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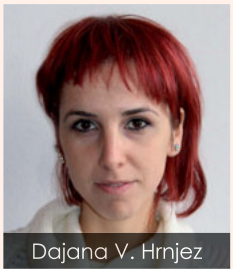
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Nutritive aspects of fermented dairy products obtained by kombucha application

Protein profile in fermented dairy products

KEYWORDS: Fermented dairy beverage, kombucha, protein profile

Abstract The aim of this study was to investigate the chemical quality and protein profile of fermented dairy products obtained by kombucha starter culture at different fermentation temperatures. Sample produced at 37°C have significantly longer fermentation time than sample produced at 42°C. Protein profile analysis revealed more stable a and β casein fractions compared to other protein fractions during milk fermentation. These results indicate that fermented dairy products produced by kombucha at 37°C and 42°C could be less allergenic than milk and suitable for special allergenic nutrition. Especially sample fermented at 37°C had lower content of a-lactalbumin and β-lactoglobulin than sample produced at 42°C.

INTRODUCTION

Research in the field of functional foods is of great interest in human nutrition. Fermented dairy products certainly hold important role in this field. Different fermented dairy products may have influence on allergenic properties of milk due to the process of fermentation by specific starter cultures. Consequently, biochemical changes of milk components during fermentation have been widely studied. Lactic acid bacteria showed only weakly proteolytic activity. Surprisingly they do cause significant degree of proteolysis in different fermented dairy products (1). Klaenhammer et al. (2) and Haandrikman (3) provided detailed review of the properties of the lactic acid bacteria proteolytic systems. The proteolytic system is composed of a proteinase which is involved in the initial cleavage of casein, peptidases which hydrolyse the large peptides thus formed and transport systems which are involved in the uptake of small peptides and amino acids.

Table 1 shows characteristics of the major proteins in cow`s milk (4). Milk contains certainly more than 100 different proteins, usually are classified into two major fractions: caseins (αs1-, αs2-, β-, and κ-casein), which are the most abundant (80%) and serum or whey proteins (20%), which include α-lactalbumin, β-lactoglobulin, blood serum albumin (BSA), and immunoglobulins (Ig). Milk proteins also include milk fat globule membrane proteins and a large variety of enzymes (about 60) and hormones (4).

Protein	Molecular mass[a] (Da)	Number of AA residues			Number of PO_4	Presence of CH_2O_2	Concentration (gl^{-1})	Genetic variants detected
		Total	Pro	Cys				
α_{s1}-Casein	23 164	199	17	0	8	0	10	A,B,C,D,E,F,G,H
α_{s2}-Casein	25 388	207	10	2	10-13	0	2.6	A,B,C,D
β-Casein	23 983	209	35	0	5	0	9.3	A^1,A^2,A^3,B,C, E,F,G
κ-Casein	19 038	169	20	2	1	+	3.3	A,B,C,E,F^1,F^2, G^1,G^2,H,I,J
B-Lactoglobulin	18 277	162	8	5	0	0	3.2	A,B,C,D,E,F,H,I,J
α-lactalbumin	14 175	123	2	8	0	0	1.2	A,B,C
Serum albumin	66 267	582	28	35	0	0	0.4	
Immunoglobulin	143000-1030000		8.4	2.3	-	+	0.8	

Table 1. Characteristics of the major proteins in cow`s milk (4)
[a] molecular mass are for the genetic variants in bold.

