

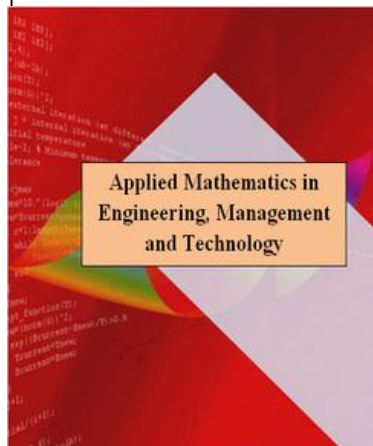
Identification of bacterial flora on the flavor of local cheese produced from the milk of cows and sheep in the city of Neyshabur and their role in the market based on sensory evaluation of cheese sales

Mohsen Vazifedoost^{1*}, Reza Majidzadeh Heravi², Jamshid Mehrzad²

¹ Department of food science and technology, Neyshabur Branch, Islamic Azad University, Neyshabur, Iran.

* Corresponding author; e-mail: m.vazifedoost@iau-neyshabur.ac.ir

² Department of food science and technology, Neyshabur Branch, Islamic Azad University, Neyshabur, Iran.



ABSTRACT

Bacterial flora of cheese the most important features is its descriptive sense. One of the main purposes of identification flora in traditional dairy products, industrial manufacturing products with similar features aromas and taste the local dairy. For this purpose, samples of cow and sheep's milk cheese traditional originating from Neyshabur were studied. Bacteria isolated from these samples by using morphological identification and sequencing of the 16S ribosomal DNA, were approved. The most frequent type of family lactic acid bacteria, isolated from cheese samples, respectively, were: Enterococcus (62.962%), Lactobacillus (25.925%), Streptococcus (7.407%) and Leuconostoc (3.703%). feeling among local cheeses accounted for the highest level of sales in the city of Neyshabur accounted for a significant difference ($p < 0.05$) to control the industrial cheese (UF) showed.

Keywords: lactic acid bacteria, identification, cheese, sensory evaluation, sales

1. Introduction

Lactic acid bacteria widely distributed in nature and their nutritional needs are very complex. Therefore most of them residents of foods rich in carbohydrates, protein analysis, and vitamins, and have reduced oxygen conditions, and therefore are found in dairy. (Abdi et al., 2006). Organoleptic characteristics such as flavor, increased demand for cheese and cheese to follow this new industry classification not be storing for months, but wanted a product that has had good organoleptic properties and results in the fastest possible time to sell (Mortazavi et al., 1991) and in terms of a greater share of the consumer market finds. Some microorganisms due to certain enzymes, the role of the initiator in the production of dairy products and adding them to the milk production of dairy products with special flavor and become more marketability. So in industrial cheese production, the microorganisms in the production process of the milk and then the milk is turned into cheese preparations (Mallesha et al., 2010).

Because of the importance of health and prevent disease, the milk used in cheese production. These bacteria were identified from prior cultures constitute started. The starter, including all bacteria naturally in the milk were not. This kind of cheese flavor in milk used in their natural bacterial flora, different flavor in pasteurized milk cheese industry that has been used. In Iran, some of which are highly popular in traditional cheeses such as cottage cheese Lighvan noted. Note that lactic acid bacteria have the ability to degrade aromatic compounds and volatile milk for this purpose may have an important role in the development of cheese flavor and aromas (Pirouzian et al., 2012). Purpose of this study is the identification of bacterial flora on the cosmetics and taste local cheese produced from cow's milk and sheep in the city of Neyshabur and their role on the sensory evaluation of the amount of cheese on the market.

2. Materials and Methods

2.1 - Sampling and bacterial isolation

Twelve samples of both a traditional cheese kg cow and sheep milk with a maturity of three months (June to August) from six different parts of the city of Neyshabur (any part of a cow and a sheep cheese samples) prepared. The process for producing the same, and the same should be done in all cases, the project manager overseeing the production process. The samples were diluted in saline solution (0.1 to 0.001), and then were transferred to MRS-Agar medium and incubate at 37 ° C for 72-36 h were. Different colonies from each plate were randomly selected to acquire. The pure colonies MRS agar medium were passaged several times. Then in terms of appearance, catalase test and Gram stain test, isolates were studied. Isolated bacteria in MRS broth cultures after centrifugation and washing the precipitate with buffered saline, phosphate sterile 200 ml sucrose solution in 15% sterile lyophilized and a temperature of - 20 ° C were maintained (Hilmi et al.,2007) .

