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Determination of Some Technological and Probiotic Characteristics of the Yeast Strains Isolated from Traditional Erzincan Tulum Cheese Produced in Turkey

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In this study, technological and probiotic characteristics of 35 yeast strains isolated from Erzincan tulum cheese, locally produced in the Eastern Anatolia region of Turkey, were investigated. Four strains belonging to *G. candidum*, *C. kefyr*, *C. lipolytica* and *C. lambica* were found to have proteolytic activities while 80% of the investigated strains had lipolytic activities. Most (69%) of the tested yeast strains had the ability to assimilate galactose. All of the strains could grow at 10°C and 83% of them had the ability to grow at 4°C, while only 34% of them were resistant to 45°C. Most of the strains had also ability to grow at pH 2.5 except one *C. colliculosa* strain. All of the isolates had the ability to grow in the medium with 5% NaCl. In the media with 10% and 15% NaCl concentration, 89% and 31% of the strains were able to grow, respectively. Tolerance to high bile salt concentrations was a rare property among the strains, of which *C. rugosa* gave the best result for growing in Ox-Bile medium. Inhibition effects of the tested yeasts on some pathogenic bacteria were generally weak, but a strong inhibition effect of a *C. krusei* strain was detected on *S. aureus*.

Key Words: Erzincan tulum cheese, Yeast, Technological characteristic, Probiotic characteristics.

Yeasts represent an important component of the microflora of many dairy products[1,2]. A large number of cheese varieties are characterized by the development of a specific surface microflora which is generally composed of molds, yeasts, micrococci and coryneform bacteria[3]. Yeasts, therefore, are frequently found within the microflora of many cheese types[3,4].

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The presence of yeasts in cheese is of major importance as they can be beneficial or detrimental, that contribute to the ripening and flavour development[5]. The major recognized action of yeasts during cheese ripening is the metabolism of lactic acid with a consequent increase of pH values. This promotes the growth and action of lactic acid bacteria, which accelerates proteolysis, consequently the ripening process. The lactose-fermenting species, for example *Kluyveromyces marxianus*, contribute to the open structure of mainly blue-veined cheeses. Their ability to ferment lactose results in the formation of CO_2 and also in flavouring compounds such as ethanol and

acetaldehyde[6]. Furthermore, due to their possible proteolytic and lipolytic activities, yeasts can become part of the overall enzymatic activity in cheese[2,7]. Some yeast species play an important role in the formation of aroma precursors such as aminoacids, fatty acids and esters. They can inhibit undesired microorganisms and excrete growth factors like B-vitamins, pantothenic acid, niacin, riboflavin and biotin[8]. The possibility of using yeast strains as adjunct starters for cheese production is proposed due to their positive attributes to cheese ripening[2]. It is reported that further investigations on yeast physiological and biochemical characteristics are needed in order to select relevant strains for starter cultures[6].

Tulum is a semi-hard cheese that can be made from whole, semi skimmed or skimmed sheep's, goat's, cow's, and buffalo's milk or their mixture. It has a crumbly texture and a strong flavour[9]. Erzincan tulum cheese is a local cheese produced mainly in the Eastern Anatolia region of Turkey, especially in the provinces Erzincan, Elazig, Tunceli and Bingöl[10]. Studies on Erzincan tulum cheese are limited and generally about its chemical properties and microbiological quality. There is no reported study about technological characterization of yeast microflora of Erzincan tulum cheese. This is the first study investigating technological and probiotic properties of yeast isolates of this cheese and also evaluating their potential for being adjunct starters.

MATERIALS AND METHODS

Yeast strains

In this study, 35 yeast strains which were previously isolated from Erzincan tulum cheese samples were used. The yeast strains were identified by API ID 32C (BioMerieux, France) and some additional identification tests in our previous study (data not shown). The yeast strains used in this study were belonging to the species of *Saccharomyces cerevisiae, Zygosaccharomyces mellis, Candida colliculosa, Candida krusei, Kluyveromyces lactis* var. *lactis, Geotrichum candidum, Candida kefyr, Candida lipolytica, Candida rugosa, Candida zeylanoides, Candida paludigena, Candida apicola, Candida japonica, Candida lambica, Pichia fermentans, Candida famata* var. *famata* and *Candida famata* var. *flareri.*

Determination of some technological and probiotic characteristics of the yeast strains

Some technological characteristics which are important for cheese production, and also some probiotic properties of the yeast isolates were investigated. For this purpose; proteolytic and lipolytic activities, fermentation and assimilation of some sugars, assimilation of some organic acids, growth at different pH and temperatures, growth at different salt concentrations, bile salt tolerance and inhibition effects of the tested strains on some pathogenic bacteria were investigated.

