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Hygienic profile and starch content of traditional yogurts in north of Iran

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Abstract

Background and objective: Traditional dairies have been produced and consumed since long time ago. However, their quality status is not routinely examined. Therefore, we determined microbial content and starch level of traditional yogurts produced in north of Iran.

Materials and methods: Number of 23 samples were collected from north, south, west, east, and center of the city. All the microbial analyses were done based on ISO directives. Detection of Coliforms and *Escherichia coli* (by MPN method), *Staphylococcus aureus* (by surface plating on Baird Parker agar), and yeast/mold (by surface plating on Dichloran Rose-Bengal Chloramphenicol Agar) were conducted. Starch inclusion was determined by addition of iodine reagent to the products and monitoring of color change.

Results and conclusion: All the samples were approved in Coliforms (<10 CFU/ml), *E. coli* (no growth), and *S. aureus* (no growth) tests. Mold was detected in five samples and the all showed yeast growth more than accepted level. According to Iranian regulation about fermented foods containing yeast as starter that exempted from maximum limit of yeast, majority of yogurts were appropriate for consumption in view of yeast contamination. Most of the samples did not contain starch except for two yogurts containing high amount of starch. Low microbial contamination and starch content in the samples was possibly due to the use of safe raw materials and prominence of healthy microbes present in traditional dairies in Iran.

Keywords: Microbial contamination, starch, traditional yogurt, Iran

1. Introduction

Dairy industry is one of the most critical and potent commerce in the world. Consumption of milk-based products per day is recommended by dietitians all the time due to their health benefits in supplying the calcium need for body that is 1000 mg per 2000 kcal energy intake per day. Calcium content of one cup of milk is accounted as 300 mg, of which approximately 32% is absorbed. Therefore, an average of three cups of milk or its equivalent in other products is required to supply the body's calcium need [1]. Among dairy products, several positive impacts are addressed to those underwent fermentation such

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as yogurt. For example, they are well tolerated by lactase deficient consumers because lactose (that is a prevalent disaccharide in milk) is fermented by indigenous or added starter species to lactic acid and other compounds [2]. Moreover, inclusion of healthy bacteria such as *Streptococcus thermophilus* and bifidobacteria through fermentation in dairy products are in favor of supplying the required folate to consumers [3].

Despite industrial production by addition of commercial starter bacteria or yeasts, traditional dairies are produced locally by people in rural or urban area by using sour dough. These products are popular in Asian countries. In Iran, many people tend to consume traditional dairies because of their natural ingredients, desired sharp flavor, and health benefits. Indeed, traditional yogurt is produced from milk and starter cultures and no processing-aid are added. Various probiotics such as Lactobacillus, Streptococcus, and Leuconostoc have been isolated from traditional yogurts, which is comparable to industrial yogurts containing two or three species of starter. This is due to the vast geographic area with natural sources in the Middle East countries that favors derivation of several microorganisms in traditional products [4]. In agreement, Tajabadi Ebrahimi et al. isolated three species of lactobacillus from traditional cheese and yogurt in Iran. They characterized the isolates as probiotic with regard to acid and bile tolerance, pathogen antagonism, and cholesterol assimilation [5]. Similar results were further reported by Faghfoori et al. about probiotic lactobacillus species isolated from traditional cheeses and yogurts prepared in north-west of Iran [6]. Other than lactobacillus, *Pediococcus acidilactici* was isolated from traditional yogurts in Iran by Sharifi Yazdi et al. that showed probiotic properties [7].

Owing to inclusion of diverse viable cells, traditional yogurts might have more positive impact on the consumers than conventional yogurts through production of several bioactive compounds by fermentation of milk nutrients. It has been reported that starter microbes are able to produce bioactive peptides through fermentation of milk proteins, which pose health benefits in lowering serum cholesterol, blood sugar control, immune regulation, and antioxidant status [2,4]. Gamma amino butyric acid (GABA) is a bioactive non-protein amino acid and differentiated from glutamic acid is able to regulate sleep disorder, lower blood pressure, stimulate immune response, and suppress stress. In the study carried out on probiotic bacteria isolated from traditional dairies in Iran, high potential of GABA production was reported for *Lactobacillus paracasei, L. plantarum*, and *P. acidilactici* [8]. Moreover, Hamzehlou et al. in 2018 characterized eleven Lactobacillus isolated from traditional yogurts produced in different regions of Iran. The bacteria had high potential in production of B-group vitamins and were suggested to be used in production of other functional foods [9]. Selenium is a vital micronutrient mainly found in marine foods and meat and can be delivered by supplements. However, its high intake can lead to some diseases such as diabetes, prostate cancer, and neurodegenerative diseases by species-dependent manner. Indeed, inorganic and some organic derivatives have higher toxic impact than the others [10]. Interestingly, biotransformation of selenite derivatives to its reduced elemental form by lower toxicity was reported for *L. brevis* LSe isolated from traditional sour yogurt in Iran. The authors found that the bacterium could grow in the presence of selenite ions and suggested that it can alleviate side effects of selenium overload in body [11]. Angiotensin I-converting enzyme inhibitory action was determined for bioactive peptides produced by active bacteria in traditional yogurts, through which an antihypertensive property is expected [12].

