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Original article

Changes of Nutritional Characteristics of Whey Fermented with Kefir Grains - A Preliminary Results

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Abstract

Whey as a major by-product of cheese production is a subject of survey in the past decade because of its nutritional value and the possibilities to make high quality dietetic beverages. Because whey is produced in high amounts and contains organic matter in high quantity its disposal can cause serious environmental pollution. Instead the whey can be used as a raw material to produce beverages which can be classified as a healthy or functional food with added value. This is especially important for the countries with poor economic development where with a single technological process all potential of whey as a raw material can be utilized. The aim of the present study was to evaluate the whey nutritional value and the possible changes as a result of whey fermentation using kefir grains. The samples of unpasteurized cow's whey were collected from a small farm where cheese was produced in a traditional way. After milk curdling with commercial enzymatic rennet the whey was collected and inoculated with kefir grains. The fermentation was carried out at room temperature (25 ℃) in the span of 24 hours. The inoculation of the whey was performed with different quantities of kefir grains (5% and 10%). The examined phisico-chemical characteristics of whey were not significantly changed. The presence of lactose is an exception. The microbiological examination showed a tendency of decreasing in the number of total aerobic bacteria, *E. coli* and *Staphylococcus aureus*. As it was expected, the number of yeasts and Lactobacillus sp. increased. Additional inoculation of fermented whey with strain cultures of E. coli ATCC 8739 and coagulase positive Staphylococcus aureus ATCC9610 was performed. After 24 hours of incubation at 37 ℃, no strains of E. coli ATCC 8739 and coagulase positive Staphylococcus aureus ATCC 9610 were recorded. These results indicated the bactericidal effects of kefir grains over the used bacterial strains but additional investigations are required.

Keywords: Whey, Kefir Grains, Microbiological Characteristics, Physico-Chemical Characteristics.

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INTRODUCTION

The constant development of the dairy industry brings a variety of new dairy products with different, characteristic, properties and improved nutritive and qualitative values. Whey beverages reckon among this kind of products. As a major byproduct of dairy industry, whey was rarely used in liquid form and it was additionally processed mostly to cottage cheese, whey powder or whey extracts (proteins and lactose). The processing of wheystarted during the 1970's and until today, different whey based beverages has been developed (Jeličić et al. 2008). In North Macedonia whey is mostly processed in cottage cheese, or used additionally in animal feed, but lately it has also appeared as a fresh beverage on the market. The nutritive value of whey, the better protein digestibility in comparison to casein, the participation in immune system activation, the bactericid characteristics as well as the positive influence in allergic reaction inhibition makes this beverage to be in high demand on the market (Barukčić, Jakopović and Božanić, 2019).

On the other hand, the dairy industry that creates a large amount of whey has to introduce processes to recover all of the milk solids. Theese processes need to be cost-effective and environmentally friendly (Londero et al., 2011). Individual or small scale cheese producers as well as farmhouses usually can't afford such expensive technologies and usually give the whey to the animals or simply discard it. Instead, whey can be used as a raw material to produce beverages which can be classified as a healthy or as functional food with added value. This is especially important for the countries with poor economic development where with a single technological process all of the potential of whey, as a raw material, can be utilized. Nutritional value and the growing awareness of consuming food with health promoting additives qualify whey as a healthy functional food. Liquid fermented whey can be a carrier of probiotics with low cholesterol and lactose content, which is an appropriate beverage for most of the human population because 5-15% of European population and up to 80% of the population from central Asia and Africa are lactose intolerant (Shukla and Kushwaha, 2017).

Characteristics of the fermented whey using kefir grains has been investigated by different authors (Londero et al., 2011, 2012; Balabanova and Panayotov, 2011; Megalhães et al., 2011; Shukla and Kushwaha, 2017) indicating that fermented whey beverages can be interesting alternative for cheese whey utilization. Kefir grains are a symbiotic association of lactic acid bacteria, yeasts and acetic acid surrounded by kefiran, water soluble polysaccharide matrix (Megalhães et al., 2011). The microorganisms from kefir grains are responsible for chemical changes of whey during fermentation like proteolysis, lactose consumption, production of volatile compounds and organic acids (Megalhães et al., 2010). They also show probiotic characteristics improving the health state of the consumers like: improving intestinal flora balance and mucosal defense, relief of the lactose intolerance symptoms, stimulation of immune system, showing antibacterial activity and antioxidant potential (Megalhães et al., 2011; Weschenfelder et al., 2018).

