

# Isolation and Identification of Yeasts from Local Traditional Fermented Camel Milk, *Chal*

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**Abstract** The yeasts in 9 samples of the Iranian traditional fermented camel milk, *Chal*, taken from farms and households were identified on the basis of their physiological and morphological properties. From the 9 samples, a total of 18 different yeast species were identified including *Pichia. anamala*, *Pichia. jadinii*, *Debaryomyces. hansenii*, *Pichia. guilliermondii*, *Kluyvermyces. marxianus*, *Candida. fermentati*, *Pichia. ciferrii*, *Torulospora. delbrueckii*, *Candida. versatilis*, *Kluyvermyces. lactis*, *Candida. kefir*, *Saccharomyces. pastorianus*, *Saccharomyces. cerevisiae*, *Candida. friedrichii*, *Kluyvermyces. polysporus*, *Rhodotorula. musilaginosa*, *Candida. lipolytica* and *Candida. lusitaniae*. All of them could assimilate the glucose as carbon source and liquefacted the gelatin, but could not production starch, tolerated 1% acetic acid, growth in the presence of NaCl 16% (except *Debaryomyces. hansenii*), anassimilateed the nitrat (except *Rhodotorula. musilaginosa*) and had not Septate hyphae. Results showed within the yeasts species isolated from Chal samples, *Kluyvermyces. lactis* (8.57%) and *Kluyvermyces. marxianus* (8.57%) were the predominant. This study revealed Chal contained a wide variety of yeasts that predominated at Chal in the 48 h fermentation.

**Keywords** Chal, Camel Milk, Fermented Milk, Yeast, Identificaton

## 1. Introduction

Chal is a nutritious fermented camel milk which is prepared by spontaneous fermentation of camel milk at ambient temperature and consume in many areas such as Africa, Arabic countries [12], and in many regions used against diseases [28]. Due to acidic pH, dairy products are favorable environment for growth of yeasts [38-30]. The yeasts usually adopt themselves to coexistence with lactic acid bacteria in dairy products. The yeasts play usefull effect upon the bacteria due to change in pH and secretion of biological substance such as vitamins, enzymes, amino acids etc [7].

Yeast species mainly the genera *Candida* (*C. sphaerica*), *Debaryomyces*, *Mycoderma*, *Saccharomyces* (*S. dairensis*, *S. unisporus*) and *Rhodotorula* by lactose assimilation decrease quality of dairy products and Some representatives of the genus *Rhodotorula* cause staining and give bitter taste to the products [17, 23, 39].

The most isolated species from different dairy products belonged to Species of *Candida*, *Debaryomyces*, *Galactomyces*, *Fellomyces*, *Pichia* and *Saccharomyces* [38, 18, 37, 32]. The yeasts isolated from dairy products are

different, but the most frequently described strains belong to the genera *Saccharomyces*, *Kluyveromyces*, *Debaryomyces*, *Issatchenkia* and *Yarrowia* [33, 34, 21]. The nature of fermented dairy products varies from one region to another.

The yeasts occur in many dairy related products [10, 14] and in the human gastrointestinal tract [16, 8], and have probiotic potential. *Saccharomyces boulardii* is the yeast commercialized as probiotic in human medicine [31, 8], but also in several studies strains such as *Kluyveromyces lactis*, *K. marxianus*, *Isaatchenkia orientalis*, *S. cerevisiae* and *Debaryomyces hansenii* have shown antifungal, antibacterial, anti-inflammatory and antitumoral activity [25, 9, 19].

*Candida albicans*, *C. parapsilosis*, *D. hansenii*, *I. orientalis*, *K. lactis*, *S. cerevisiae* and *S. boulardii* were isolated from infants' gastrointestinal tract and feta cheese [36, 2, 26]. Many *in vitro* investigation proved the potential probiotics of the *S. cerevisiae* 832 and *S. boulardii* KK1 due to acid and bile tolerance and cholesterol removal ability [27].