The approved health benefits of indigenous microorganisms derived from traditional yogurts have resulted in a great tendency to their consumption in recent decades. On the other hand, the consumers are not aware of hygienic status or unknown additives of traditional yogurts and it may lead to unexpected adverse outcomes especially in sensitive people. Therefore, the aim of current study was determination of microbial profile (Coliform, *Escherichia coli*, *Staphylococcus aureus*, yeast and mold) and starch content of traditional yogurts produced in north of Iran. At the end of research, we could conclude that whether the products can help people to intake functional ingredients or might put the consumers at risk of unexpected diseases.

2. Materials and methods

2.1. Materials

Sterile glass and plastic wares, wire loops, and inoculating needles were used during experiments. The glass wares were washed and rinsed with distilled water, sterilized, and underwent endotoxin removal at 400 °C for 3 h. The culture media were accurately weighed and dissolved in distilled water according to the manufacturer's direction and were stored in refrigerator until use. The culture media of Lauryl Sulfate Tryptose broth, EC broth, and Tryptone Water and Indole reagent (mixture of isoamyl alcohol, para-dimethylaminobenzaldehyde, and concentrated hydrochloric acid) were used for Coliform and *E. coli* enumeration. The selective medium of Baird Parker agar was used for *S. aureus* determination. Yeast and mold determination was conducted on Dichloran Rose-Bengal Chloramphenicol agar. Iodine and potassium iodide were used for preparation of iodine reagent for starch determination test. All the media and chemical reagents were purchased from Merck Company (Germany).

2.2. Sample size and sampling procedure

Samples were collected from local markets in north or Iran (Rasht city). The following formula was used for detection of sample size.

$$n = \frac{z^2 \times \delta^2}{d^2}$$

Where, "n" is sample size, "z" is 1.96 for 95% confidence interval, "δ" is standard deviation (average of 0.16 was used based on the results of previous study [13]), and "d" is acceptable error (a factor between 0.01 and 0.1; the average of 0.066 was used). Therefore, a sample size of 23 was achieved. In practice, the city was divided to north, south, west, east, and center and 23 samples were collected randomly. The collected samples were labeled numerically (1-23), and then were transported to the microbiology lab at low temperature by a portable cool chamber.

2.3. Microbial analysis

All experiments were conducted under sterile condition to avoid cross-contamination. Positive control of bacterial and fungal isolates were prepared and used for comparison. For bacterial enumeration, serial dilutions were prepared firstly by adding 1 g of homogenized sample to 9 ml sterile saline solution (0.85% w/v in distilled water). Then, 1 ml of the first dilution was added to 9 ml of the dilutant in series up to reach the dilution of 10^{-6} [14]. Coliform/*E. coli*, coagulase positive staphylococcus, and yeast/mold were enumerated by ISO 11866-1 | IDF 170-1, ISO 6888-1, and ISO 21527-1, respectively [15-17]. Incubation conditions were 37 °C for 24-48 h for the bacteria and 25 °C for five days for yeast/mold. The colonies grown after incubation were counted and expressed as colony forming unit per gram of the samples. In the next step, bacterial isolates were characterized by morphological and biochemical tests including IMViC series, evaluation of color and shape of colonies, gram staining, monitoring of motility, and ability to produce oxidase, catalase, urease, coagulase, and sulfide.

2.4. Starch determination

Approximately, 1-2 ml of iodine reagent (2.6 g iodine and 3 g potassium iodide in 200 ml of distilled water) was dropped into 5 ml of sample in test tube. The mixture was boiled and then left to cool to ambient temperature. Disappearance of blue color in the boiling sample and its further appearance in the cooled sample was indicator of starch in yogurt [18].

3. Results and discussion

Iran is a vast country with several natural foods assessed for their functional and health properties. Endemic traditional foods are of concern sometimes because they do not undergo hygienic processes that industrial products do. Traditional products are not monitored regularly and not-allowed additives may be added by producers.

