Microbiological Aspects Considering the Production of Nutraceutical Curd Containing Onion

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Abstract
The curd is a traditional Romanian dairy product highly appreciated by the consumers. The purpose of this study was to produce and analyze from the microbiological point of view a nutraceutical curd containing onion, as a prebiotic source. The synbiotics generate among the organoleptic benefits also health improvement due to the different actions taken by probiotics and prebiotics, working together. The curd was produced by the traditional method using buffalo milk with reduced cholesterol content due to the treatment with crosslinked β-ciclodextrin with adipic acid.The curd prepared by rennet adding and coagulation at 30°C during 30-45 minutes was subjected to the maturation at a temperature of 12-16°C. Considering the microbiological tests, the curd recorded a pozitive evolution, being characterized by a lower microbial load compared with a control curd, prepared without onion.

Keywords: β-ciclodextrin, curd, microorganisms, nutraceutic, probiotics, prebiotics.

1. Introduction

Probiotic means "for life" and it refers to different species and strains with a diverse range of clinical and immunological capacities [1]. The research conducted in the field of emphasizing the benefits for health of probiotic dairy products has revealed a series of conclusions: produce short-chain fatty acids, enhance the minerals absorption, improve the intestinal microbial balance and consequently enhance the immune system by reducing the proportion of pathogenic bacteria due to the production of lactic and acetic acids, reduce the phenomena of lactose intolerance, suppression of damaging microbial enzymes associated with colon cancer in animals, restoration of intestinal microflora after gastrointestinal diseases, inhibitory effect of mutagenicity [2, 3].

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Lately, probiotics are found in combination with prebiotics in the products called nutraceutical or synbiotic products used to manage the gut microflora [4]. Prebiotics are dietary ingredients, usually nondigestible food carbohydrates (if late, functional alternatives such as galacto-oligosaccharides, GOS, or cheap sources of carbohydrates like onion, sago are under investigation) which ordinarily stimulate bifidobacteria development [5-7].
Thus, nutraceuticals are gaining popularity during the present decade due to their role in prevention and treatment of different diseases [8].
Also, consumers became more careful about their eating habits and therefore more aware about the diseases that can be generated or influenced by their diet. Some experiments undertaken on humans and animals have shown that plasma cholesterol can be raised at an increased intake of cholesterol and saturated fat, generating the risk of coronary heart diseases [9]. Thus, a lot of studies have been made considering different methods of cholesterol reduction from a lot of products: the use of supercritical CO_2, of organic solvents, of

saponin, etc., but they all implied a series of disadvantages (organic solvents, residues, etc.) [10]. Further researches had opened the way to the use of powder of β-cyclodextrin (β-CD), a nontoxic, edible and chemically stable compound which demonstrated its efficiency in cholesterol elimination [11-18]. But powder β-CD is too expensive and not efficient considering a recycling process, so it can be bonded efficiently with the adipic acid for its recovery and recycling [19, 20]. Although these studies demonstrated the efficiency of the cholesterol removal, there is a lack of information considering the microbiological aspects of the cholesterol removal from milk and dairy products. Also, the majority of the researches apply the cholesterol removal method directly from the dairy products, while in this study the cholesterol is removed from the raw milk further processed in products due to the danger of postproduction contamination. So, this study was conducted in order to analyze a nutraceutical curd, obtained from low cholesterol buffalo milk, considering the microbiological point of view.

2. Materials and methods

Preparation of the crosslinked β-cyclodextrin (β-CD) with adipic acid

This compound was obtained as follows: 15 g adipic acid was dissolved in 100 ml of distilled water, then 100 g β-CD was added and the mixture was stirred at 700 rpm for 2 hours at 80 ° C; the pH was adjusted to 12 with 1N NaOH and the solution was stirred at 60 ° C for 24 hours; a new re-adjustment of the pH was made, this time to 5 using 10 % HCl; the β-CD linked with adipic acid solution was kept at 4 ° C for 48 hours for crystallization, then the compound was recovered by filtration on paper and drying in a vacuum oven at 60 ° C for 20 hours, ending with a sieving operation for its uniformity [20].

Cholesterol removal from buffalo milk

The buffalo milk was treated with β-cyclodextrin linked with adipic acid at a rate of 1 % (wt / vol) and stirred at 800 rpm with an electromagnetic stirrer at 10 ° C for 10 minutes. After stirring, the mixture was centrifugated at 6000 rpm for 10 minutes (cholesterol removal was about 95%) and the supernatant fraction containing cholesterol-reduced milk was decanted and used for the curd preparation [20].

