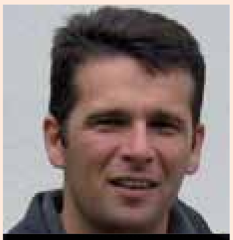
RADE POPOVIĆ[1]*, SPASENIJA MILANOVIĆ[2], MIRELA ILIČIĆ[2], MARIJAN RANOGAJEC[2], KATARINA KANURIĆ[2], VLADIMIR VUKIĆ[2], DAJANA HRNJEZ[2]
*Corresponding author
1. University of Novi Sad, Faculty of Economics of Subotica, 24000 Subotica, Serbia
2. University of Novi Sad, Faculty of Technology Novi Sad, 21000 Novi Sad, Serbia


Rade Popović

Nutritive characteristics and market prospects
of kombucha fermented milk beverages

KEYWORDS: Fermented milk, kombucha, nutritive characteristics, rheology, market prospects.

Abstract The aim of this study was to compare nutritive, microbiological and rheological characteristics in fermented milk samples produced from milk with different fat content; 0.9% (w/w) and 2.2% (w/w) by kombucha inoculum application. Also, the average cost structure of both beverages was analysed and compared with the cost structure of plain yoghurt with 2.8% fat content. Kombucha fermented milk beverages contain an average 80% of lactic acid bacteria (LAB), and the rest 20% of microorganisms were yeast and acetic acid bacteria (AAB). The samples are a good source of minerals (Ca, K, Na, Mg, P) and vitamins (B1, B2 and B6). Low fat kombucha fermented milk contained a 6.0% lower atherogenic index than beverage with 2.2 % (w/w) fat content. The Sample with 2.2% (w/w) fat content showed higher values of rheological characteristics than the low fat sample. Low fat kombucha fermented milk beverage has a lower average cost for both packaging types. Both drinks can be classified as a high nutritive, valuable and price competitive food that is intended for special consumers categories.

INTRODUCTION

Kombucha is a symbiosis of bacteria and yeast embedded within cellulosic pelliculs. Major bacterial genus in most of five different samples from distinct geographic locations was *Gluconobacter*, present in 85% of the samples. *Lactobacillus* population was identified in up to 30% of the samples, while the yeast population was dominated by *Zygosaccharomides* (95% of samples) (1- 4).
Kombucha is traditionally cultivated on a sweetened black and green tea. KALLELA *et al.* (2012) investigated the biochemistry of kombucha fermentation by using green (GTK) and black tea (BTK). Authors found that carbon sources, glucose and fructose, resulting from sucrose were consumed faster in BTK. The biochemical changes were more rapid in BTK than in GTK, and the levels of cellulose, acetic acid equivalents, ethanol, phenolics, caffeine and proteins were higher (5).
Novel researches have shown that kombucha inoculum can be cultivated as a non-conventional starter culture and used for the production of fermented milk beverages (6-12).
In addition to the nutritional and technological characteristics of the product, the economic analysis of production also occupies an important place. Economists usually investigate the models of yoghurt consumption in the EU. It is a generally shared view of these models that a number of factors influence decision-making, including the properties of the products, individual consumer differences, socio-demographic characteristics and phychological factors, as well as previous experience with the product, and environmental influences ranging from cultural and economic factors (13). The level of consumption depends on types, quality of products (chemical composition, rheology, texture, microstructure and sensory properties) and cost of production.

The aim of this study was to investigate the effect of non-conventional starter culture-kombucha on microbiological and nutritive characteristics (lactose, protein, fat, vitamins B_1, B_2, B_6, minerals Ca, Mg, K, Na, total fatty acids and atherogenic index of products), and viscosity of fermented milk beverages produced from milk with 0.9% and 2.2% (w/w) fat. Also, the average cost structure of both beverages was analysed and compared with the cost structure of plain yoghurt with 2.8% fat content.

MATERIALS AND METHODS

Milk

Homogenized (130 bar, 55°C) and pasteurized cow milk (82°C, 40s) with different fat content was taken from AD Imlek, Division Novi Sad Dairy, Serbia for the production of the kombucha fermented milk beverages. The composition of milk with 0.9 g/100g milk fat (sample M1) was as follows: total solids (TS) 9.85g/100g, total proteins (TP) 3.15 g/100g and lactose 4.74g/100g. Milk with 2.2g/100g milk fat (sample M2) contained total solids (TS) 11.60 g/100g, total proteins (TP) 3.27 g/100g and lactose 4.53g/100g.