2-2 - DNA extraction

DNA extracted from 24-h cultures of selected strains, the cell sediments were washed first with 300 ml of lysis buffer (containing 50 mM sucrose, EDTA, and 25 mM Tris-HCL with pH = 8)and 10 microliters of enzyme lysozyme concentrations (mg / ml) 10 was added and incubated for 10-15 min at 37 ° C. Gzdyd rest extraction by Kate Bayvnyrh Korea (Bioneer, Daejeon 306-220, Korea) and performed according to the instructions provided took. qualities extracted DNA by gel electrophoresis (1%) and were analyzed by spectrophotometer (Hilmi et al.,2007).

2-3 - SrDNA16 amplification and identification of bacteria

To reproduce the SrDNA16, forward primer: 5'-AGA GTT TGA TCC TGG CTC AG -3 'and reverse primer 5'-CCG TCA ATT CCT TTG AGT TT -3' Universal was used (11,14), the use of Primers listed in the S16 ribosomal DNA nucleotides 1 to 1498 of lactic acid bacteria to multiply. PCR reactions were performed in the following conditions: temperature 95 ° C for 2 min followed by 35 cycles of 95 ° C temperature for 45 seconds, 53 ° C for 45 s, and 72 ° C for 60 s and a final cycle of 73 ° C for 3 min (22). PCR products were cut from agarose gel electrophoresis and gel extraction kit (Bioneer, Daejeon 306-220, Korea) was isolated. Extracted fragments for sequencing to determine the concentrations of the company SeqLab (Gttingen, Germany) was added. Samples with the genomic library of Skvns International Center of Biotechnology Information (NCBI) were compared BLAST. High similarity (97%) were classified as the same species and bacterial species were identified.

2-4 - sensory evaluation

Cheese sensory evaluation by a panel group of 16 people and microbial tests were conducted using the same grading scale. 1 and 2 were applied (Sany et al.,2006).

2-5 - Statistical Plan

Results obtained by SAS software and then completely randomized design with 2 × 6 factorial with two levels of whey (bovine and ovine origin) and 6 sites were analyzed in three replicates. Duncan multiple comparison procedure was performed and graphs were drawn by EXEL software.

3. Results and Discussion

3-1 - lactic acid bacteria isolated from traditional cheese Neyshabur

In this study, lactic acid bacteria were isolated from 12 samples of traditional cheese Neyshaburian. Predominant bacterial flora of sheep and cow 's cheese enterococci and lactobacilli , streptococci and Leuconostoc constitute messenger Pirouzian and colleagues (2008) and Lotfi al (2009) are consistent(Pirouzian et al., 2012; lotfi et al., 2009).

3.1.1 - Evaluation of morphological

Isolates of catalase test , Gram staining and morphology were evaluated . Cocci and 20 strains isolated from 27 of the 16 isolates of cocci and bacilli 7, isolate the cheese of sheep and cattle isolates is 4 . The five strains of Bacillus isolated from 7 Cheese cow and two sheep isolates .

3.1.2 – Sequencing

The results determine the sequence of the S16 ribosomal samples in Table 1 has been determined , as we will see 62.9 % of lactic acid bacteria isolated from the genus Enterococcus that six samples of cheese were obtained and 50 % of bacteria in all samples included will . 62.9 percent of enterohaemorrhagic Cocos and 29.6 percent of the genus Enterococcus faecium strain HN-N35 , which was about 4 samples of cheese . Enterococcus dominant flora of raw milk cheese made from sheep, constitute almost 63 % of the study was to identify the Enterococcus bacteria make up 59.26 percent of the bacteria associated with enterococci is Faecium . Because most of the Enterococcus traditional cheese have in common is that enterococci can account for the hypo tonic environments , hyper tonic, acidic and alkaline grow and Enterococcus faecalis during cheese ripening can be in wide temperature range (10-45 ° C) , acidic conditions (at pH 4 to 9.6) and 6.5 % salt -containing environment to carry out their activities (Tserovska et al., 2002).

