

**Enumeration and identification of yeasts isolated from Kurdish traditional fermented food**<sup>1</sup>Darvishi N., <sup>2</sup>Darvishi Sh., <sup>3</sup>Moradi-BageHendi S.<sup>1</sup>General Practitioner, Researcher, Research Center for Gastroenterology and Liver Disease, Kurdistan University of Medical Sciences, Tohid Hospital, Sanandaj, Iran.<sup>2</sup>Assistant Professor of Food Science and Technology Dept., Sanandaj Branch, Islamic Azad University, Sanandaj, Iran.<sup>3</sup>Assistant Professor Microbiology Dept., Razi Institute, Tehran, Iran.

Darvishi N., Darvishi Sh., Moradi-BageHendi S.; Enumeration and identification of yeasts isolated from Kurdish traditional fermented food

**ABSTRACT**

The yeasts in 30 samples of the Kurdish traditional fermented product, Doooina, taken from farms and households were enumerated and identified. The yeast counts ranged from  $<10^2$  to  $8.8 \times 10^5$  cfug<sup>-1</sup>. Yeast isolates were identified using the API ID 32C test kits and the simplified identification Method (SIM) as well as reference to the standard taxonomic keys. From the 30 sample, a total of 5 different yeast species were identified. *Saccharomyces (S) cerevisiae* (6 isolates) and *Candida (C) colliculosa* (4) were the predominant species identified. *C. tropicalis*, *S. dairensis* and *S. pastorinus* were identified less often. Two of the *S. cerevisiae* isolates were able to assimilate DL- lactate. The analyzed doooina samples contained a wide variety of yeasts, but only a few species predominated and these could possibly contribute to the characteristics of the fermented product in the fermentation.

**Key words:** yeasts, Fermented product, Doooina, Enumeration, Identification.**Introduction**

Fermented food is an important part of the traditional diet in Kurdistan of Iran. The fermented food produced at household level is called *Doooina*. This product consists of traditional drink yogurt, salt, parboiled wheat meal, and added oregano which are then allowed to ferment for 10 days and then sun dried. Organic acids formed during fermentation period and after fermentation excess moisture is removed by drying. It is usually reconstituted with water and served as a hot soup.

There are similar products with different names such as askishk in Egypt, Syria, Lebanon and Jordan, Tarhana (Turkish) trahanas (Greek), tarkhīneh (Persian), trahana (Albanian), трахана/тархана (Bulgarian) and kushuk (Iraq) [4,15,1,2]. Apart from causing spoilage in products such as yoghurt and sour milk, yeasts are also important because they produce desirable flavors as in cheese ripening [12,7]. The fermentative and spoilage activities of yeasts are well known in many modern food, but little consideration has been given to the specific occurrence and significance of yeasts in traditional dairy products. There is increasing interest in the role of yeasts in dairy fermentations and especially their

potential use as starter cultures [6,7,11]. To establish the role of yeasts in doooina, the yeasts have to be isolated and identified and then their technological properties studied. The aim of this study was to isolate, enumerate and identify the yeasts in the Kurdish fermented food, Doooina. This is the first report on the characterization of yeasts of Doooina in Iran.

**Materials and Methods***Sampling:*

Thirty samples of doooina, which had been dried, were collected from small farms and homes in Iran in sterile plastic pouches and brought to the laboratory in a cooler box.

*Enumeration and isolation:*

The yeasts were enumerated on spread plates of yeast extract glucose chloramphenicol agar.

Colonies with distinct morphological differences such as colour, shape and size were picked and purified by streaking at least three times on malt extract agar.

**Corresponding Author**

Darvishi Sh., Assistant Professor of Food Science and Technology Dept., Sanandaj Branch, Islamic Azad University, Sanandaj, Iran.

Tel: +98-871-6661436

E-mail: sho.darvishi@yahoo.com

**Identification:**

The yeast were identified based on their physiological and morphological properties as described by Deak and Beuchat [3], Van der Walt and Yarrow, and also by using the API ID 32C test strips for yeast (bio- Merieux, Marcy l'Etoile, France). The tests included the fermentation of sugars, liquid assimilation of carbon compounds, liquid assimilation of nitrogen compounds, growth at 37°C, growth on 50% (W/V) glucose yeast extract agar, growth in vitamin - free media, growth in media containing 16% NaCl, resistance to 0.01% cycloheximide, and urease activity. API ID32 C strips have 30 coupules containing different carbohydrates and one containing actidione (cycloheximide). The strips were used according to the manufacturer's instructions.