Kombucha inoculum

Before application of kombucha inoculums (KI) as a starter culture, they were prepared according to the following steps: 1 L of boiled tap water with 70 g of sucrose and 1.5 g of black tea leaves ("Adonis", Jagodina, Serbia) was heated at 100°C for 5 min. The tea was cooled to room temperature and inoculum from previous fermentation was added in concentration of 100ml/L. pH value of kombucha inoculum was 3.72.

Production of kombucha fermented milk beverages

Two samples were produced: the first sample from milk with 0.9% fat (KFM1) and the second sample from milk with 2.2% fat content (KFM2). Kombucha inoculum in concentration of 100mL/L was added in pasteurized milk cooled at 43°C. Fermentation lasted until pH reached 4.6, and then samples were cooled at 8°C. The fermentation of milk with kombucha lasted beetwen 9.5 and 10 hours. After cooling, the gel was stirred and stored in a refrigerator at 4°C. Each trial was repeated three times.

Chemical and microbiological analysis

The following physicochemical characteristics were determined in all samples: total solids (TS) by oven drying, method ISO 6731, IDF 21:2010 (14); total proteins (TP) by Kjeldahl method ISO 8968-1:2001(15), fat by Gerber method ISO 488:2008 (16). pH was measured with a pH-meter (EcoScan pH 6 Eutech Instruments, Netherlands). The content of lactose (K-LACGAR 12/05) was determined by using enzymatic tests Megazyme, Ireland (17). Minerals (Na, Ca, and K) were analyzed with atomic absorption spectroscopy (GBC 932 plus) (18).The content of vitamins B_1, B_2 and B_6 was analyzed with reversed-phase liquid chromatography with a fluorescence detector-method HCTM-01, HCTM-02, HCTM-03 (Shimadzu C-R4A, CROMATOPAC).

The fatty acids content examination was performed using gas chromatography (VARIAN, model 1400), with a flame-ionization detector - ISO 5509 2000 (19). Saturated fatty acids (SFA); monounsaturated fatty acids (MUFA); unsaturated fatty acid (UFA), polyunsaturated fatty acids (PUFA) and atherogenic index (AI) were calculated in kombucha fermented milk beverages on the basis of fatty acids composition (20).

Statistical analysis of results was carried out with the computer software program Statistica version 6 (StatSoft Inc, Tulsa, OK, USA) and OriginPro 8.5.1.

Total lactic acid bacteria, acetic acid bacteria and yeasts were monitored after the production of kombucha fermented milk beverage. For this purpose, 20g portions of fermented milk product samples were blended with 180 mL of sterile physiological solution. 1mL of the obtained solution was transferred into a Petri plate (q 9 cm), in which the medium was GYC substrate, prepared as a basal medium containing: 10g yeast extracts, 50 g glucose, 30 g CaCO3, 25g agar agar (21). Samples were incubated for 3 days at 30°C. Yeasts were isolated using selective medium Saburov malt agar ("Torlak"), prepared as a basal medium containing 10 g peptone and 40 g maltose. Substrates are sterilized at 120°C for 20 minutes, and all colonies were counted on Saburov malt agar as colonies of yeast, and GYC agar for only those that provide enlightened zone around the colonies formed (22).

Viability of lactic acid bacteria were monitored on selective substrate for both fermented milk products:

- *Lactococcus* sp. on M17 agar in aerobic condition at 30°C during 48h;
- *Lactobacillus* sp. on MRS agar in facultative anaerobic incubation at 30°C during 48 h (23).

Rheology measurement

Rheological properties of fermented milk samples were measured at 5°C using a viscometer HAAKE RheoStress 600HP (Karlsruhe, Germany) fitted with sensor PP60Ti (gap 1mm) (24, 10-11).

Estimation of production cost of kombucha fermented milk beverage

The average cost structure was analysed in two dairy companies during September of 2014. Theis estimate is done for two types of dairy products, with 0.9 and 2.2 % of milk fat. On the Serbian market two packaging sizes with 1 and 0.18 litre are most common for liquid fermented milk products in the last decade. After many decades of use PE bags, over cartons, today dairy companies use mostly PET bottles and PP glasses (25). Structure of expecting costs for kombucha fermented milk beverage is compared with the cost structure of plain yoghurt with 2.8% milk fat.