Among Enterococcus faecalis and Enterococcus Faecium because it is able to produce bacteriocins , to prevent the growth of other susceptible species and the environment is predominant , as the initiator of the bacteria used in cheese production , the bacteria and the effects of probiotics there . results obtained with Bulut Research (2003) and Jokovic et al (2008) show consistent(Jokovic et al., 2011; Bulut, 2003)

Rasouli victories and colleagues (2008) Different strains of Enterococcus isolated from traditional cheese and Lighvan in the dominant flora Enterococcus faecalis and Fasyvm said . The bacterial flora isolated from E. Cheese Local mirage of the dominant flora are presented .

Enterococcus due to proteolyticactivity , and production Astrvlytyky January acetyl citrate metabolism caused by maturation and development cheese aromas such as cheddar , feta , Muzarella , Sbryrv and is Vnakv . Due to Enterococcus role in the investigation , development and production of bacteriocins in cheese aromas , it has been suggested that enterococci with desirable characteristics and metabolic process can be used along with various cheese starters .

Genera Lactobacillus and Enterococcus were ranked second . Lactobacilli predominant flora cheese made from raw cow 's milk to make up about 71 percent of lactobacilli in this study is related to cow cheese samples Bulut and colleagues is consistent with the results . (Bulut, 2003).

Table 1. Isolated lactic acid bacterium from Neyshaburian cheese.

Species	GenBank no.	Similarity percentage	Bacterial no. (%)	Sample no.
Enterococcus				
Enterococcus faecium strain HN-F23	FJ378708.2	100	1(3.7)	1
Enterococcus faecium strain HN-N35	FJ378690.1	100	2(7.4)	2
Enterococcus faecium strain R8	EU483112.1	99	1(3.7)	1
Enterococcus faecium strain HN-N35	FJ378690.1	99	8(29.6)	4
Enterococcus faecium strain 5-19	HQ384298.1	99	2(7.4)	1
Enterococcus faecium strain 133	EU418442.1	100	1(3.7)	1
Enterococcus faecium strain IMAU60169	FJ749883.1	99	1(3.7)	1
Enterococcus durans strain NM156-5	HM218621.1	99	1(3.7)	1
TOTAL			17(62.962)	6 (50)
Streptococcus				
Streptococcus lutetiensis strain 907	EU163503.1	99	2(7.4)	1
TOTAL			2(7.407)	1(8.3)
Leuconostoc				
Leuconostoc mesenteroides strain NM195-3	HM218802.1	99	1(3.7)	1
TOTAL			1(3.703)	1(8.3)
Lactobacillus				
Lactobacillus plantarum strain IMAU80161	GU125581.1	99	3(11.1)	2
Lactobacillus plantarum strain NM28-2	HM218143.1	99	1(3.7)	1
Lactobacillus helveticus strain NM100-1	HM218419.1	99	1(3.7)	1
Lactobacillus gasseri strain ATCC 33323	NR041920/1	100	1(3.7)	1
Lactobacillus brevis strain MGC8-1	HM058398.1	99	1(3.7)	1
TOTAL			7(25.925)	4(33.3)
TOTAL			27	12

3-2 - sensory evaluation

According to Figure 1 , a significant difference between the different regions of sheep cheese flavor characteristics exist between bovine and ovine cheeses of this difference is not significant .

Also, according to Figure 2 , a significant difference between sheep cheeses in terms of features , there is no significant difference between the scent of cow cheese does not see these comments . But the cheese, cow cheese and sheep products produced in the central region and the Takhte jolge and Tagonkooh the difference is statistically significant at the 95 percent confidence level and cow cheese produced in the central region has the highest rating of the notes feature .

As can be seen from Figures 1 and 2 , a total of 12 samples from different areas of Neyshabur cheese , cow cheese produced in the central region, the highest rating in terms of flavor and aroma to your is not allocated.