Caseins are synthesized in the mammary gland and contain ester-bound phosphate with relatively high content of proline. The amino acid and the gene sequences of the four casein fractions (with molecular mass from 19 038 for κ-casein to 25 388 for α_{s2}-casein), have been established. Immunoglobulins are the largest fraction (143 000–1 030 000 Da), while α-lactalbumin is the smallest (14 175Da) whey protein. The complete amino acid sequence of β-lactoglobulin, α-lactalbumin, and serum albumin, with molecular masses of 18 277, 14 175, and 66 267 Da, respectively, are also known. The immunoglobulins are extremely heterogeneous and are classified based on their immunochemical properties. In bovine milk five classes of immunoglobulins (IgG, IgA, IgM, IgE, and IgD) have been identified. They have similar structure with other immunoglobulins and contain two heavy and two light polypeptide chains covalently linked by disulfide bonds (4). Lactoferrin is ironbinding glycoprotein with high biological activity. It is present in both bovine and human milk. It has

important physiological functions with antimicrobial, antiviral and antioxidant activity, as well as immunomodulatory agent. Furthermore, its pharmacological influence on suppressing the tumors transfer has been established (5, 6). Lactoferrin concentration in human milk is usually at relatively high levels ranging from 1 mg/mL in mature milk to 7 mg/mL in colostrum whereas only minor amounts exist in bovine milk (0.02–0.2 mg/mL) (7, 8).

Kombucha is a association of yeasts (*Pichia, Zygosaccharomyces, Saccharomyces, Schizosaccharomyces, Saccharomycodes, Brettanomyces, Torulaspora* and *Candida*) and acetic acid bacteria (*Acetobacter* and *Gluconobacter*). It has been applied for fermentation of sweetened black and green tea (*Camellia sinensis*) for centuries (9, 10). Kombucha`s positive impact on human health has been investigated as well as confirmed by many authors. Kombucha was found to be helpful in cases of digestive aliments, kidney stones, gall bladder problems, diabetes, arteriosclerosis, high level cholesterol, high blood pressure, angina, gouty eczema, arthritis, rheumatism, irritability, anxiety, headaches, dizziness, fatigue, tiredness, in combating stress and cancer as well as vitalizes the physical body, etc. (11-18). Novel researches investigate the possibility of kombucha application as non-conventional starter culture, and its technological and nutritional aspects in dairy products (19, 20). As a result of milk fermentation by kombucha, new refreshing fermented dairy product with mild sour taste is obtained (20- 22). Sensory analysis of the product, showed high quality characteristics and good potential for human nutrition (21).

The aim of this study was to investigate the chemical quality and protein profile of new fermented dairy products obtained by kombucha starter culture at different fermentation temperatures.

EXPERIMENTAL

Sample production

Samples were produced from homogenized and pasteurized milk (AD Imlek, Division Novi Sad Dairy) with pH value 6.07. Kombucha was cultivated on a black tea (*Camellia sinensis* – oxidized, 1.5 gL^{-1}) with saccharose concentration of 70 gL^{-1}. The tea was cooled at the room temperature, after which inoculum from a previous fermentation was added in concentration of 10%. Incubation was performed at 29°C for 7 days. Kombucha inoculums (pH=3.17) in concentration of 10% (30 mL) was added for milk fermentation (20). Samples were fermented at 37°C (sample K37) and 42°C (sample K42). Fermentation was stopped after pH 4.6 was reached.

Chemical composition

Chemical quality was tested in fermented dairy products after production using following methods (23): dry matter (DM) (24) milk fat (MF) according to Gerber (25); total proteins (TP) (26) and ash (A) (27).

Determination of protein profile

Sample preparation was carried out according to Tidona et al. (28) with some modifications. Samples were diluted in buffer (0.125 M TrisHCl, 4% SDS, 2% glycerol, 5% β-mercaptoethanol, pH=6.8) in ratio sample:buffer = 1:1.5 v/v, and heated for 5 min at 100°C. The chip-based separations were performed on the Agilent 2100 bioanalyzer (Agilent Technologies, Santa Clara, CA, USA). Protein 80 Plus LabChip kit was used as capillary gel system and results were analyzed using Protein 80 software assay on 2100 expert software. Chips were prepared according to manufacturer's protocol (Protein 80 LabChip kit). Bovine serum albumin was used as the standard for quantification of the sample proteins. All samples were analyzed in triplicate.