The aim of this study was to prepare the fermented whey beverage and to determine its chemical and microbiological characteristics after being treatedwith different amount of kefir grains. The whey used for this study was obtained after the manufacturing of traditional cheese using unpasteurized milk. It was challenging to determine the possibilities of making quality healthy drink using simple technological process and to evaluate its possible antibacterial activity.

MATERIALS and METHODS

Whey obtained from the production of traditional cheese from unpasteurized cow milk was a subject of this research. The whey samples collected from the farm with small production capacity were kept and transported to the laboratory in a transport refrigerator at a temperature of 4°C. Samples who couldn't be analyzed immediately, were kept at a temperature of -18℃. The used kefir grains were first activated by putting them in milk and after 12-24 hours were washed with sterilee water and then the whey was inoculated. Kefir grains can be composed of different species of lactic acid bacteria as well as yeasts. Kefir grains usually consist of *Lactobacullus delbrueckii* subsp *bulgaricus*, *L. helveticus, L. kefiranofaciens* subsp. *kefiranofaciens*, *L. kefiranofaciens* subsp. *kefirgranum*, *L. acidophilus*, *L. fermentum*, *L. brevis*, *L. kefir* and *L. parakefiri*. From Lactococcus species contains: *L. lactis* subsp. *lactis*, *L. lactis* subsp. *cremoris* and the yeasts population consists of *Sacharomysec cerevisiae*, Kazachstania *exigua, K. turicensis, Kliveromyces marxianus, Pichia fermentans, Debariomyces occidentalis* (Londero et al. 2012, de Oliviera Leite et al., 2013)

The process of whey fermentation was carried out in one-liter sterile glass bottles filled with 500 ml of whey. The whey in the bottles was inoculated with 5% and 10% kefir grains in sterile conditions and left to ferment 24 hours at room temperature (25℃). After the fermentation, the whey samples were filtered through a sterile plastic sieve. The fermented whey was collected in sterile Erlenmeyer flasks and microbiological analyses were performed the same day. The chemical analyses of whey and fermented whey were performed in the following days or were frozen.

Antimicrobial characteristics of fermented whey were examined with additional inoculation of *Escherichia coli* ATCC 8739 and *Staphylococcus aureus* ATCC 9610 strains in fermented whey after removing grains.

Chemical analyses of whey

The following analyses were performed on not-fermented and fermented whey: determination of total solids by drying on a temperature of 105 °C till a constant weight was reached; determination of crude proteins by Kjeldahl method (Nx6.38); determination of lipids by method of Gerber; determination of ash by incineration for 8 hours at temperature of 600 °C; lactose was determinate by the Lane-Eynon method. The results were expressed as percents. pH was measured by a pH-meter and the titratable acidity was determinate by Soxhlet-Henkel titrated with n/10 NaOH. Results were expressed as SH°

Microbiological analyses

Microbiological analyses of fermented and not-fermented whey were performed by determination of: total aerobic bacteria, coagulase positive Staphilococcus, *Escherichia coli*, Yeasts and *Lactobacillus* sp. Analyses were made according following methods:

Table 1. Methods for microbiological analyses

Parameters	Methods
Total number of aerobic bacteria	ISO 4833: 2013
Coagulase positive *Staphylococcus*	ISO 6888-1:1999
E.coli	ISO 16649-1,2:2017
Yeasts	ISO21527-1,2:2008
Lactobacillus	On Rogosa agar at 30°C

After additional incubation with *E. coli* ATCC 8739 and *Staphylococcus aureus* ATCC 9610 strains, the whey samples were cultivated on Chromogenic coliform agar and Baird Parker respectively and incubated 24h at 37°C.

Statistical analyses

The statistical analysis of the obtained results was performed using the computer program Excel 2007. The statistical significance between the groups was determined by one-way analysis of variance (ANOVA). The values are presented as mean(s) ± statistical error for each variable.