Determination of proteolytic activity

Proteoliytic activity was determined according to Harrigan[11]. In this method, activated cultures were inoculated on 10% (w/v) reconstituted skim milk added Milk agar media by streaking across the surface of the agar medium. Petri dishes were incubated at 28°C for 2-14 days. After incubation, positive results were recorded when colonies were surrounded by a clear zone.

Determination of lipolytic activity

For determination of lipolytic activities, activated cultures were inoculated on Tributyrin agar by streaking across the surface of the medium and then incubated at 28°C for 2-14 days[11]. Hydrolysis of tributyrin results in clearing of the medium and formation of a clear zone. After incubation, positive results were recorded.

Determination of some sugar fermentation and assimilation characteristics

Fermentation of glucose, galactose and lactose were investigated by using media with Durham tubes including 2% (w/v) of each tested sugar. After inoculation of activated yeast cultures to the media and incubation of them at 28°C for up to 28 days, positive results were evaluated according to the growth and gas formation into the Durham tubes[12].

Assimilation of the same sugars were recorded from the assimilation test results of API ID 32C strips (BioMérieux, France).

Determination of some organic acid assimilation characteristics

Assimilation of citric and succinic acid were investigated by using auxanographic method in Yeast Nitrogen Base (YNB) agar[12-14]. Assimilation of lactic acid (DL-lactate) was recorded from the assimilation profiles of API ID 32C (BioMerieux, France) test results. In auxanographic method,

activated yeast cultures were suspended in distilled water and suspensions having 2 McFarland turbidity were obtained. 0.5 mL suspensions were inoculated into YNB agar with poured plate method and after solidification small amount of granules of the organic acid salt were deposited aseptically on the surface of the agar. Glucose granules was also added onto each plate as positive control. Media were incubated at 28°C for 2-3 days and growth around the organic acid granules were evaluated as positive.

Determination of growth at different pH

Growth of the yeast strains at different pH values was investigated in Malt Extract (ME) broth[15]. The media, pH level of which were adjusted to 2.5 and 4.0, were used for this purpose and the same medium having pH 5.4 was also used as control medium. These pH values were selected concerning cheese production conditions and also desired resistance of probiotics to gastric pH.

Determination of growth at different temperatures

For investigation of growth at different temperatures, activated cultures were inoculated to ME broth and then incubated at 4°C, 10°C, 28°C, 37°C, and 45°C for upto seven days[12]. These temperature values were selected concerning the conditions in production and storage of cheese, and also desired resistance of probiotics to human body temperature.

Determination of growth at different salt concentrations

Salt tolerance of the yeast strains were evaluated by using ME broth including NaCl at different concentrations[12]. NaCl concentrations in the media were used as 5%, 10% and 15% (w/v). The used salt concentrations were selected according to the properties of cheese. ME broth without salt was used as control. Water activity of the media were also determined. Activated yeast cultures were inoculated to ME broth with NaCl and incubated at 28°C for 7 days.

Determination of the tolerance to bile salt

Bile salt tolerance was determined according to Psomas *et al*.[15]. Firstly, growth of the yeast strains in ME agar including 0.5% and 1% (w/v) bile salt (No.3, Lab M, UK) , were investigated. For this purpose, 24-48 hours old yeast cultures were inoculated onto the media containing bile salt by streaking to the surface of the agar medium and incubated at 37°C for 72 hours. After incubation growth of the yeasts were examined. In the second stage, yeast strains resistant to 1% (w/v) bile salt concentration were chosen and after activation at YM broth, inoculated to ME broth with 0.3% (w/v) Ox-Bile (Merck, USA) at the level of 10^5 cfu/mL and incubated at 37°C. Yeast counts were performed by using ME agar at the beginning and after 72 hours and tolerance of the tested yeast strains to Ox-Bile were evaluated. The strains which were not be able to grow at 37°C, could not be tested for bile salt tolerance. In these experiments, a strain of *Saccharomyces boulardii* was also used as control strain because of its known probiotic characteristics.