Nutraceutical curd production

Buffalo milk with reduced cholesterol level was heated to 30-31 ° C; then the probiotic culture was added and also the finely chopped onion tails in a 10 % share, the salt and the rennet and after they were stirred, the milk was left at 30 ° C for about 30-45 minutes for coagulation; the obtained curd was cut into cubes and passed in the forms for the whey separation; then the curd was poured into some gauze bags, which remain hanging for 3 hours until all the whey drained freely; the obtained curd was subjected to the ripening operation at temperatures of 12-16 ° C for 2-3 days [21]. The obtained nutraceutical curd is shown in figure 1.

Figure 1. Nutraceutical curd

Microbiological methods

Microbiological analyses were performed on the nutraceutical curd. Samples were collected and homogenized in aseptic conditions, immediately after which the decimal dilutions were made in sterile saline. Forwards, to determine the total number of germs it was applied the plate count method (STAS ISO 4833-2003), using as a culture medium the Plate Count Agar (PCA). The inoculated Petri plates were stored at 30-35^{0}C for 24-48 hours, after which the pair of corresponding plates was selected, and the average value of the total number of aerobic mesophilic germs/g of the product was calculated. To test the hygiene conditions during the obtaining and storage of nutraceutical curd, the total number of yeasts and molds which could spoil the product was performed according to STAS ISO 7954-2001. For this determination, in each Petri plate over inoculum was distributed Sabouraud Choloramphenicol Agar Medium. The inoculated

Petri plates were kept at room temperature, in a dark place for 4-5 days. Thus, by average value calculation, the number of yeasts and molds/g of product was determined. To detect the fecal contamination of the product, the number of coliform bacteria was carried out through the Most Probable Number (MPN). To each test tube for coliforms cultivation on the Brilliant Green Bile medium, a Durham tube was introduced to track the gases evolution. By using the Mac Cready's table the most likely number of coliform bacteria/g of product was reported, after incubation at 37^0C for 24-48 hours, depending on the number of positive reaction tubes. The number of staphylococci coagulase-positive colony forming units was established by inoculation from each dilution of a three plates with Baird Parker Agar medium, followed by incubation at 37^0C, for 24-48 hours. The black or gray typical colonies were counted, bright and convex (between 1 and 1.5 mm in diameter after incubation for 24 hours, and between 1.5 and 2.5 mm in diameter after incubation for 48 hours), surrounded by a clear zone (STAS ISO 6888-1/2002).

3. Results and discussion

Yeasts and molds present in dairy products lead to the excretion of side metabolic products, causing odors and flavors in addition to the visible changes in color or texture. Also, yeasts, coliform bacteria, heterofermentative lactic acid bacteria and spore-forming bacteria can all cause gassing defects in the cheeses [22].

The degree of the microbial contamination with aerobic mesophilic germs was studied in the analyzed samples, due to the fact that it can cause defects on the buffalo milk curd with low cholesterol level. The presence of the lactic bacteria play an important role in the fermentation and maturation of the product, but curd is also an excellent medium for microbial agents including the pathogens which are capable of food poisoning production.

The addition of onion in the curd had the purpose of obtaining a nutraceutical product, properly from the microbiological point of view. Onion (*Allium cepa* L.) is a prebiotic and also has antimicrobial action, and according to Ali et al. [23] the effect of fresh onion homogenates is due to both methylcysteine sulfoxide and S-n-propyl cysteine sulfoxide from which the corresponding thio-sulfinates are formed enzymatically [24, 25]. Onion also contains numerous phenolic compounds with antimicrobial activity and particularly red onion water extract, has the potential to prevent some pathogens in foods in natural ways [26].

Thus, the two types of β-cyclodextrin-treated curd (simple and that with the addition of onion) were analyzed regarding the content of aerobic mesophilic germs, yeast and molds, coliforms and coagulase-positive staphylococci. Their presence was detected by microbiological analyses that certify the macroscopic appearance of the obtained colonies (Figures 3, 4, 5, 6).

The results of the microbiological analyses are presented in the following table.

Table 1. Values of the analyzed microbiological parameters

Samples	The microbiological parameters (CFU/g)			
	Total number of germs	Yeasts and molds	Coliforms	Coagulase-positive staphylococci
Simple curd	3.25×106	4.75×103	45	5.36×102
Onion curd	2.24×106	4.15×103	35	4.63×102

In order to highlight the differences considering the analyzed microbiological aspects, the average logarithmic values of the number of colony forming units are shown in Figure 2.