Statistical analysis

Statistical analysis of results was carried out with the computer software program "Statistica" and were expressed as average, standard deviation of values obtained at three independent experiments STATISTICA version 9.1 (2009) (StatSoft Inc. Tulsa, OK, USA). The results were statistically processed by analysis of variance at the significance level $\alpha = 0.05$.

RESULTS AND DISCUSSION

Chemical composition

Our results revealed that dry matter, milk fat, total proteins and ash of samples produced at 37°C and 42°C have not been significantly changed during fermentation process (Table 2). Furthermore, differences are not significant between samples produced at different temperatures.

Chemical characteristic (g/100 g)[a]	Milk	K37	K42
Dry matter	10.34±0.02	9.98±0.24	10.34±0.03
Fat	2.00±0.00	1.98±0.00	1.98±0.00
Total proteins	3.12±0.12	2.82±0.17	3.14±0.08
Ash	0.69±0.05	0.62±0.05	0.68±0.01

Table 2. Chemical composition of milk and produced samples
[a] Mean value (n=3), ± standard dev

Fermentation flow

It is evident that milk fermentation started at pH=6.07 after addition of 10% of kombucha inoculums and reached pH value of 4.6 after 13 hours and 40 minutes in sample K37 (Figure 1). Sample K42 have shorter fermentation time than sample K37 and it lasted for 8 hours and 15 minutes. This could be result of extended lag phase and slower activity of starter culture on lower temperature. The pH value of K37 was relatively stable for 9 hours, when it rapidly dropped and after 3 hours reached pH value of 4.95.The pH value of K42 increased in the first 2 hours of fermentation (up to pH=6.2). At the following 2 hours pH value slowly decreased and then rapidly dropped to pH 5.1 in the next 1 hour and 30 minutes. Further decreasing of pH values of both samples continue until they reached pH 4.6. These results are in accordance with previous results (29).