RESULTS AND DISCUSSION

The nutritive characteristics and rheological properties of kombucha fermented milk products

The total number of lactic acid bacteria in kombucha inoculum was 10^3 cfu/ml, while the number of acetic acid bacteria and the number of yeast was the same - $6x10^4$cfu/ml. It is evident from Figure 1 that the ratio of yeast is the highest (80%) in kombucha inoculum and milk samples with inoculum, while the ratio of LAB was 18%, and these results are in accordance with literature data (1). After fermentation, both samples of kombucha fermented milk products have a significantly lower ratio of yeasts (5%), while lactococcus was presented in a much higher ratio (70%). The number of acetic acid bacteria and yeast are in accordance with results of CHEN & LIU (2000), GOH *et al*. (2012).

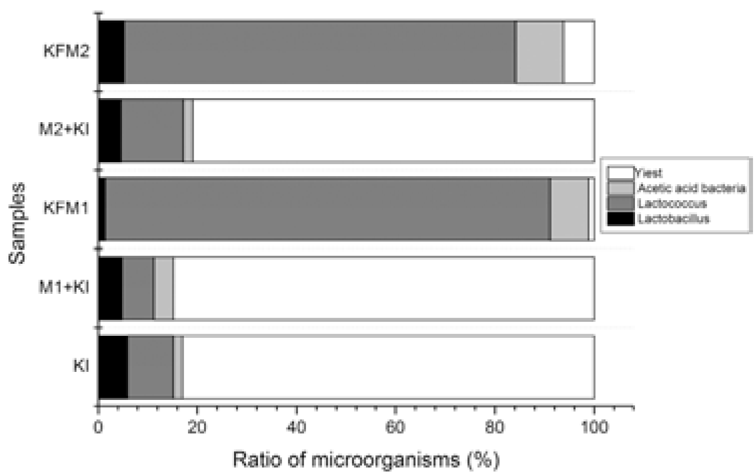


Figure 1. The ratio of microorganisms in kombucha inoculums (KI), milk with addition of kombucha inoculum (M1 + KI and M2 + KI) and kombucha fermented milk beverages (KFM1,KFM2) produced from milk with 0.9%(w/w) and 2.2% (w/w) fat content.

Characteristics [g/100g]	SAMPLE KI	M1	M2	KFM1	KFM2
Total Solids (TS)	6.68±0.07	9.85±0.33	11.60±0.21	10.92±0.33	11.52±0.30
Lactose	-	4.74±0.15	4.53±0.21	4.14±0.13	3.79±0.19
Fat	-	0.9	2.2	0.8	2.1
Protein	0.70±0.01	3.15±0.04	3.27±0.06	2.56±0.22	2.79±0.15
Minerals [mg/kg]					
Na	27.14±0.50	587±11.74	576.3±11.50	721.0±14.42	661±13.22
Ca	97.38±1.95	1195.67±23.90	1133±22.66	1210.0±24.20	1154±23.08
K	40.84±0.80	1545.0±31.0	1515.0±29	1794.0±35.88	1846.0±36.09
Vitamins					
B_1 [mg/L]	0.09±0.01	35.43±0.71	43.03±0.80	37.42±0.75	43.79±0.88
B_2 [µg/100g]	0.075±0.002	104.47±2.09	131.33±2.63	108.39±2.15	136.68±2.51
B_6 [µg/100g]	<0.03	79.34±0.15	81.36±1.62	81.36±1.5	106.52±2.13
Energy value [kJ/100g]	116	174	225	199	235

Table 1. Composition of kombucha inoculum, milk and fermented milk beverages

Total solids in kombucha beverages produced from milk with 0.9% (KFM1) and 2.2% (KFM2) of fat content is 10.92 % and 11.52%, respectively. Lactose content in kombucha fermented milk beverage has varied from 3.79 to 4.10 g/100g, and that was 13% -16.3% less than in the milk used for production of this beverage (Table 1). In addition, chemical characteristics of kombucha beverages are in accordance with literature data (7, 10, 11). The highest content of minerals are in KFM_1: calcium – 1210 mg/kg, sodium content was 721 mg/kg, and magnesium was 125 mg/kg, which are in accordance with literature data (26).Milk of 2.2% (w/w) fat content had a higher content of vitamins B_1, B_2 and B_6 than milk of 0.9% (w/w) fat content (Table 1). The content of vitamin B_2 is the highest in both of samples: 108 µg/100g (KFM1) and 136 µg/100g (KFM2). These average values are higher by approximately 90% compared to content of vitamin B_2 in traditional yoghurt (27). Energy value of sample KFM1 was 199 kJ/100g and 15% lower than the energy value of sample KFM2. Consumption of 150g/day of low fat kombucha fermented milk beverage (0.9% fat) and 112.5g/day of beverage with 2.2% fat content can satisfy a childs' daily requirement for vitamins B_1, B_2 and B_6 (Table 2). As for adults, the requirement of the forementioned vitamins is greater so they must ingest 300 g of the beverage per day (28) to meet the daily requirement.