Determination of the inhibition effects of the yeast strains on some pathogenic bacteria

For investigation of inhibition effects of the yeast strains on some pathogenic bacteria, modified agar-cup diffusion method was used[16]. In these experiments, *Escherichia coli* ATCC 35218, *Staphylococcus aureus* GATA and *Listeria monocytogenes* 1462 strains were used. Bacterial cultures were activated on Brain Heart Infusion (BHI) agar at 37°C, while yeasts were activated on ME broth at 30°C, for 24 hours. Then, suspensions of bacterial cultures which have 0.5 McFarland turbidity were prepared in 0.85% NaCl and spread on BHI agar in petri dishes. Then, three wells were formed on BHI agar and ME agar was placed at the bottom of the wells. After that, 0.2 mL yeast cultures were added in the prepared wells. After incubation at 30°C for 24-48 hours, inhibition zones around the wells were examined. The yeast *S. boulardii* was also used as control strain in these experiments.

RESULTS AND DISCUSSION

Proteolytic and lipolytic activities

Proteolytic and lipolytic activities of the yeast strains were represented in Table 1. Of 35 tested yeast strains, four strains (11%) belonging to *G. candidum* (T138), *C. kefyr* (T120), *C. lipolytica* (T122) and *C. lambica* (T103) were found to have proteolytic activity. It was determined that 28 (80%) of the investigated strains had lipolytic activities.

Proteolysis is reported as the most important of the primary biochemical events that

occur in most cheeses during ripening. It is known that cheese microflora is a good source of proteolytic enzymes. Although enzymes of starter and nonstarter lactic acid bacteria contribute to the ripening of all cheeses, proteolysis in varieties in which a secondary flora is encouraged to grow, is often affected greatly by enzymes from these secondary organisms[17]. Therefore, proteolytic activity is an important technological characteristic for microorganisms to be used as adjunct starters. In a study performed by Wit *et al*.[18], effects of using *Y. lipolytica* and *D. hansenii* as adjunct starters in Cheddar cheese production were investigated. By using these yeasts, proteolysis and lipolysis during ripening accelerated, leading to better aroma formation.

In another study, identification of yeast microflora of Rokpol cheese was investigated and *C. famata* was reported as predominant yeast species. It was determined that most of the *C. famata* strains had low proteolytic activity[19]. Suzzi *et al*.[20] reported that eight *Y. lipolytica* strains isolated from water-buffalo mozzarella and goat cheeses had high proteolytic activity at 25°C, and low proteolytic activity at 10°C. In a study performed by Klein *et al*.[21], peptidase activities of

Table 1. Proteolytic and lipolytic activities of the yeast strains

Isolate no.	Yeast species	Proteolytic activity	Lipolytic activity
T141	*Saccharomyces cerevisiae*	-	-
T50	*Saccharomyces cerevisiae*	-	-
T106	*Saccharomyces cerevisiae*	-	+
T125	*Saccharomyces cerevisiae*	-	-
T75	*Saccharomyces cerevisiae*	-	-
T110	*Zygosaccharomyces mellis*	-	+
T104	*Candida colliculosa*	-	-
T132	*Candida krusei*	-	+
T2	*Kluyveromyces lactis* var. *lactis*	-	+
T138	*Geotrichum candidum*	+	+
T154	*Geotrichum candidum*	-	+
T147	*Geotrichum candidum*	-	+
T90	*Geotrichum candidum*	-	+
T78	*Geotrichum candidum*	-	+
T107	*Candida kefyr*	-	+
T112	*Candida kefyr*	-	+
T120	*Candida kefyr*	+	+
T122	*Candida lipolytica*	+	+
T168	*Candida lipolytica*	-	+
T82	*Candida rugosa*	-	+
T29	*Candida zeylanoides*	-	+
T176	*Candida zeylanoides*	-	+
T60	*Candida zeylanoides*	-	+
T170	*Candida paludigena*	-	+
T51	*Candida paludigena*	-	+
T81	*Candida apicola*	-	-
T166	*Candida japonica*	-	+
T127	*Candida lambica*	-	+
T103	*Candida lambica*	+	+
T139	*Candida lambica*	-	+
T9	*Pichia fermentans*	-	-
T7	*Candida famata* var. *famata*	-	+
T144	*Candida famata* var. *famata*	-	+
T98	*Candida famata* var. *famata*	-	+
T42	*Candida famata* var. *flareri*	-	+

six yeast strains belonging to four species (*K. lactis, S. cerevisiae, D. hansenii, P. anomala*) originated from cheeses were compared to those of six lactic acid bacteria. All of the yeasts were reported to have the same or higher peptidase activity when compared to the activity of *Lactobacillus helveticus*, the most peptidolytic of the bacteria tested. In another research, six *Y. lipolytica* and six *D. hansenii* strains originated from blue-veined cheeses were investigated for their potential as adjunct starters in production of Danablu cheese. None of the *D. hansenii* strains had proteolytic activity at 10°C, while four *Y. lipolytica* strains determined as proteolytic at this temperature[22]. In another study, proteolytic and lipolytic activities of three different yeast strains; *D. hansenii, Y. lipolytica, Cryptococcus laurentii* isolated from Picante cheese were investigated. It was reported that proteolytic and peptidolytic activity of *Y. lipolytica* was considerably higher than those of the other strains[23].