According to Table 2, the bacterial flora in the central region include Cow Cheese Ingredients: Lactobacillus helveticus, Streptococcus lutetiensis and Lactobacillus gasseri 50% and 50 % of the time it lactobacilli constitute Streptococcus.

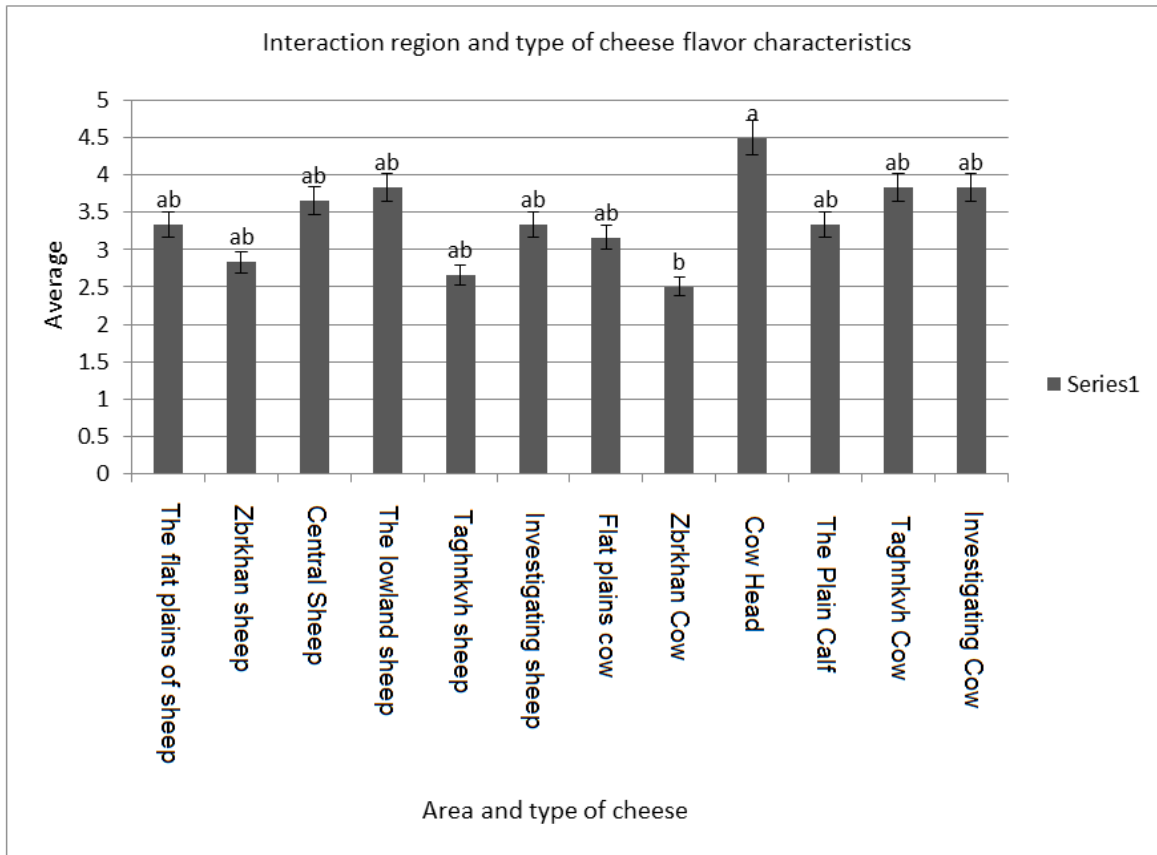


Figure 1 - Descriptive sensory profiles 12 traditional Neyshabur cheese on cheese flavor (Similar letters were statistically not significant at 95% confidence level)