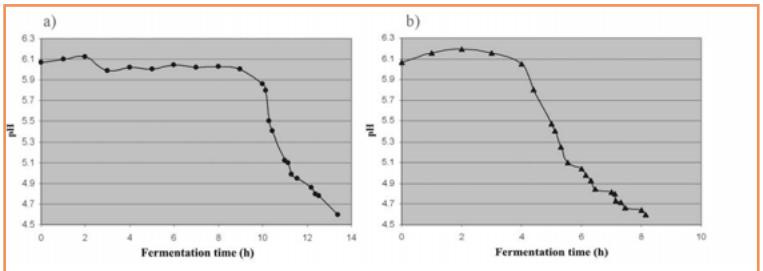

Figure 1. Changes of pH value during fermentation: a) K37, b) K42

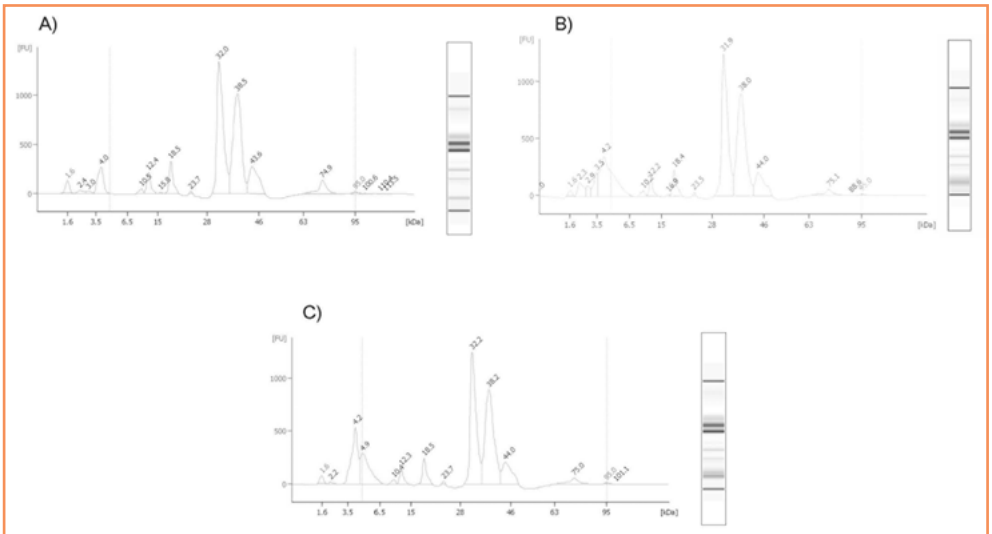

Figure 2. Quantification of the protein bands (1 -α lactalbumin, 2- lysozyme, 3- β- lactoglobulin, 4- β casein, 5- α casein, 6- κ casein, 7- lactoferrin) detected in the SDS-PAGE: A) milk sample; B) K37; C) K42

Protein profile

Using Agilent Bioanalizer 2100 all caseins migrate at higher molecular weights (Figure 2) than it was expected (4). This migration time shifts are due to caseins tendency to form aggregates, or their other chemical properties (glycosylation, phosphorylation pattern and overall hydrophobicity), that influence protein structure and interaction with the gel matrix during separation. Migration time shift of k-casein is especially emphasized, as it is shifted from an expected 19 kDa to approximately 46 kDa.

Relative content of α- and β- casein fractions increased in all samples, ($p<0.05$) while all other fractions showed decreasing of their relative content (Table 3). These results indicate better stability of α and β casein fractions compared to other protein fractions during fermentation. Lysozyme was detected only in milk sample, as it is cow`s protein with antimicrobial activity (28). In the final products lysozyme was not recorded, which could be result of starter cultures metabolic activities during fermentation process. As well as lysozyme, lactoferrin (protein with antimicrobial activity produced by cow), α lactalbumin (α-lac), β- lactoglobulin (β-lg) and κ casein relative content decreased. But these protein fractions were detected in the final products in significantly ($p<0.05$) lower concentrations. Milk whey proteins, α-lactalbumin and β-lactoglobulin, are among the main milk allergens. These results indicate that fermented dairy products produced by kombucha at 37°C and 42°C could be less allergenic compared to milk and suitable for special nutrition for people. Especially sample produced at 37°C had lower content of both allergenic fractions (α-lactalbumin and β-lactoglobulin) than in sample K42.

Protein fraction	Sample		
	M (%)	K 37 (%)	K42 (%)
α lactalbumin	4.86±0.04^{a}	3.26±0.04^{b}	3.88±0.05^{c}
Lysozyme	0.13±0.02	nd	nd
β lactoglobulin	6.92±0.04^{a}	5.48±0.05^{b}	5.81±0.09^{c}
β casein	35.66±0.18^{a}	40.57±0.20^{b}	38.54±0.01^{c}
α casein	35.17±0.16^{a}	38.81±0.14^{b}	39.19±0.05^{c}
κ casein	12.04±0.03^{a}	8.80±0.02^{b}	9.15±0.05^{c}
lactoferrin	2.93±0.02^{a}	1.42±0.07^{b}	1.42±0.05^{b}

Table 3. Protein fractions of produced samples
nd – not detected
^{abc}Means in the same row with different alphabets are significantly different ($p<0.05$)

CONCLUSION

Fermented dairy products obtained at 37°C have significantly longer fermentation time than sample produced at 42°C. Protein profile analysis revealed more stable α- and β-casein fractions compared to other protein fractions during milk fermentation. These results indicate that fermented dairy products produced by kombucha at 37°C and 42°C could be less allergenic than milk and more suitable for special allergenic nutrition, especially sample fermented at 37°C where content of α-lactalbumin and β-lactoglobulin were lower than in sample K42.These products could be proposed for special nutrition for consumers with proteins allergies.

