

Evaluation of Microbiological quality and determination of starch, gelatin and natamycin content in yoghurt from Turkish market[#]

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Abstract: Yoghurt is one of the most popular dairy products, is considered as a healthy food with its high protein and fat content. Starch and gelatin may be used as gelling, thickening agent in dairy products. Natamycin, a prohibited preservative in yoghurt, is effective on yeasts and moulds. In this study, the presence of gelatin, starch and natamycin was investigated and microbiological quality of yogurts were examined in 14 national and 6 local brands available in the Turkish market. No gelatin and starch were found in yoghurt, and natamycin was detected in 5 of the 20 samples. Natamycin concentrations observed in samples ranged from 1,3 to 16,5 ppm. In 4 of the 5 natamycin using brands also revealed higher yeast counts than non-using ones. No *E. coli* was detected. 4 samples of yoghurts were contaminated by total coliforms, within only one sample was above the critical limit of official Turkish legislation.

1. Introduction

A fermented product yoghurt gains popularity increasingly with its large number of health benefits. The origin of yoghurt is contradictive and according to different sources; it is originated from ancient Greeks and Romans or Asia or Balkans. In the 8th century, the first Turkish name appeared as 'Yogurut' [1]. This phenomenon intensifies ancient Turks were the originator of this milk-based fermented product. Yoghurt is produced from lactic acid fermentation of milk by *Streptococcus thermophilus* and *Lactobacillus delbrueckii* ssp. *bulgaricus*. Yoghurt can be referred as 'probiotics' cause it has beneficial physiological effect by the consumption of live cultures of *S. thermophilus* and *L. bulgaricus*. As yoghurt consumption improves lactose digestion, lactase deficient people can benefit from it smoothly. Regular consumption of yoghurt may also provide a significant decrease in allergic symptoms [2]. World Health Organization recommended yoghurt for management of acute diarrhoea [3]. Natamycin is a polyene antibiotic which is used as a preservative in processed meats and dairy products. It has no effect on the bacteria of the starter culture or viruses whereas it has a wide antimicrobial spectrum against yeasts and moulds [4]. Gelatin and starch are used as stabilizer in yoghurt production. Starch is a polysaccharide from

plants and gelatin is a kind of proteinaceous hydrocolloid produced from collagen [5]. The interaction of gelatin with the casein matrix of yogurt provides a stronger three-dimensional network leading fewer tendencies to syneresis. Despite both of them being responsible for gelling and thickening, gelatin was proven to be more effective in preventing syneresis [6]. They provide retardation of syneresis and create a good sensation in the mouth [7]. According to studies, creaminess and mouth-feel intensities increase with their addition [6]. The aim of this work was to evaluate the detection of natamycin, starch and gelatin contents and determination of microbiological quality of yoghurt which are sold in Turkish market.

2. Materials and Methods

2.1. Materials

14 national and 6 local brands of yoghurt were purchased which have high availability in the major markets in Afyonkarahisar and Isparta regions in Turkey. The analyses were carried out immediately in Isparta Food Control Laboratory within a cold chain.

2.2. Methods

The pH of samples was measured by CYBERSCAN 510 pH meter. Gelatin contents of samples were determined according to AOAC (2005) [8] method and starch content was measured with the mixture of filtrate of sample and a few drops of lugol solution. Blue color formation indicates that there is starch in media [9].

2.2.1 Natamycin Analysis

Sample preparation was made according to TS EN ISO 9233-2. [10]. The chromatographic analysis was carried out in a HPLC system (Shimadzu, Japan). HPLC was equipped with an autosampler, a diode-array detector. Separation was performed by injecting 10 µL onto a C18 column. Mobile phase used was 60 methanol+ 40 H₂O+ 5 acetic acid (v/v). Flow rate was 0.7 ml min⁻¹. Chromatograms were recorded at 303 nm. Standard solutions were prepared by dilution in water-methanol at five concentration levels in the range of 0,1 0,8 µg / ml. According to these concentrations a calibration curve was plotted.

2.2.2 Microbiological Analysis

The microbiological analysis; coliform, *E.coli*, yeast and mould were carried out according to Anonymous (2002) [11] and Anonymous (2001) [12], respectively.

3. Results

pH values ranged between 4.32- 4.82. There is not enough studies to compare the pH values in the literature. Mısırlılar et al. [13] concluded that pH values were decreased significantly not in control samples but in samples with the addition of nisin, natamycin and propyl paraben during storage period. In present study, It was determined that 5 of the 20 samples contain natamycin. The chromatogram of a natamycin standard at 10 mg/L is shown in figure 1. Retention time is about 5.0 min. The limit of detection (LOD) and limit of quantification (LOQ) were determined as 0.050 mg /kg and 0.15 mg/ kg, respectively. The natamycin content of the samples were ranged between 1.03 ppm – 16.5 ppm. There are several studies about natamycin in cheese but there is only a few studies about natamycin content of yoghurt. Dervisoglu et al. [14] searched the natamycin content of yoghurt samples from Turkish market. They determined that more than half of 60 samples from the small-scale brands use natamycin and it ranged between 0.1 ppm – 9.9 ppm. They also indicated that 2 of the 18 large-scale brands use natamycin ranges between 0.11 ppm- 1.7 ppm.