**Results:****Enumeration of yeasts:**

The yeasts counts ranged from less than  $10^2$  –  $8.8 \times 10^5$  cfug<sup>-1</sup> (Table 1).

**Identification:**

Thirteen strains of yeast were isolated and identified. Table 2 shows the yeast species identified and their distribution among the samples. Seven different yeast species were identified. The predominant species were *S.cerevisiae* (2 strains) and *C. colliculosa* (2), While *C. tropicalis*(1), *S. pastorianus*, (1) and *S.dairensis* (1) were identified less often.

**Table 1:** Colony counts of yeasts isolated from Kurdish traditional fermented product.

Sample	Source	Strain ref. number	Cfug <sup>-1</sup>
101	ShahrakSaadi- Sanandaj	- <sup>b</sup>	<10 <sup>2</sup>
102	ShahrakSaadi- Sanandaj	- <sup>b</sup>	<10 <sup>2</sup>
103	Koseihajj - Uraman <sup>a</sup>	1	4 × 10 <sup>5</sup>
104	Kola hasareh	- <sup>b</sup>	<10 <sup>2</sup>
105	Najaf abad	-	<10 <sup>2</sup>
106	Chaharbagh-sanandaj	2	2.8 × 10 <sup>5</sup>
107	Govazmarivan <sup>a</sup>	3	2.4 × 10 <sup>5</sup>
108	Payegalan- marivan <sup>a</sup>	- <sup>b</sup>	<10 <sup>2</sup>
109	Takhte - Uraman <sup>a</sup>	- <sup>b</sup>	<10 <sup>2</sup>
110	Ta- Kamyaran <sup>a</sup>	4	5 × 10 <sup>4</sup>
111	Uraman- Takht <sup>a</sup>	5	5 × 10 <sup>4</sup>
112	Tangisar - Kamyaran <sup>a</sup>	6	5.3 × 10 <sup>5</sup>
113	Moochesh - Kamyaran <sup>a</sup>	7	8.8 × 10 <sup>4</sup>
114	Farsiabad - Uraman <sup>a</sup>	- <sup>b</sup>	<10 <sup>2</sup>
115	Tayne - - Kamyaran <sup>a</sup>	8	2.8 × 10 <sup>5</sup>
116	Palebar - Uraman <sup>a</sup>	9	2.7 × 10 <sup>5</sup>
117	Kherkah <sup>a</sup>	10	2.4 × 10 <sup>5</sup>
118	Lown <sup>a</sup>	- <sup>b</sup>	<10 <sup>2</sup>
119	Kamaleh <sup>a</sup>	11	8.8 × 10 <sup>5</sup>
120	Khoshkedole <sup>a</sup>	- <sup>b</sup>	<10 <sup>2</sup>
121	17- Shahrivarsanandaj	- <sup>b</sup>	<10 <sup>2</sup>
122	Marenj <sup>a</sup>	- <sup>b</sup>	<10 <sup>2</sup>
123	kilagalan <sup>a</sup>	12	10 <sup>3</sup>
124	Dooshan	- <sup>b</sup>	<10 <sup>2</sup>
125	Hawanlah <sup>a</sup>	- <sup>b</sup>	<10 <sup>2</sup>
126	Toodarsahraii <sup>a</sup>	- <sup>b</sup>	<10 <sup>2</sup>
127	Kanisavaran <sup>a</sup>	- <sup>b</sup>	<10 <sup>2</sup>
128	Boraban <sup>a</sup>	- <sup>b</sup>	<10 <sup>2</sup>
129	Bonsie - olya	- <sup>b</sup>	<10 <sup>2</sup>
130	Gashki- kamiaran <sup>a</sup>	13	7.2 × 10 <sup>5</sup>

(a) Samples were taken from household.

(b) No isolations were carried out.

**Table 2:** The diversity of yeasts isolated from Kurdish traditional fermented food

Yeast strain	Number of isolated	Number of samples
<i>S. cerevisiae</i>	6	2
<i>C. colliculosa</i>	4	2
<i>S. pastorianus</i>	1	1
<i>C. tropicalis</i>	1	1
<i>S. dairensis</i>	1	1
<i>C. spp</i>	1	1
<i>S. spp</i>	1	1
Unknown	1	1