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REFERENCES AND NOTES

1. Dutta S. M., Kuila R. K., Ranghanathan B., Laxminarayana H., *Indian Journal of Dairy Science*, **24,** 107-114 (1971).
2. Klaenhammer T. R., *Int Dairy J*, **5**, 1019–1058. (1995).
3. Law J., Haandrikman, A. *Int Dairy J*, **7**, 1–11. (1997).
4. Ng-Kwai-Hang K.F., *Heterogeneity, Fractionation, and Isolation* in Encyclopedia of dairy sciences, Edited by John W. Fuquay, Patrick F. Fox, Paul L. H. McSweeney . London, UK: Elsevier Ltd. (Vol 3 pp.751-764 (2011).
5. Tsuda H., Sekine K., Fujita K.-I., *Biochem. Cell Biol,* **80 (1)**, 131-136 (2002).
6. Brock J., *Immunology Today,* **16(9)**, 417-419 (1995).
7. Brisson G., Britten M., Pouliot Y., *J Membr Sci,* **297**, 206-216. (2007).
8. Wakabayashi H., Yamauchi K., Takase M., *Int Dairy J,* **16**, 1241-1251(2006).
9. Dufresne C., Farnworth E., *Food Res Int,* **33**, 409–421 (2000).
10. Teoh A. L., Heard G., Cox J., *Int J Food Microiol,* **95**, 119–126 (2004).
11. Thummala S., Ramachandran A., Jagadeesan

A., Uppala S., *Biomedicine & Preventive Nutrition,* **3**, 53–58 (2013).
12. Semantee B., Ratan G., Parames C., Sil., *Food Chem Toxicol,* **60**, 328–340 (2013).
13. Houda B., Amina B., Emna A., *LWT- Food Sci Technol,* **47**, 71-77 (2012).
14. Balentine D. A., Wiseman S. A., Bouwens L. C., *Food Science and Nutrition,* ***37***, 693–704(1997).
15. Petrović S., Lončar E., *Microbiology,* ***33***, 101–106 (1996).
16. Guttapadu S., Yang Z., Wieger K., *J Agric Food Chem,* **48**, 2589–2594 (2000).
17. Pasha C., Reddy, G., *Food Chem,* ***89***, 449–453 (2005).
18. Sreeramulu G., Zhu Y., Knol W., *Acta Biotechnol,* **21**, 49–56 (2001).
19. Malbaša R., Lončar E., Milanović S., & Kolarov Lj.,*Acta Periodica Technologica*, **40,** 47–53 (2009a).
20. Malbaša R., Milanović S., Lončar E., Đurić M., Carić M., Iličić M., Kolarov Lj., *Food Chem,* **112**,178–84. (2009b).
21. Iličić M., Kanurić K., Milanović S., Lončar E., Djurić M., Malbaša R., *Rom Biotech Lett*, **17 (1)**, 7013-7021(2012).
22. Milanović S., Lončar E., Djurić M., Malbaša R., Tekić M., Iličić M., Duraković K., *Acta Periodica Technogica*, **39,** 37-46 (2008).
23. *Standard methods of milk and dairy products analyses,* Edited by Carić. M., Milanović. S. et al.. Prometej & Faculty of Technology. Novi Sad. Yugoslavia, p. 204 (2000).
24. International Dairy Federation, Brussels, Belgium. IDF Standard 21A IDF (1982).
25. Brussels, International Dairy Federation, Belgium. IDF standard 105, IDF (1981).
26. International Dairy Federation, Brussels, Belgium. IDF Standard 20, IDF (1962).
27. International Dairy Federation, Geneva, Brussels, Belgium IDF Standard 90, IDF (1979).
28. Tidona F., Sekse C., Criscione A., Jacobsen M., Bordonaro S., Marletta D., *Int Dairy J,* **21**, 158-165 (2011).
29. Milanović S., Carić M., Lončar E., Panić M., Malbaša R., Dobrić D, *Milk and milk products,* **(1-2),** 8-13 (2002).

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