RESULTS AND DISCUSSION

Results from chemical analyses of not-fermented whey and whey fermented by using 5% and 10% kefir grains are shown in Table 2. Significant changes ($p<0.01$) can be noticed in the content of total solids and lactose, and also in the pH and titratable acidity value ($p<0.001$), as a result of kefir grains microbiota activity. Significant decrease of the pH value from the initial 6.63 to 3.63 and 3.44 are noticed after 24h of fermentation. These results are similar or lower than the results of Megalhães et al. (2011 a, b) and Weschenfelder et al. (2018) who recorded 4.3 to 4.0 pH values after 48 hour of whey fermentation. The authors also emphasize the sharp increase of lactic acid concentration after 48 h of kefir grains incubation. In this study, significant changes were noticed in the concentration of lactose, as a result of lactic acid fermentation, which decreased from 4.75-4.20%, while titratable acidity increased from 4.43 to 54.60 SH° (Table 2). According to Megalhães et al. (2011 a, b) total utilization of lactose in cheese whey can be expected after 72h and after 60 h in milk. This is because, milk is richer with nutrients, especially proteins than whey, and because the kefir grain's microbial community needs more time to adapt to cheese whey, as milk is commonly used media for their growth.

Table 2. Chemical composition of whey after fermentation with different amount of kefir grains

	Total solids %	Crude protein %	Lipids %	Lactose %	Ash %	pH	Titratable acidity SH°
Whey 0% k.g.	7.53±0.14A	1.39±0.31	0.63±0,05	4.75±0,06A	0.51±0,01	6.63±0,04A	4.43±0,21A
Whey 5% k.g.	6.64±0.14B	1.09±0.10	0.53±0.06	4.40±0.04B	0.49±0.02	3.65±0.03B	40.47±0.50B
Whey 10%k.g.	6.55±0.19B	1.01±0.22	0.57±0.06	4.20±0.04B	0.51±0.04	3.44±0.02B	54.60±0.40B
P value	<0.01	n.s	n.s	<0.01	n.s	<0.001	<0.001

k.g. - kefir grains; n.s – not significant; SH° - Soxlet Hencel degrees;

Different letters in the column indicate statistical differences among the data ($p<0.01$) ($p<0.001$)

The concentration of total crude proteins, lipids and ash was not significantly changed after the fermentation process. From Table 2 it can be seen that, protein concentration is decreasing from 1.39% to 1.01%. Lipids content is in the range of 0.53-0.63% and the ash is in the range of 0.49-0.51%. These results are similar with the results of Jeličić et al. (2008); Londero et al (2011) and Weschenfelder et al. (2018). In fact, the whey composition depends of the milk characteristic, technology of cheese making and renneting and the whey fermentation conditions Megalhães et al. (2010).

The obtained results of microbiological analyses of the fresh whey are shown in Figure 1. From the results it can be seen that non-fermented whey is characterized with high load of microorganisms, among which pathogenic bacteria such as *S. aureus* and *E. coli*. This is expected because, the whey was not pasteurized, but still indicates necessity of improving sanitary conditions in the farm. After the 24h of whey fermentation with 5% and 10% kefir grains the number of *S. aureus* and *E. coli* starts to decrease, getting a final value of 2.15 log cfu/ml and 0.95 log cfu/ml respectively. The number of total aerobic bacteria, lactobacilli and yeasts increased reaching their higher value (4.73, 4.09 and 4.66 log cfu/ml) after whey fermentation with 10% kefir grains (Fig.1).