**Discussion:**

One sample (Number 119) had viable yeast counts of  $8.8 \times 10^5$ . This sample could have been fermented over more than seven days. Particularly low counts of yeasts ( $10^2 \text{cfu/g}^{-1}$ ) were recorded for seventeen sources and the low yeast counts could possibly be explained by better hygienic practices at these sources compared to other sources of samples. Karagozlu *et al.* [8] found that the yeasts count was  $3.66 \pm 0.27 \log \text{cfu/g}$  in tarhana. The identification was carried out using API ID 32C kits (bio Merieux) and SIM key [3] as well as reference to standard taxonomic key out lined by Kurtzman and Fell [10]. Comparing to other studies, it seems that different yeast species predominate in different fermented milk products. For example, *S. cerevisiae* together with *G. geotrichum* and *Kluyveromyces marxianus* were the predominant strains in *makamo*, a Ugandan traditional fermented milk [13], while other strains such as *C. holmii*, *S. dairensis*, *C. stellata* and *Zygosaccharomyces* spp. were isolated in lesser numbers. *Debaromyces hansenii*, *Torulaspordelbrueckii* and *Kluyveromyces marxianus* were the predominant species found in South African traditional fermented milk [11].

*S. cerevisiae* was a common species many reports agreed on the dominance of *S. cerevisiae* in yoghurts [6]. The strains successfully identified as *S. cerevisiae* by both the API and SIM procedures could ferment sucrose and raffinose in addition to fermenting glucose and galactose. They were not able to utilise lysine and cadavarine. Other reactions, which are considered diagnostic for *S. cerevisiae*, were lack of assimilation of ethylamine hydrochloride, D-ribose, D-mannitol and their inability to grow in vitamin – free medium [10].

**Conclusions:**

The analysed dooina samples contained a wide variety of yeasts, but only a few species predominated and these could possibly contribute to the characteristics of the fermented product in the fermentation.

**References**

1. Alnouri, F.F. and C.L. Duitschaever, 1974. The Use of Pure Cultures for the Preparation of Kshuk. *Journal of Institution of Canadian Science and Technology Alimentary*, 7: 228-229.
2. Darvishi, Sh., 2009. *Dictionary of Food Microbiology*. Ahmad Press, Sanandaj, Iran.
3. Deak, T. and L. R. Beuchat, 1996. *Handbook of Food Spoilage Yeasts*. Boca Roton, FL, CRC Press.
4. Economidou, P.L., K.H. Steinkraus, 1983. Acid-Fermented Milk and Milk/Cereal Foods. In: Steinkraus, K.H., (ed) *Handbook of Indigenous Fermented Foods*, Marcel Dekker, New York.
5. Fleet, G.H. and M.A. MIAN, 1987. The Occurrence and Growth of Yeasts in Dairy Product. *International Journal of Food Microbiology*, 4: 145-155.
6. Fleet, G.H., 1990. Yeasts in Dairy Products-A Review. *Journal of applied Bacteriology*, 68: 199-211.
7. Jakobsen, M. and J. Narvhus, 1996. Yeasts and their Possible Beneficial and Negative Effects on the Quality of Dairy Products. *International Dairy journal*, 6: 775-768.
8. Karagozlu, N., B. Ergonul and C. Karagozlu, 2008. Microbiological Attributes of Instant Tarhana during
9. Fermentation and Drying. *Bulgarian Journal of Agricultural Science*, 14(6): 535-541.
10. Kurtzman, C.P. and J.W. Fell, 1998. *The Yeasts: A Taxonomic Study*. Elsevier, Amsterdam.
11. Loretan, T., B.C. Viljoen, J.F. Mostert, A.M. Vogeland, H.F. du, P. Jordan, 1998. A Preliminary Study of the Diversity and Technological Properties of Indigenous Traditional South African Fermented Milk (Note). In Jakobsen, M., J. Narvhus and B.C. Viljoen, *Yeasts in the Dairy Industry: Positive and Negative Aspects*, Proceeding of the symposium organized by Group F47, 2-3 September 1996, pp: 178-182. Brussels, Belgium.
12. Marshall, V.M., 1987. Fermented Milks and their Future Trends: I. Microbiological aspects. *Journal of Dairy Research*, 54: 559-547.
13. Sserunjogi, M.L., 1999. Ugandan- indigenous fermented dairy products with particular, focus on ghee. Ph.D. thesis, Agricultural University of Norway, As, Norway.
14. Van der Walt, J.P. and D. Yarrow, 1984. Methods for the Isolation, Maintenance, Classification and Identification of Yeasts. In N.J.W. Kreger- Van Rij. *The yeasts: A taxonomic study*, pp: 45-105.
15. Youssef, M.M., 1990. Instantization and Evaluation of Some Traditional Egyptian Foods. *Food Chemistry*, 38: 247-254.