The aim of this study was isolation and identification of yeasts from Chal sold in Golestan province of Iran.

## 2. Materials and Methods

### 2.1. Sampling

A total of 9 sour camel milk (Chal) samples (A to I) were randomly collected from different house holds and retail

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markets with different sanitation levels in the original container from Golestan province. The areas under investigation were Gonbad, Aghghala and Bandar Torkman cities.

## 2.2. Compositional Analysis

The Chal samples were analyzed for Fat [29], ash [20], pH, NaCl, Alcohol (after 48 h fermentation) [6], Protein, TS and Acidity content [3].

## 2.3. Isolation and Identification of Yeasts

One milliliter from each samples was serially diluted in distilled water and spread- plated onto YGC agar (Mirmedia, Iran) to isolate the yeasts. The plates were incubated at 25°C for 5 days. Colonies with distinct morphological differences such as colour, shape and size were picked and purified by streaking. The purified isolates were stored on YGC agar slant at 4°C under liquid parafin before identification.

The colonies were tested and described based on morphological characteristic on Yeast- Mold Agar (YMA) and YM broth as following:

Yeast fermentation base medium containing 2% one of Carbohydrates (glucose, fructose, galactose, maltose, lactose, sucrose, xylose, arabinose, trehalose, manitol, melezitose and raffinose) as a sole carbon source for testing the assimilation of carbon sources for a month, gas production from carbohydrates 5% (glucose, fructose, galactose,

maltose and lactose) by using durham tube for three weeks, production of extracellular starch compounds, urease test for hydrolyzation activity, growth in the presence of D-glucose (50 and 60%) and NaCl (10 and 16%), gelatin liquefaction and tolerance of 1% acetic acid according to [35], and investigation of the assimilation of nitrat, growth in the presence of cycloheximide (0.1% and 1%) with modified method according to [15], using disk inoculum- solid medium method were achieved. The isolate were identified according to protocol described by [13, 4, 32, 18, 17].

In all tests sensitive compounds to heat such as carbohydrates, cycloheximide, yeasts nitrogen base medium, yeasts fermentation base medium were sterilized using 0.23µ Millipore Filter apparatus.

## 3. Results and Discussion

### 3.1. Copositional Analyses

Chemical properties of chal given in Table 1.

Our results revealed that the pH was ranged from 3.8 to 4.5. Although lower pH has inhibitory effect on the vegetative cells of pathogenic microorganisms, but it is favorable for growth of yeasts [1].

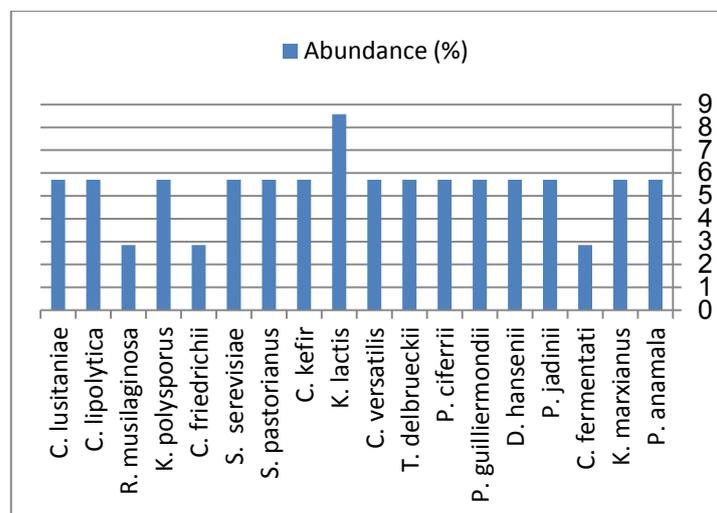
### 3.2. Identified Yeasts

All the results have been shown in Tables 2, 3 and Figure 1.