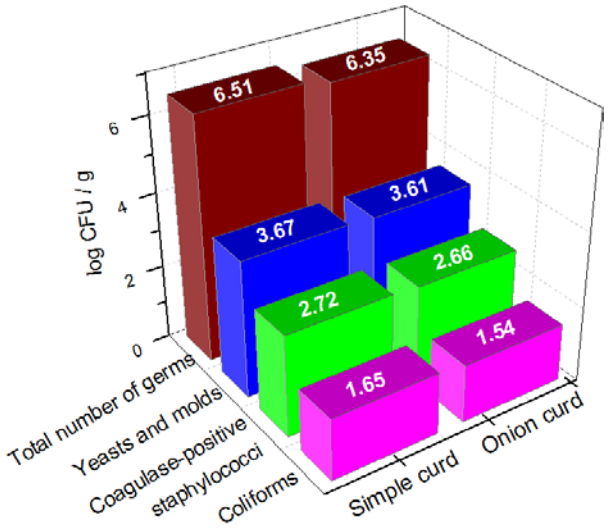

Figure 2. Microbiological analyses on samples of nutraceutical cheeses

As can be seen in the above figure, in the case of the nutraceutical onion curd the average values of the main groups of the analyzed microorganisms are lower compared with the simple curd.

The total number of germs is 3.25×10^6 CFU/g for simple curd, and for the onion curd is with almost 31 % lower, having a value of 2.24×10^6 CFU/g.

The presence of germs in a number having a 10^6 order is due to the addition of probiotics with the purpose of improving the product quality.

However there can be also observed a slight inhibitory action of the β-cyclodextrin, fact confirmed by Martin Del Valle [27] who highlighted the preservative action of β-cyclodextrin on apple juice.

Determination of coagulase-positive staphylococci (*S. aureus*) and coliforms in cheese are commonly used as indicators of hygienic quality. In the simple curd there were identified 5.36×10^2 CFU coagulase-positive staphylococci / g and 45 coliforms / g, while the addition of onion generated a reduction of these bacteria potentially pathogenic to 4.63×10^2 CFU coagulase-positive staphylococci / g and 35 coliforms / g. Thus, in the onion curd case the coagulase-positive staphylococci value was lower with 13.61% and the coliform bacteria value with 22.22% compared to the simple curd.

Yeasts and molds presence in the finished product may be the result of improper hygiene conditions [28]. As for the results in the case of buffalo curds, there were found no significant differences, the values being 4.75×10^2 CFU / g for simple curd and 4.15×10^2 CFU / g for the onion curd.

Macroscopic aspects of the main types of microorganisms present in the curds are represented in the figures 3-6.

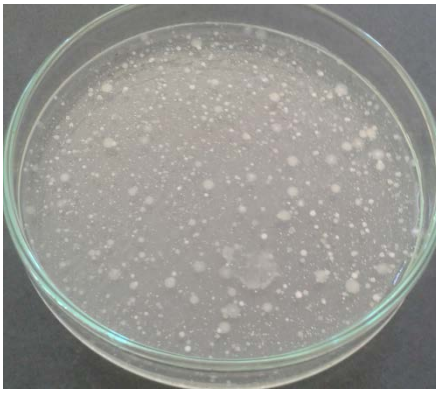

Figure 3. Specific bacterial microbiota

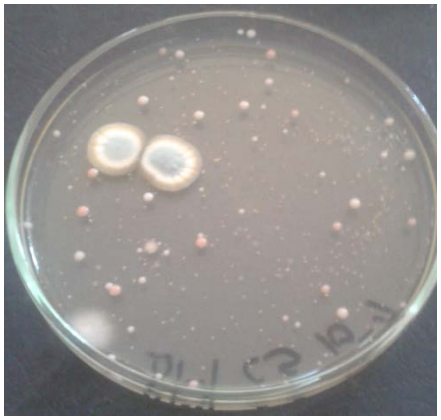

Figure 4. Yeasts and molds

Figure 5. Coagulase-positive staphylococci

Figure 6. Coliforms positive test tube

4. Conclusions

Microbiological analyses of the curds obtained from buffalo milk indicate an increased bacterial load which can be explained by the fact that they were obtained from raw milk unsubjected to any heat treatment. Also, probiotic bacteria were inoculated to improve the quality of the finished product.

The total number of germs registers an average between 2.24-3.25 × 10^6, with a reduced charge in the onion curd case which is due to the onions bacteriostatic effects.

The content of yeasts and molds which can alter the curds organoleptic characteristics is also lower in the curd containing onion. The same thing can be observed in the case of coliform bacteria, which are indicators of fecal contamination.

Coagulase-positive staphylococci number does not exceed the maximum allowable of 1000 CFU / g product.

The addition of onion in the dairy products production generated a nutraceutic curd less microbiologically contaminated with beneficial properties for the health of the consumers.

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