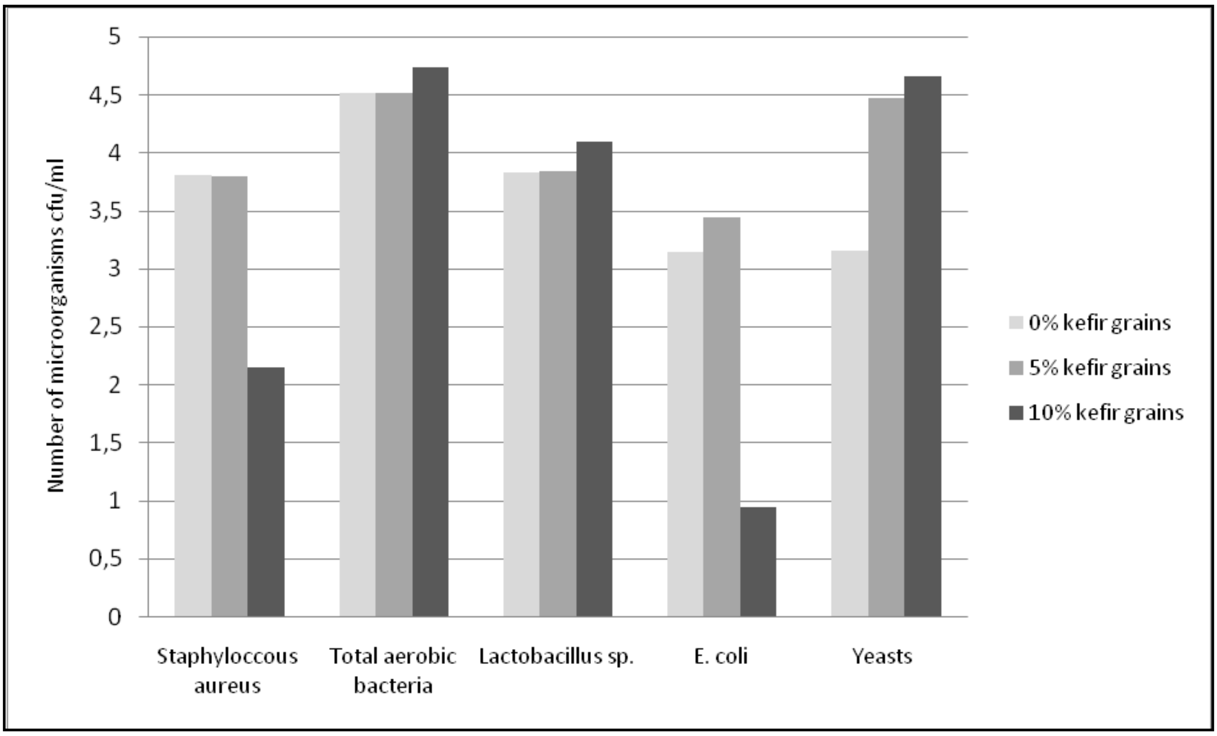

Figure 1. Microbiological characteristics of whey fermented with different amount of kefir grains

The additional inoculation with *E. coli* ATCC 8739 after filtration of kefir grain results with an absence of *E. coli* and *S. aureus* in the fermented whey (Fig.2). The other groups of microorganisms (lactobacilli, total aerobic bacteria and yeasts) remain in the same quantity in all of the investigated whey samples. After the addition of *Staphylococcus aureus* ATCC 9610 strain (Fig.3), the absence of *E. coli* and *S. aureus* was recorded in whey fermented with 10% kefir grains. In fermented whey with 5% kefir grains, the number of the above mentioned bacteria was 3.73 log cfu/ml and 2.59 log cfu/ml respectively. The number of total aerobic bacteria, lactobacilli and yeasts remain in the same range in the fermented whey (Fig.2 and Fig. 3) or decreased in comparison to the not-fermented whey (Fig 1.).

