFarland, L.V. Biotherapeutic agents in the treatment of infections diarrhoca, *Gastroenterol Clin North Am.*. **2001**, *30*(3) : 837-854.
9. Gaon, D., Garcia, H., Winter, I.,, Rodriguez,N., Quintas,R.,I ,. Gonzalez, N. , Oliver, G., Effect of lactobacillus strains and *Saccharomyces boulardii Medicina (Buenos Aires),* **2003**, *63*(4): 293-298
10. Czerycka, D., Rampal, P., Experimental effect of *Saccharomyces boulardii* on diarrheal pathogens, *Microbes and infection*, **2002**, *4*(7): 733-739, http://dx.doi.org/10.1016/S1286-4579(02)01592-7
11. Martins, F.S., Nardi, R.M.D., Arantes, R.M.E., Rosa, C.A., Neves,. M., Nicoli, J.R., Screening of yeasts as probiotic based on capacities to colonize the gastrointestinal tract and to protect against enteropathogen challenge in mice. *J. Gen. Appl. Microbiol.*, **2005**, *51*(2): 83-92
12. Abeer A.A. Aabdel All, H.A.Dardir, Hygienic quality of local traditional fermented skimmed milk (laban rayb) sold in Egypt, *World Journal of dairy & Food ciences*, **2009**, *4*(2):205-209
13. Paucean, A.Socaciu, C.,, Vodnar D., Mudura, E., A new functional dairy product containing an optimized mixture of *Lactococcus bacteria*, kefir and brewer's yeasts; *Romanian Biotechnological Letters, Bucharest,* **2010**, *15*(6), 5793-5800
14. Varga, L., Microbiological quality of commercial dairy products, *Communicating Current Research and Educational Tpoics and Trends in Applied Microbiol*., 2007, 487-494.
15. Garcia Fontan, M.C., Martinez, S.,Franco, I., Carballo, J., Microbiological and chemical changes during the manufacture of kefir made from cow's milk, using a comercial starter culture, *International Dairy Journal*, **2006**, *16*(7), 762-767, http://dx.doi.org/10.1016/j.idairyj.2005.07.004
16. Pfleger, R., Zum hygienischen Status von Milch und Milchprodukten aus der Direktvermarktung = The hygienic status of ex farm milk and milk products, Wiener Tierarzliche Montsschrift, **2002**, *89*(8), 227-236