The ratio and fatty acids composition in kombucha fermented milk beverage presented in Table 2, are in accordance with the literature data (29, 9, 30).The saturated, mono- and polyunsaturated fatty acids content in KFM1 and KFM2 were within ranges of 66.7-68.1, 22.5-25.5 and 2.0-2.7, respectively. It is evident that lower fat kombucha fermented milk product (0.9% fat) shows the most desirable fatty acid composition with the lower saturated fatty acids and higher polyunasaturated fatty acids compared to beverage produced from higher fat content (2.2%). The atherogenic index (the sum of concentration of 12:0 (lauric acid), 16:0 (palmitic acid) and 4*14:0 (myristic acid) divided by the concentration of total unsaturated fatty acids) was calculated on the basis of these results, and are in accordance with the literature data (9, 20). Atherogenic index of kombucha fermented milk beverage was 2.41, and it was lower by 6% when compared to the sample produced from 2.2% fat content milk.

Fatty acids	*Fatty acid content (% of total fatty acids)*	
	KFM1	***KFM2***
4:0 (butyric)	*4.2±0.08*	*3.8±0.07*
6:0 (capric)	*3.1±0.06*	*4.0±0.07*
8:0 (caprylic)	*1.7±0.03*	*2.3±0.04*
10:0 (caproic)	*3.3±0.06*	*3.3±0.05*
unidentified	*-*	*0.5±0.01*
12:0 (lauric)	*3.3±0.06*	*3.3±0.06*
unidentified	*0.8±0.01*	*0.5±0.01*
unidentified	*6.6±0.11*	*3.3±0.05*
14:0 (myristic)	*11.7±0.20*	*12.1±0.22*
14:1	*0.6±0.01*	*1.2±0.02*
15:0	*0.6±0.01*	*1.2±0.01*
16:0 (palmitic)	*29.9±5.90*	*29.3±5.89*
16:1 (cis+trans)	*2.4±0.04*	*1.9±0.03*
17:0	*-*	*0.2±0.01*
18:0 (stearic)	*8.5±0.17*	*8.5±0.15*
18:1 (cis+trans) (oleic)	*20.1±0.03*	*22.1±0.04*
18:2 (linoleic) (cis+trans+conug.)	*2.1±0.04*	*1.7±0.03*
18:3 (linolenic)	*0.6±0.01*	*0.3±0.01*
20:0 (arachidonic)	*0.4±0.01*	*0.4±0.01*
SFA[a]	*66.7*	*68.1*
MUFA[b]	*22.5*	*25.2*
PUFA[c]	*2.7*	*2.0*
UFA[d]	*33.20*	*31.8*
AI[e]	*2.41*	*2.55*

[a]SFA-Saturated fatty acids; [b] MUFA-monounsaturated fatty acids; [c]PUFA-polyunsaturated fatty acid; [d] UFA - polyunsaturated fatty acid [e]AI-atherogenic index.

Table 2. Fatty acids composition of kombucha fermented milk beverages (% of total fatty acids)

When comparing the presented flow curves at Fig. 2, it can be seen that shear stress in both samples has similar trend in shear rate function. The value of hysteresis loop area in sample obtained from milk with 2.2% fat content was 275 Pa/s, while it was significantly lower in sample produced from milk with 0.9% fat (102 Pa/s). The results of apparent viscosity and the value of hysteresis loop area are in accordance with HERNANDEZ (1996) who stated that a thyxotropic fluid of higher viscosity must have a greater hysteresis area than a fluid with lower viscosity, even when an increase occurs in the breaking of the structure of the less viscous product (30).