**Table 1.** Composition of chal samples

Sample Code	A	B	C	D	E	F	G	H	I
Protein%	1.0	1.0	1.2	1.1	1.5	2.2	1.3	1.5	1.4
Fat%	2.2	2.1	2.0	1.7	1.8	1.6	2.0	1.9	1.6
TS%	4.0	3.9	4.1	3.7	4.0	4.6	4.1	4.0	3.8
Ash%	0.23	0.31	0.32	0.27	0.32	0.38	0.31	0.32	0.29
Acidity (D*)	32.0	33.0	31.0	34.0	31.0	34.5	35.0	35.5	31.0
pH	4.0	4.2	4.5	4.4	4.5	4.0	3.9	3.8	4.5
NaCl%	0.80	0.75	0.90	0.85	0.70	0.80	0.76	0.60	0.80
Alcohol% (w/v)	0.80	0.70	0.40	0.70	0.40	0.50	0.80	0.60	0.60

\* Dornic



**Figure 1.** Estimation of yeasts species isolated from Chal samples

Yeasts commonly associated with traditional fermented dairy products and have been reported in several studies [22, 13, 11, 5].

The morphological data of the isolated strains are represented in Tables 2 and 3 and identified strains in Figure 1. Septate hyphae were formed by none of them and *Kluyvermyces marxianus*, *Torulospora delbrueckii*, *Kluyvermyces lactis*, *Candida lipolytica* and *Candida lusitanae* formed ascospores. A part of the detected strains belonged to order *Ascomycotina* and the rest to order *Deuteromycotina* – Imperfect yeasts.

The most microorganisms utilized galactose, fructose and maltose. Except four of the strains, the lactose was assimilated as a single carbon source by rest of them.

All of isolates assimilated the glucose and liquefacted the gelatin, but starch production, tolerance to 1% acetic acid, growth in the presence of NaCl 16%, septate hyphae and nitrat assimilation were not seen for all, except

*Debaryomyces Hansenii* which grown in the presence of NaCl 16% and *Rhodotorula Musilaginosa* assimilated the nitrate.

From the Chal samples, 35 yeasts species including, 8 *Pichia* (22.85%), 8 *Kluyvermyces* (22.85%), 10 *Candida* (28.57%), 2 *Debaryomyces* (5.71%), 2 *Torulospora* (5.71%), 4 *Saccharomyces* (11.42%) and 1 *Rhodotorula* (3.50%) were identified. Among the yeasts species, *Kluyvermyces lactis* (8.57%) *Kluyvermyces marxianus* (8.57%) were the predominant (Fig 1).

Yeast species including *Pichia anamala*, *P. jadinii*, *P. guilliermondii*, *Deb. hansenii*, *Kluyveromyces polysporus*, *Kluyveromyces Marxianus* which were isolated from samples of Chal, were also isolated from dairy products in the previous study [24-32]. However *Candida fermentati* was isolated from Chal only and was n't found previously in dairy products.