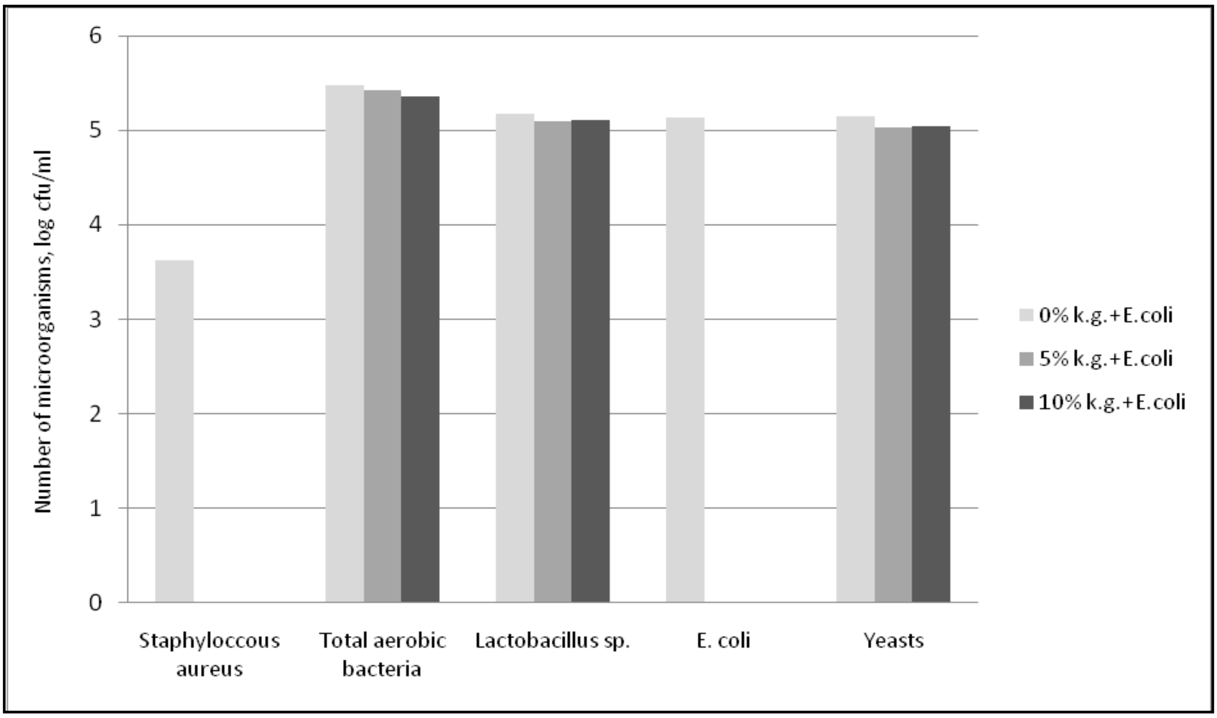

Figure 2. Microbiological characteristics of whey fermented with different amount of kefir grains and *E. coli* ATCC 8739 added