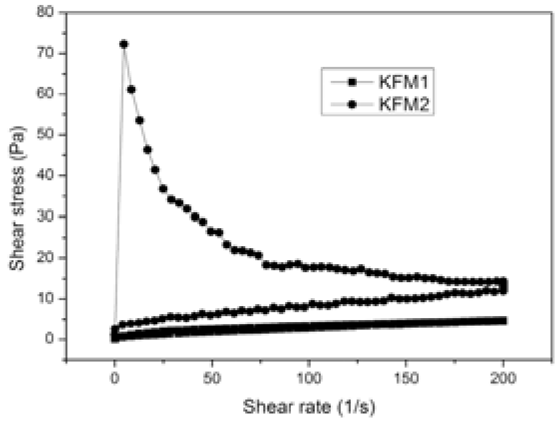


Figure 2. Flow curves of kombucha fermented milk beverages

Market prospects of of kombucha fermented milk beverage

Kombucha is well known drink in Serbia, but it is mostly in home production. Since that, here is assumed that production of kombucha inoculums is done in dairy plant. The average cost of kombucha inoculums is estimated at 0.14 EUR/litre. It takes in account all inputs with market prices during September 2014, calculated in EUR by average exchange rate in same period, as well as specifics of production process. Estimation of production cost of kombucha fermented milk beverage is done based on current technology of yoghurt production, applied in middle size dairy plants in Serbia. Commonly, daily capacity of such type of dairies is from 100 to 200 thousand litres per day. Average cost structure was analysed in two dairy companies, during September of 2014.

Differences in production technologies are taken in account, since kombucha fermented milk beverage have a slightly longer fermentation process, and the starter culture is different. Besides that, other inputs are the same. Estimate is done for two types of dairy products, with 0.9 and 2.2 % of milk fat.

	Unit	Quantity	Price EUR/unit	Cost
Black Tea	kg	0.0015	8.3030	0.0125
Sucrose	kg	0.07	0.5597	0.0392
Depreciation		1	0.0211	0.0211
Overhead cost		1	0.0674	0.0674
TOTAL:				0.1401

Table 3. Estimated cost in kombucha inoculums production

On Serbian market two packaging types: 1 litre bottle from PET material and 0.18 litre glasses from PP material are most common for liquid fermented milk products. The structure of expect cost for kombucha fermented milk beverage are presented in Table 3 and compared with the cost structure of plain yoghurt with 2.8% milk fat. In cost structure, raw milk is the main input with the biggest share that ranges from 46% for 0.9 milk fat beverage in 0.18 litres packaging to 59% for plain yoghurt in 1 litres packaging. Cost of kombucha inoculums is higher than starter culture for plain yoghurt, but still lower than most starter cultures for flavoured yoghurts. Packaging costs include cost for PET bottle of 1 litre and PP glass of 0.18 litres. Overhead cost include: labour, energy, water, other supplies, etc. Appraisal production costs of two new type milk beverages are slightly different than production costs of plain yoghurt with 2.8% milk fat. In the first case, kombucha fermented milk beverage with 0.9% milk fat has lower average cost for both packaging types (Table 4).
It is mainly because of the cost of milk with lower fat content. Cost of kombucha inoculums and overhead cost are higher, than in case of plain yoghurt. In case of second beverage with 2.2% milk fat average cost are slightly higher because of kombucha inoculums and overhead costs.

Item	Kombucha milk fermented beverage				Plain yoghurt	
	0.9% milk fat		2.2% milk fat		2.8% milk fat	
	1/1	0.18	1/1	0.18	1/1	0.18
Raw milk	0.2192	0.0394	0.2487	0.0448	0.2579	0.0464
Kombucha inoculums / starter culture	0.0140	0.0025	0.0140	0.0025	0.0051	0.0008
Packaging	0.0902	0.0211	0.0902	0.0211	0.0902	0.0211
Depreciation	0.0194	0.0037	0.0202	0.0038	0.0202	0.0042
Overhead cost	0.0674	0.0194	0.0717	0.0211	0.0624	0.0177
TOTAL:	0.4102	0.0861	0.4448	0.0932	0.4358	0.0902

Table 4. Estimated cost in production of kombucha fermented milk beverage and cost of plain yoghurt in EUR per product

CONCLUSIONS

Two samples of kombucha fermented milk beverage with 0.9% (w/w) and 2.2% (w/w) milk fat were successfully produced by application of 10% (w/w) kombucha inoculums.
Based on the research, it can be concluded that this kind of beverages can be classified as a highly valuable functional food intended for all categories of consumers. Higher nutritional values of new products with competitive production cost could be main reasons for dairy industry to increase investment and include them in the production portfolio. An aging population with changes of consumption habits and preferences could be other reasons to introduce new functional dairy product on market.