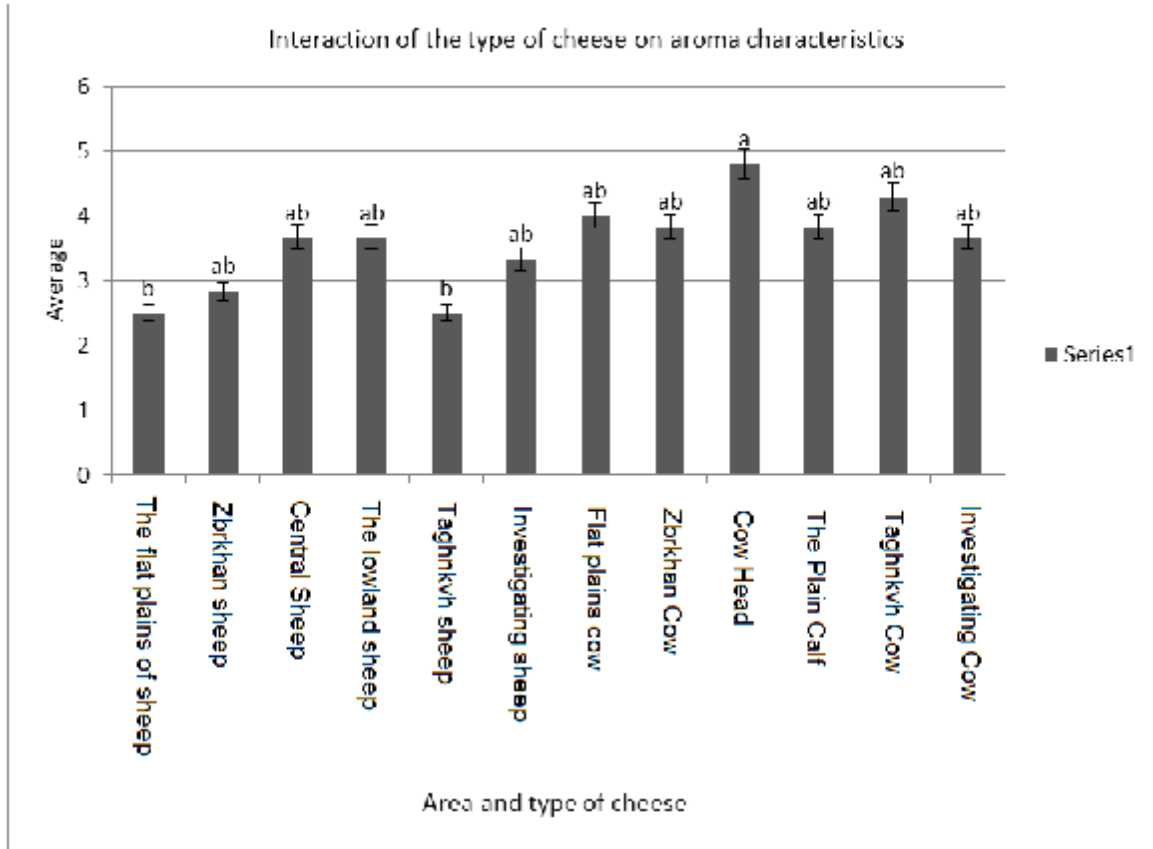
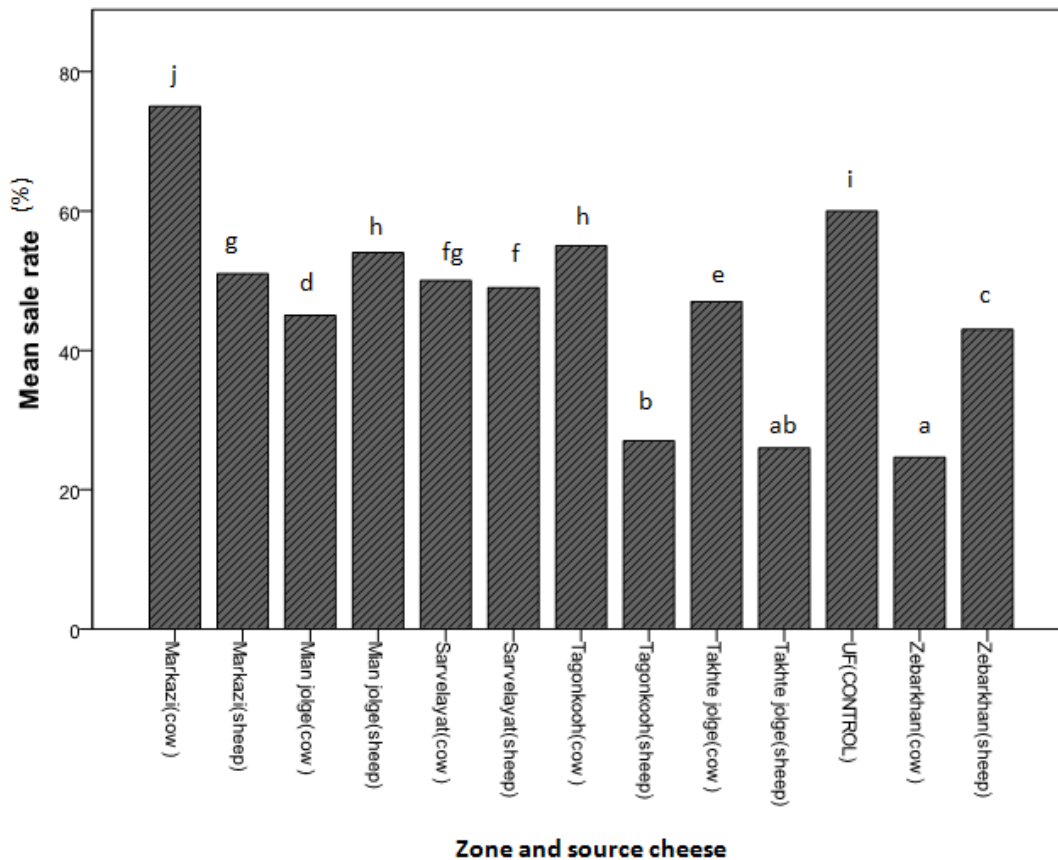