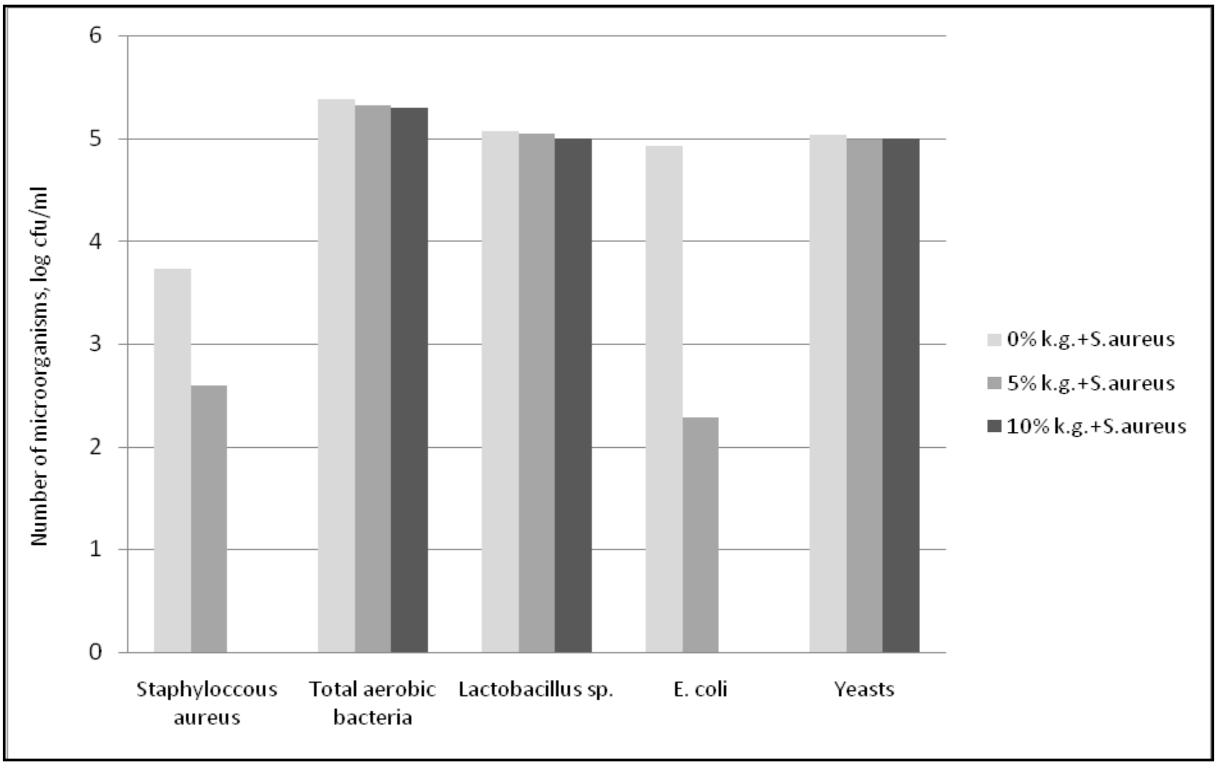

Figure 3. Microbiological characteristics of whey fermented with different amount of kefir grains and *Staphylococcus aureus* ATCC 9610 added

The inhibitory potential of fermented whey can be seen from the microbiological analyses. Londero et al (2011, 2012) emphasized that inhibitory activity of the fermented whey is due to organic acids, lactic and acetic acid produced by the kefir grains during fermentation processes or bioactive peptides, which are result of kefir grains proteolitic activity. The reduction of the pH value itself, as a result of fermentation, doesn't have an inhibitory effect on pathogenic bacteria. According to authors, lactic acid dissociation in bacteria cell lowers the intracellular pH, which affects cellular metabolic functions and membrane osmolarity. On the other hand, the inhibitory activity also depends on the initial number of pathogenic bacteria. If the initial number is lower, the pathogenic bacteria can't survive the inhibitory effect of fermented whey. Antibacterial activity of fermented whey is probably associated with microbial constitution of kefir grains, their ability to produce bacteriocins, the quality and composition of the whey as a substrate of fermentation, handling, packaging and storage of the end product. The chemical processes during fermentation also affect the antibacterial activity and the characteristics of evaluated microorganisms (Weschenfelder et al., 2018). According to the authors, behavior of the microorganisms in food can be associated with intrinsic characteristic of food. Antimicrobial activity of kefir community can be the result of antagonistic behavior of lactobacilli from kefir grains against pathogenic bacteria such as *E. coli*, *L.* monocyotgenes, *Samonella typhimurium, Y. enterocolitica, S. aureus, B. subtilis* (Leite et al., 2013). The authors emphasize that organic acids, bacteriocins, carbon dioxide, hydrogen peroxide, ethanol and diacetyl, produced from kefir microbiota, have possible impact over pathogen bacteria growing or over deteriorating microorganisms growing.

Conclusion

Fermented whey with kefir grains seems to be a good and simple way of getting quality beverages with growth-inhibitory activity against some pathogenic bacteria, providing the consumer with necessary nutritional ingredients, having potential probiotic characteristics. Chemical characteristics of fermented whey depend on milk quality and did not change significantly, except in the content of lactose pH and titratable acidity. The microbiological characteristics of whey indicate an increase in total number of aerobic bacteria, lactobacilli and yeasts after fermentation with 10% kefir grains. In contrast the number of anlised pathogenic bacteria decreased. *E. coli* and *S. aureus* were not detected in fermented whey incubated with additional *E.coli* strain. Fermented whey with 10% kefir grains was inhibitory for *E.coli* and *S. aureus* when cultivated with additional strain of *S. aureus*. With this single technological process, the whey as a by-product has a potential as a new beverage with good quality for animal and human nutrition. Althought fermented whey shows antibacterial activity against pathogenic bacteria such as *E. coli* or *S. aureus*, the food still can't be defined as safe for use, because of the bacteria's capability of producing toxins. Further investigations are needed to be sure that gained product is safe for human and animal consummation.

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