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REFERENCES

1. Marsh, A.J., O'Sulliva, et al. Food Microbiology, 38: 171-178 (2014).
2. Debon, J., Schwinden Prudêncio, E. et al. J Food Eng., 99: 128-135 (2010).
3. Chen, C., Liu, B. Y. J Apply Microbiol., 89: 834-839 (2000).
4. Goh, W., Rosma, A. et al. Food Res. J, 19: 109-117(2012).
5. Kallela, L., Desseauxb, V. et al. Food Chem. 49: 226-232 (2012).
6. Milanović, S., Lončar, E. et al. APTEFF, 39: 37-46 (2008).
7. Malbasa, R., Milanovic, S. et al. Food Chem., 112: 178–184 (2009).
8. Ilicic, M. Optimisation of functional fermented dairy beverage technology", Ph.D. Thesis, Univ. of Novi Sad, Faculty of Technology (in Serbian). 49-50(2010).
9. Milanović, S., Kanurić, K. et al. Determination of fatty acids during milk fermentation by kombucha cultivated on black tea. 6th Central European Congress on Food, CEFood2012, 23-26 May, 1024-1028 (2012).
10. Iličić, M., Milanović, S., et al.. J Text. Stud., 44: 160-168 (2013).
11. Vukić,V., Hrnjez, D. et al. J Text. Stud., 45: 261-273 (2014)
12. Hrnjez, D., Vastag, Ž. et al. J Funct. Foods., I0, 223-245,(2014).
13. Valli, C., Traill W. B. Food Qualty and Prefer., 16: 291-304 (2005).
14. ISO 6731IDF 21. Milk, cream and evaporated milk-Determination of total solids content (Reference method), Brussels, Belgia (2010).
15. ISO 8968-1. Milk – Determination of nitrogen content-Part 1: Kjeldahl method, Brussels, Belgia (2001) .
16. ISO 488. Milk –Determination of fat content-Gerber butyrometers, Brussels, Belgia (2008).
17. BERGMEYER, H.U. Methods of Enzymatic Analysis. 3rd ed., vol. VI, PP.VCH Publishers (UK) Ltd., Cambridge UK.p.1150 (1988).
18. FAO Mannuals of food quality control: 2. Additives, contaminents, techniques. FAO Food and Nutrition paper, 1980, 14/2, Rome (1980).
19. ISO 5509. Animal and vegetable fats and oils –Preparation of methyl esters of fatty acids. General guidance. International Organization for Standardization (2000).
20. Brezo, T., Kravić, S. et al. Food ind.-Milk and dairy prod., 22: 21-24 (2011).
21. De Lay, J. Family VI Acetobacteriaceae. In: Bargeys Manual of Systematic Bacteriology , ed. R.N. Kreig, G.J. Holt, Wiliams&Wilkins Co., Baltimore, 267-278 (1984).
22. Lodder, J. The yeasts. North Holland Publishing Company, Amsterdam(1974).
23. Škrinjar, M. Microbiological control of foodstuffs, Faculty of Technology, Novi Sad, p.245 (2001).
24. Hassan, A.N., Ipsen, R. et al. J Dairy Sci., 86: 1632-1638 (2003).
25. Popović, R. Food industry-milk and dairy prod., 18: 65-68 (2007).
26. Renner, E., Schaafsma, G. et al. Micronutritiens in Milk and Milk Based Food Products, E. Renner, Ed. Elsevier Applied Science, London, 1-9 (1989).
27. TAMIME, A.Y., ROBINSON, R.K. Yoghurt, Science and Technology, Woodhead Publishing Limited, Cambridge England, p.619 (2004).
28. Smolin, L., Grosvenor, M. (2000): Nutrition, Science and Application, saunders College Publishing. A Division a Harcourt College Publishers, Third edition. 597.
29. Guler, Z. Milchwissenchaft, 63: 73-77(2008).
30. Vitas, J., Malbaša, R. et al. Food Ind.-Milk Dairy Prod., 22: 25-28 (2011).
31. Hernández, M.J. Caracterizacion reologica de hidrigeles de MCC-NaCMC+almidon.Tixotropia y sinergismo.PhD. Thesis. Universitat de Valencia, Spain (1996).