FUNGAL AND YEAST EVALUATION OF YOGHURT IN MISURATA CITY, LIBYA

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ABSTRACT

Yoghurt is one of the most well-known food products that contain a variety of microbes that has the ability to render the customers into danger. The aim of this study is to evaluate the microbial content of fermented milk products in Misurats's markets, 20 samples have been collected for plain, fruit, and flavored yoghurt. These samples were transported to the lab to perform microbial examinations. First of all, the pH was measured in the yoghurt samples which were 4.58±0.27 in plain yoghurt, 4.6±0.23 in fruit yoghurt and 4.61±0.22 in flavored yoghurt. In addition to bacteria, some yeasts and fungal species isolated including *Saccharomyces cerviceae*, *Candida parapsilosis*, *Pichia*, *Rhodotorula rubra*, *Penicillium*, *Aspergillus*, and *Rhizopus* were isolated from the examined plain yoghurt samples in the percentage of 50%, 37.5% and 12.5% respectively. While in fruit yoghurt only *Mucor* and *Penicillium* were isolated giving 33.3, 66.6% respectively, while in flavored yoghurt only *Penicillium* was isolated by the percentage 100%. When our results were compared with the Libyan standard 4 out of 20 samples were not acceptable in all types of examined yoghurt.

KEYWORDS: Yeast, Fungi, yoghurt.

INTRODUCTION

The generic name of fermented milk was derived from the fact that the milk destined for the product is inoculated with a starter culture which converts part of Lactose into Lactic acid. Carbon dioxide, Acetic acid, Diacetyl acetaldehyde and several other substances are formed in the conversion process, and these impart to the products their characteristic fresh taste and aroma[(7)]. Fermented milk helps to maintain food and water intakes which are often reduced in elder people. Beyond their good taste, fermented milk and especially yoghurts have unique nutritional attributes. These foods contain high levels of proteins, calcium, phosphorus and vitamins B_2 and B_{12}[(18,20)]. The digestive system of some people lacks of the lactase enzyme. As a result of that, the Lactose will not be broken down in the digestive process into simpler types of sugars and those people cannot drink ordinary milk. On the other hand, they can eat cultured milk in which the lactose is already partially broken down by the bacterial enzymes[(17)]. Yeasts and fungi can enter foods through inadequately sanitized equipment or as air-borne contaminants, moreover, can tolerate the psychrotrophic temperature, therefore they become a problem in dairy products[(21)].

MATERIALS AND METHODS

Collection of samples:

A total of 60 random samples of plain yoghurt, fruit yoghurt and flavored yoghurt, (20 for each) collected from different localities in Misurata city, from January 2017 to March 2017.

Preparation of samples:

On arrival to the laboratory each sample was perfectly mixed before being divided into two sub-samples. The first one used for determination of pH, while the second was examined bacteriologically.

Determination of pH:

Standard method[(4)] was used to determine pH value by using pH meter (Jenway- model:3505, Made in UK).

Yeast and fungi count[(4)]:

One ml from the previously prepared dilutions was inoculated into duplicate plates. Then about 15 ml sterile Sabaroud Dextrose agar cooled to 45°C, were poured into each petri-dish. Inoculated plates, after being mixed and solidified, were incubated at room temperature (25°C) for 5 days, then yeast and fungi count ml was calculated.

Identification of isolated fungi:

The incubated plates were examined visually and microscopically. The individual colonies of fungal isolates were selected depending upon their morphological characters and microscopical examination. Members of the Genus *Aspergillus* were classified according to the keys of[(19)].

Stock cultures were made from the isolates and examined on Saboroud Dextrose agar slopes for further identification. Identification of the colonies were carried out by careful observation of the macroscopic and microscopic characteristics of the fungal colonies as follow:

Macroscopic examination:

This depends on the observation of the rate growth of colonies, texture and pigmentation on the surface and reverse side over a period of one week.

Microscopic examination:

Direct microscopic examination:

From 4-6 days old colonies, a piece was transferred to a clean glass slide, and then one drop

of lactophenol cotton blue was added. The slide was covered with a glass cover and examined microscopically.

Identification of isolated yeasts:

Examination for morphological characters of the isolated yeast facilitated the identification and classification of them into genera.

Gross appearance of yeast colonies:

The colonies characteristics were described considering the rate and pattern of growth, its size, texture and surface color.

Direct microscopic appearance:

Carried out by placing a part of the colony on a slide with one drop of distilled water, then cover with cover slip and examined under 100X of magnification power for the presence of yeast cells.