Figure 2 - Descriptive sensory profiles 12 traditional Neyshabur cheese on cheese smell
 (Same initials statistically significant at the 95 percent confidence level are

4. Market study

According to the results obtained from sensory evaluation of cheese into the central region of bovine Neyshabur To compare the results obtained with samples of cheese sales in the market city of Neyshabur , Random sample of 10 supermarkets in the city of Neyshabur cheese market were selected for review . In order to compare the sales of local cheeses produced in different parts of the city of Neyshabur industrial cheese UF (control) of each type of cheese that is sold in supermarkets at a rate of 5 kg and from the supermarket , they were asked within a week of its sales of the Notes , and accordingly any kind of cheese (3) , respectively.



(Similar letters were statistically not significant at 95% confidence level)
 Figure 3 - cheese sales (%) by region and type of cheese

According to Figure 3, we see that cow cheese produced in the central region had the highest percentage of sales and a significant difference ($p < 0.05$) among the control group shows with other cheeses. After seeing the second place, which still shows a significant difference with other cheeses. Zebarkhan the Cow cheese has the least.

5. Conclusions

As previously mentioned The purpose of this study Identification of bacterial flora Affecting local cheese flavor Manufactured from cow and sheep milk The city of Neyshabur And their role on the sensory evaluation of cheese sales on the market. Therefore, the selected species were identified by morphological methods and by DNA sequencing of the ribosomal S 16 were approved. This study also evaluated the sensory attributes of cheese samples In total cheese ,cow cheese Heartland Neyshabur higher score than the other samples tested organoleptic (flavor) accounted for The predominant bacterial flora Lactobacillus helveticus example, Lactobacillus gasserii and Streptococcus was lutetiensis.

Overall, the most common family type lactic acid bacteria, isolated from cheese samples, respectively, were: Enterococcus (62.962%), Lactobacillus (25.925%), Streptococcus (7.407%) and Leuconostoc (3.703%). It also has been determined based on the results of the market study The results of the sensory analysis of cheese samples The results largely matched cheese And the largest percentage of sales (about 75 percent) belong to the typical cow cheese produced in the central region And significant differences ($P < 0.05$) showed the witness and other cheeses.

6.Appreciate

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