Codex Alimentarious has classified yogurt as fermented milk and defined it as "a milk product obtained by fermentation of milk, by the action of suitable microorganisms and resulting in reduction of pH with or without coagulation (iso-electric precipitation). These starter micro-organisms shall be viable, active, and abundant in the product to the date of minimum durability" [19]. Several adulterations are addressed in dairies to make the products cheaper, more palatable, and resistant to microbial contamination but they threaten the consumers' health in some cases [20]. They include addition of water, vegetable or animal protein, non-dairy fat, urea, melamine, starch, ammonium sulfate, formalin, and non-declared species of milk to the final products [21-24]. These can be fully detected by instrumental

and complex analytical methods such as Raman spectroscopy, high performance liquid chromatography-mass spectroscopy, and chemometrics approaches [23-25].

Because of people's concern about adverse effects of the non-permitted additives that are not detected visually in industrial products, some of them prefer to use traditional dairy products. In this regard, evaluation of microbial load as health index and starch level that is harmless but may cover technical faults were done in traditional yogurts collected from local markets in north of Iran.

3.1. Microbial load

Traditional dairies do not usually expose to pasteurization and may contain unacceptable level of pathogens. Although, it is supposed that the harmful microbes are destroyed in the products under fermentation process in the presence of healthy starters. A descriptive list of potential bacteria, yeasts, and molds in fermentation of dairies, beverages, cereals, coffee, cocoa, fishes, fruits, meats, sourdough, soy, tea, vegetables, vinegar, and wine was reported by European Food and Feed Cultures Association [26].

According to Table 1, no growth of *E. coli* and acceptable number of coliforms were detected in all samples. Interestingly, no *S. aureus* was detected in the samples. Other than good hygienic status of the products, it might be due to the metabolism of healthy bacteria that led to pathogens' suppression, if existed. In corroboration, inhibitory effect of lactic acid bacteria active in fermented milks was reported by Gomez-Gallego et al. against *E. coli* and *Salmonella enteriditis* [3]. Cutrim et al. inoculated traditional yogurts containing *Streptococcus thermophilus* and *Lactobacillus bulgaricus* with *E. coli* O157:H7 and found that the pathogen was not detectable up to 10 days [27]. In addition, suppression of *S. aureus* might be due to its inability in catalase production to degrade H_2O_2 produced by the starters under acidic condition [28].

Table 1- Microbial count (CFU/g) of traditional yogurts

Sample	Coliform	*E. coli*	Mold	Yeast	*S. aureus*
1	<10	0	<100	>100	0
2	<10	0	<100	>100	0
3	<10	0	<100	>100	0
4	<10	0	>100	>100	0
5	<10	0	>100	>100	0
6	<10	0	<100	>100	0
7	<10	0	>100	>100	0
8	<10	0	>100	>100	0
9	<10	0	<100	>100	0
10	<10	0	<100	>100	0
11	<10	0	<100	>100	0
12	<10	0	<100	>100	0
13	<10	0	<100	>100	0
14	<10	0	<100	>100	0
15	<10	0	<100	>100	0
16	<10	0	<100	>100	0
17	<10	0	<100	>100	0
18	<10	0	<100	>100	0
19	<10	0	<100	>100	0
20	<10	0	<100	>100	0
21	<10	0	<100	>100	0
22	<10	0	<100	>100	0
23	<10	0	>100	>100	0

Our results showed high level of yeast in all samples (Table 1). In agreement, Guzeler et al. found non-significant difference in count of yeast/mold compared to lactic acid bacteria in kefir yogurt [29]. In accordance to several studies that examined diversity of beneficial microorganisms including yeasts in traditional dairies from different regions of Iran [30-32], the high

yeast level in our samples was expected. Importantly, Faghihi Shahrestani et al. reported yeast species of traditional milk in Iran through which *Yarrowia lipolytica*, *Saccharomyces cervisiae*, and *S. paradoxus* were detected [33] that none of them is pathogen. Kluyveromyces, another fungus that has been detected in yogurt, adopts with high and low temperatures of fermentation and storage, respectively, and considered as harmless organism for human [34]. Importantly, its metabolites such as rennet and lactase are listed in GRAS list of US FDA [35]. On the other hand, Afzali et al. found antagonistic activity of *L. brevis* isolated from traditional cheeses in Iran against pathogenic yeasts thanks to the organic acids produced by the bacteria at high concentration [36].