Biochemical properties of yeast:

For identification of yeast into species, biochemical tests have been done.

1- Sugar fermentation[6]:

Media containing sugar were inoculated with the isolates suspension and incubated at 25°C for 3 days.

Positive case indicated by production of acid (medium turn to yellow color).

2- Sugar assimilation test[23]:

Sugar assimilation medium was cooled at 45°C poured into sterilized petri dishes and mixed with 0.2ml saline suspension of the isolates. The plates were left to dry at room temperature. Filter paper discs soaked in 20% solution of different sugars were placed on the surface of the media, and then incubated for 5 days at 25°C. Presence of growth around the disc indicated the utilization of sugars.

Statistical analysis: Done by ANOVA one way and t- test

RESULTS AND DISCUSSION

The results described in (table 1) show that the pH in examined plain yoghurt samples was ranged from 4.2 to 5.1 with a mean value of 4.58±0.27 and in examined fruit yoghurt samples was ranged from 4.2 to 5.1 with a mean value of 4.61± 0.23. while in examined flavored yoghurt, samples were in a range from 4.3 to 5.0 with a mean value of 4.61±0.22. Nearly similar data were obtained by[14,15].

(Table 1) Statistical analytical results of pH in examined yoghurt samples.

Product	No.	Min.	Max.	Mean ± S. D	p. value
Plain	20	4.2	5.1	4.58 ± 0.27	0.902
Fruit	20	4.2	5.1	4.61± 0.23	
Flavored	20	4.3	5.0	4.61± 0.22	

A careful inspection of (table 2) shows that according to frequency distribution of examined yoghurt samples based on pH, the highest frequency distribution of the examined plain yoghurt samples (25%) lies within the range of 4.31-4.4, and the highest frequency distribution of examined fruit yoghurt samples (20%) lies within the range of 4.3-4.4 and 4.4-4.5 while (25%) of examined flavored yogurt samples were lies within the range 4.5-4.6.

(Table 2) Frequency distribution of examined yoghurt samples based on their pH.

interval	Plain yoghurt		Fruit yoghurt		Flavored yoghurt	
	No.	%	No.	%	No.	%
4.21-4.3	4	20%	2	10%	3	15%
4.31-4.4	5	25%	4	20%	2	10%
4.41-4.5	1	5%	4	20%	2	10%
4.51-4.6	3	15%	2	10%	5	25%
4.61-4.7	2	10%	2	10%	3	15%
4.71-4.8	1	5%	3	15%	1	5%
4.81-4.9	1	5%	1	5%	3	15%
4.91-5.0	2	10%	1	5%	1	5%
5.01-5.1	1	5%	1	5%	0	0%
Total	20	100%	20	100%	20	100%

(Table 3) demonstrate that the total yeast and fungi count in examined plain yoghurt samples was ranged from 0.1X10 to 1.2X10 with a mean value 0.48X10± \0.29X10 and in examined fruit yoghurt samples was ranged from 0.1X10 to 1.6X10 with a mean value 0.56X10±0.36X10. While it was ranged from 0.1X10 to 1.0X10 with a mean value 0.49X10±0.28X10 in examined flavored yoghurt samples. Nearly similar data were reported by (1, 2, 5, 11). While higher counts were obtained by (3, 10, 12, 16, 22, 24).

Table (3) Statistical analytical results of total yeast and fungi count\ml in examined yoghurt samples.

Examined yoghurt samples	No. of examined samples	Positive samples		Count\ml		Mean ± S. D	p. value
		No.	%	Min.	Max.		
Plain	20	20	100%	0.1X10	1.2X10	0.48X10± 0.29X10	0.979
Fruit	20	20	100%	0.1X10	1.6X10	0.56X10± 0.36X10	
Flavored	20	20	100%	0.1X10	1.0X10	0.49X10± 0.28X10	

The results present in (table 4) indicate that most of the examined plain yoghurt samples (95%), (90%) of examined fruit yoghurt samples and (95%) of examined flavored yoghurt samples based on their total yeast and fungi count lies within <10.