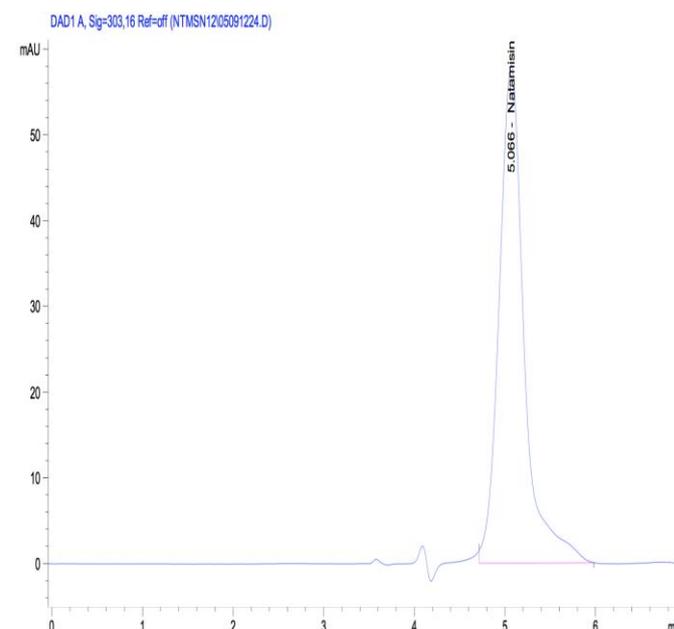


Figure 1. Chromatogram of natamycin standard (10 mg /kg)

From the 20 yoghurt samples, as shown in Table 1., none of them were found to contain starch or gelatin.

Table 1. Results of chemical analysis

Sample	pH	Starch	Gelatin	Natamycin (ppm)
1	4.38	None	None	-
2	4.54	None	None	-
3	4.61	None	None	-
4	4.47	None	None	-
5	4.47	None	None	-
6	4.36	None	None	-
7	4.63	None	None	7.65
8	4.33	None	None	-
9	4.52	None	None	-
10	4.32	None	None	-
11	4.61	None	None	-
12	4.76	None	None	8.85
13	4.66	None	None	-
14	4.82	None	None	-
15	4.49	None	None	-
16	4.38	None	None	-
17	4.52	None	None	16.5
18	4.56	None	None	1.03
19	4.57	None	None	-
20	4.76	None	None	10.65

According to table 2., no cases of *E.coli* was reported. For the 4 cases were positive to coliform. One of them (sample 12) is above the Turkish Food Codex limit for coliforms. Yeast counts of 4 samples (7,12, 17 and 20) were detected to be above the legal limitations in codex. These samples are also natamycin using brands. Only sample 2 was determined to contain much mould counts above legal limitations in codex. Teymori et al. [15] explained they detected coliform bacteria in 4 of 23

yoghurt samples in West Azerbaijan province, 3 of these samples, *E.coli* was also detected.

Table 2. Results of microbiological analysis

Sample	Coliform Bacteria (log cfu /g)	E.coli	Yeast (log cfu /g)	Mould (log cfu /g)
1	< 0.48	N.D	< 1	< 1
2	< 0.48	N.D	< 1	3.92
3	< 0.48	N.D	< 1	< 1
4	< 0.48	N.D	2.95	< 1
5	< 0.48	N.D	< 1	< 1
6	< 0.48	N.D	< 1	< 1
7	< 0.48	N.D	3.77	< 1
8	< 0.48	N.D	< 1	< 1
9	< 0.48	N.D	< 1	2.47
10	< 0.48	N.D	< 1	< 1
11	< 0.48	N.D	< 1	< 1
12	4.04	N.D	3.50	< 1
13	< 0.48	N.D	< 1	< 1
14	0.95	N.D	< 1	< 1
15	< 0.48	N.D	< 1	< 1
16	< 0.48	N.D	< 1	< 1
17	< 0.48	N.D	3	< 1
18	1.36	N.D	< 1	< 1
19	< 0.48	N.D	< 1	< 1
20	1.63	N.D	3.53	< 1

N.D.:Non-detected

4. Conclusion

Natamycin has been used for decades in food industry. It was also approved in the European Union and known as E235. In Turkey, according to Turkish Food Codex, it is only allowed to used in the surfaces of dried and cured sausages and also in the surfaces of cheeses [16]. Some dairy producers use natamycin as preservative for improving shelf life of yoghurts, however it is not acceptable according to Turkish Food Codex [17]. 5 of the 20 brands were detected to add natamycin. These 5 brands were also detected to have higher coliform and/or yeast counts. It can be concluded that natamycin using brands are aware of their own unhygienic process conditions. To minimise contamination of yoghurt with microorganisms, heat treatment of milk should be sufficient, post-production contamination, especially by staff, should be avoided. Aseptic fermentation tank may be used to eliminate contamination and any unhygienic conditions in the plant must be annihilated. Legal surveillance by the state authorities should also be more frequent to prevent adulterations.

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