Considering the all results reported by other studies, because microbial load of traditional products is region-specific, a complementary evaluation is needed to determine the yeasts' type in our study. However, a total count up to 100 CFU/g of yeast-mold is acceptable according to Iranian regulation that is limited to mold in the products containing yeast as starter [37]. To the contrary, what is concern is that count of mold was more than acceptable level in approximately 22% (fine out of 23) of samples (Table 1). Dairy products may be source of aflatoxin contamination that mainly produced by *Aspergillus* species. Molds or their toxins enter to milk through consumption of contaminated silage by lactating animals [33]. Therefore, monitoring of animal farms to alleviate fungal contamination in further products is recommended.

3.2. Starch content

Starch may be added to dairy products to increase viscosity or mimic fat perception in consumers [38]. Starch granules consist of amylose and amylopectin in form of helices and branch, respectively. Several hydrophobic molecules such as fatty acids can enter to internal space of amylose [39]. Dispersion of lipid structures in starch molecules leads to a same sensory perception as pure fat that is important in preparation of dietary foods. In addition, water interaction with starch molecules through hydrogen bonds limits water mobility and increase viscosity of the mixture [40]. In this regard, Singh and Byars observed that addition of starch-lipid complex to yogurt up to 4% made a consistent texture without syneresis [41]. This was corroborated in the study of Lobato-Calleros et al. about studying the effect of starch on syneresis of yogurt so that phase separation was decreased as a result of H_2O molecules bounded to hydrophilic sites of starch [42]. Another study reported that addition of starch-lipid complex up to 2% induced a dense phase of casein within a reduced coagulation time. The authors showed that starch-lipid complex jointed to the casein aggregates and might act as nucleus of casein agglomeration in yogurt [43]. Similar result in viscosity enhancement and creaminess was observed by Ares et al. after addition of starch to stirred yogurt [44]. Although, starch addition is a technical point that promotes healthiness of low-fat products for obese people, it is not desirable by the consumers interested in intake of dairy fat especially from traditional products. Our investigation showed that majority of the samples were free of starch and just two out of the all contained high amount of starch (Figure 1).

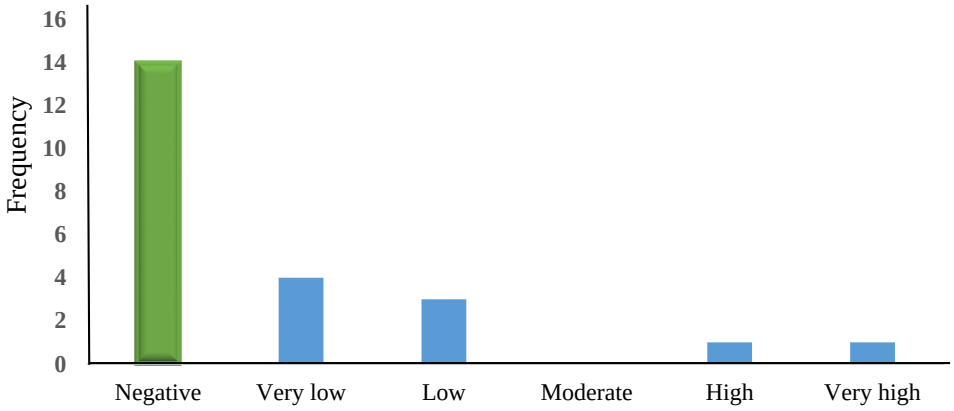

Figure 1- Qualitative determination of starch in traditional yogurts

4. Conclusion

A large number of traditional foods are produced in Iran of which dairy products are popular because they are easily prepared and distributed within the country. However, the products' qualification is of concern for home-made products not underwent thermal or chemical processes. Although, acidic nature of fermented dairies is a good point in repression of spoilage bacteria, cross-contamination or viability of yeasts and molds that are relatively resistant to low pH should not be neglected. According to our results, there was no concern about coliform, *E. coli*, and *S. aureus* in the yogurts. In comparison, yeasts level was higher than acceptable limit in the all samples. Based on several reports of other scientists, Iranian traditional dairies are source of healthy microorganisms and the high yeast count would not be concerning. However, characterization of the yeasts in the samples by molecular approaches is undertaken. Furthermore, five yogurts were contaminated by molds, which should be ignored for consumption. More than 90% of the samples contained no or little starch. It shows the desirable raw materials and appropriate methods used in yogurt preparation. In general, there was no widespread concern about consumption of the traditional yogurts in north of Iran.

5. Conflict of interest

There is no conflict of interest to be declared.

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