**Table 2.** Different characteristics of yeast isolates

Characteristics	1,13	2,4,6	3	5,11	7,25	8,17	14,9	10,18	15,22
Assimilation									
glucose	+	+	+	+	+	+	+	+	+
fructose	+	+	+	+	+	+	+	+	+
Galactose	+	+	+	+	+	+	+	+	-
Maltose	+	+	+	+	+	+	+	+	-
Lactose	+	+	+	v	+	+	-	-	+
Xylose	+	W	W	w	+	w	V	+	-
Arabinose	+	W	+	W	+	+	+	-	-
Manitol	V	+	+	+	+	+	+	-	+
Melezitose	+	+	W	+	+	+	+	-	-
Rafinose	+	+	+	+	+	+	+	-	-
Fermentation									
Glucose	+	+	+	+	+	-	v	+	+
Fructose	+	+	+	+	+	+	+	V	-
Galactose	-	+	+	+	-	+	v	+	-
Maltose	+	+	-	+	-	+	v	V	-
Lactose	-	-	+	-	-	+	-	v	-
Starch produc	-	-	-	-	-	-	-	-	-
Urea	-	-	-	-	-	-	-	-	-
Glucose50%	+	+	+	+	+	+	+	+	+
Glucos 60%	+	+	+	+	+	+	+	+	+
Nacl 10%	-	-	+	-	+	+	-	+	-
Nacl 16%	-	-	-	-	+	-	-	-	-
Gelatin lique	+	+	+	+	+	+	+	+	+
1% Acetic acid	-	-	-	-	-	-	-	-	-
Nitrat	+	+	+	+	+	+	+	-	+
Cyclohex0.1%	-	-	+	+	-	+	-	-	+
Cyclohex1%	-	-	+	+	-	+	-	-	+
Colony color*	wh-c	c	wh-c	wh-c	wh-c	wh-c	c	c	wh-c
Shape of cell*	sp-o	o-e	g-o	sp-o	r-o	r-o	o	r-o	r-o
Septate hyphae	-	-	-	-	-	-	-	-	-
Ascospor	-	+	-	-	-	-	-	+	-

+ means positive, - means negative, W means weak and V means variable  
wh = white, c = cream, e = ellipsoidal, o = oval, r = round, sp = spherical, g = globose, \*according to reference [24]

**Table 3.** Different characteristics of yeast isolates

Characteristics	12,19,26	20,23	21,27	24,29	30	31,16	32	33,35	34,36
Assimilation									
glucose	+	+	+	+	+	+	+	+	+
fructose	+	v	+	v	V	+	+	+	+
Galactos	+	+	+	+	+	+	+	+	+
Maltose	+	-	+	+	+	+	+	-	+
Lactose	+	+	-	-	-	-	-	-	+
Xylose	-	-	+	-	+	+	+	-	+
Arabinos	-	V	+	W	+	+	+	+	v
Manitol	+	-	-	-	-	+	+	+	+
Melezitose	+	-	+	-	+	+	+	-	+
Rafinose	+	-	V	-	+	-	-	-	-
Fermen									
Glucose	+	+	+	+	+	+	+	-	+
Fructose	+	V	+	V	-	-	+	+	V
Galactos	-	+	+	+	+	+	+	-	+
Maltose	+	+	+	+	+	+	V	-	+
Lactose	+	-	-	-	-	-	-	-	+
Starch produc	-	-	-	-	-	-	-	-	-
Urea	-	-	-	-	-	-	+	-	-
Glucose50%	-	-	-	-	+	-	-	-	+
Glucos 60%	-	-	-	-	-	-	-	-	-
Nacl 10%	-	-	-	-	V	-	+	-	+
Nacl 16%	-	-	-	-	-	-	-	-	-
Gelatin lique	+	+	+	+	+	+	+	+	+
1% A. acid	-	-	-	-	-	-	-	-	-
Nitrat	-	+	-	-	-	-	-	+	+
Cyclohex0.%	-	+	-	-	-	-	+	+	+
Cyclohex1%	-	+	-	-	-	-	+	+	+
Colony color*	Sm-wh	c	c	whc	whc	wh-c	pn	c	c
Shape of cell*	r	o-e	o-e	e	o-e	r	r	o-e	o-e
Septate hyphae	-	-	-	-	-	-	-	-	-
Ascospor	+		-	-	-	-	-	+	+

+ means positive, - means negative, W means weak and V means variable

wh = white, c = cream, e = ellipsoidal, o = oval, r = round, sm = smooth, pn = pink, \*according to reference [24].

## 4. Conclusions

Thirty five yeast species belonging to 18 genera were isolated and identified from samples of chal. Results showed Chal contained a wide variety of yeasts that are predominant at Chal after the 48 h fermentation.

Chal was made from raw camel milk, but the presence of yeasts indicates the contamination of the product by air, water or persons who are engaged in the preparation and transportation. This study recommended that increasing the hygienic level in Chal production be effective to decrease the yeast contamination.

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