(Table 4) Frequency distribution of examined yoghurt samples based on their yeast and fungi count.

interval	Plain yoghurt		Fruit yoghurt		Flavored yoghurt	
	No.	%	No.	%	No.	%
10>	19	95%	18	90%	19	95%
10-10^2	1	5%	2	10%	1	5%
Total	20	100%	20	100%	20	100%

The results reported in (figure 1) show that *Saccharomyces cerviceae* was predominant yeast by 43.7%, *Pichia, Rhodotorula rubra*, *Candida parapsilosis*, and *candida albicans* were isolated from the contaminated examined plain yoghurt samples in the percentage of 18.7%, 18.7%, 12.5% and 6.2% respectively. While in fruit yoghurt *Saccharomyces cerviceae* was isolated in 38.4% of positive samples then *Candida parapsilosis, Pichia*, *Rhodotorula rubra* and *Cryptococcus uniguttualtus* isolated by the percentage of 23.07%, 15.3%, 15.3% and 7.6% respectively. While in flavored yoghurt also *Saccharomyces cerviceae* was predominant yeast by 50% then *Candida parapsilosis* 21.4%, *Rhodotorula rubra* 14.2%, *Pichia* and *Cryptococcus uniguttualtus* were giving similar results (7.1%). *Candida albicans* was not isolated in fruit and flavored yoghurt samples. These data were nearly with[9,18].

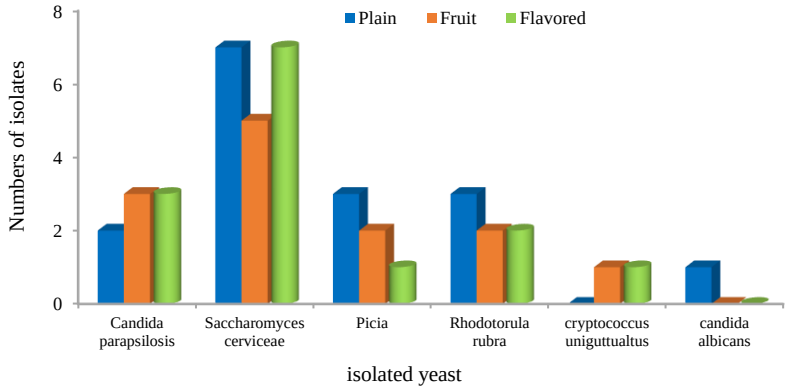
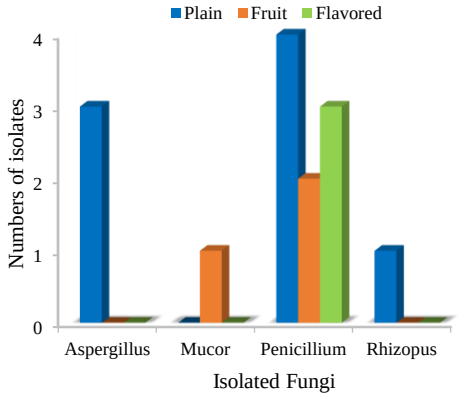


Figure (1) Incidence of isolated yeast in examined yoghurt samples.

The results reported in (figure 2) demonstrate that *Penicillium*, *Aspergillus*, and *Rhizopus* were isolated from the examined plain yoghurt samples in the percentage of 50%, 37.5% and 12.5% respectively. While in fruit yoghurt only *Penicillium* and *Mucor* were isolated by the percentage of 66.6% and 33.3% respectively. While in flavored yoghurt only *Penicillium* was isolated by the percentage 100%. These results were agreed with[8,12,13].



(Figure 2) Incidence of isolated fungi in examined yoghurt samples.

(Table 5) reports that just 1 plain yoghurt samples unacceptable with the yeast count, while in examined fruit yoghurt samples there were 2 samples unaccepted with yeast count in compare with Libyan standard, and in flavored yoghurt samples most of the samples were going with the Libyan standard according to yeast count which were 19 accepted with the Libyan standard (2005\559).

(Table 5) Comparison between Libyan standard criteria and examined yoghurt samples.

	Standard	Plain samples				Fruit samples				Flavored samples			
		Unacceptable		Acceptable		Unacceptable		Acceptable		Unacceptable		Acceptable	
		No.	%	No.	%	No.	%	No.	%	No.	%	No.	%
Yeast	<10	1	5%	19	95%	2	10%	18	90%	1	5%	19	95%

CONCLUSION AND RECOMMENDATIONS

The information given by the results of microbiological examination allow to conclude that most of yoghurt in Misurata city accepted with Libyan standard criteria and satisfy the consumer's demand in obtaining such products in good sanitary condition and retaining as far as possible their nutritive value. According to all of our findings and to save the consumer from being infected and to save a lot of products from being spoiled on the market, the following suggestions are recommended:

- A suitable sanitizer should be used to prepare animals and ensure that their udders are clean.
- Strict hygienic measures should be applied during milking, collection and transportation.
- Only healthy persons who have sanitary certificates should be employed and should pass periodical medical examination.

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