In the present study, proteolytic activity was noticed in only four strains which may have important effects on proteolysis during cheese

Table 2. Assimilation and fermentation characteristics of some sugars

Isolate no.	Yeast species	GlucoseA/F	GalactoseA/F	LactoseA/F
T141	*Saccharomyces cerevisiae*	+/+	+/+	-/-
T50	*Saccharomyces cerevisiae*	+/+	+/+	-/-
T106	*Saccharomyces cerevisiae*	+/+	+/+	-/-
T125	*Saccharomyces cerevisiae*	+/+	+/+	-/-
T75	*Saccharomyces cerevisiae*	+/+	+/+	-/-
T110	*Zygosaccharomyces mellis*	+/+	-/-	-/-
T104	*Candida colliculosa*	+/+	+/+	-/-
T132	*Candida krusei*	+/+	-/-	-/-
T2	*Kluyveromyces lactis* var. *lactis*	+/+	+/+	+/+
T138	*Geotrichum candidum*	+/-	+/-	-/-
T154	*Geotrichum candidum*	+/-	+/+	-/-
T147	*Geotrichum candidum*	+/-	+/-	-/-
T90	*Geotrichum candidum*	+/-	+/-	-/-
T78	*Geotrichum candidum*	+/-	+/-	-/-
T107	*Candida kefyr*	+/+	+/+	+/+
T112	*Candida kefyr*	+/+	+/+	+/-
T120	*Candida kefyr*	+/+	+/+	+/-
T122	*Candida lipolytica*	+/-	-/-	-/-
T168	*Candida lipolytica*	+/-	-/-	-/-
T82	*Candida rugosa*	+/-	+/-	-/-
T29	*Candida zeylanoides*	+/-	-/-	-/-
T176	*Candida zeylanoides*	+/-	-/-	-/-
T60	*Candida zeylanoides*	+/-	-/-	-/-
T170	*Candida paludigena*	+/-	+/-	-/-
T51	*Candida paludigena*	+/-	+/-	+/-
T81	*Candida apicola*	+/-	+/-	+/-
T166	*Candida japonica*	+/+	+/+	-/-
T127	*Candida lambica*	+/+	-/-	-/-
T103	*Candida lambica*	+/+	-/+	-/+
T139	*Candida lambica*	+/+	-/-	-/-
T9	*Pichia fermentans*	+/+	-/-	-/-
T7	*Candida famata* var. *famata*	+/-	+/+	+/+
T144	*Candida famata* var. *famata*	+/-	+/-	+/-
T98	*Candida famata* var. *famata*	+/-	+/+	+/-
T42	*Candida famata* var. *flareri*	+/-	+/+	+/-

A: assimilation, F: fermentation

ripening. According to the results, while some strains of a certain species had proteolytic activity, others belonging to same species did not have this property. For example, only one *Y. lipolytica* and one *G. candidum* strain were found to have proteolytic activity, although those species were reported to have high proteolytic activities in the literature. These results confirm that proteolysis is a strain-dependent characteristic.

Lipolysis is one of the important reactions occuring during cheese ripening and directly contributing to aroma and texture formation. Secondary flora and adjunct starters are given among sources of lipases besides milk, rennet and starter bacteria. The results concerning lipolytic activity of the strains in this study may be valuable, demonstrating adjunct starter potential of the yeast strains. In several studies about use of yeasts as adjunct starters, usually *C. lipolytica* (telemoph: *Y. lipolytica*), *D. hansenii* (anamorph: *C. famata*), *G. candidum* and *K. lactis* strains have been selected[19,23,24]. The main technological properties

Table 3. Growth of the yeast strains at different temperature and pH values

Isolate No.	Yeast species	Temperature (°C)					pH		
		4	10	28	37	45	2.5	4.0	5.4
T141	*Saccharomyces cerevisiae*	+	+	+	+	-	+	+	+
T50	*Saccharomyces cerevisiae*	-	+	+	+	-	+	+	+
T106	*Saccharomyces cerevisiae*	-	+	+	+	-	+	+	+
T125	*Saccharomyces cerevisiae*	-	+	+	-	-	+	+	+
T75	*Saccharomyces cerevisiae*	+	+	+	+	+	+	+	+
T110	*Zygosaccharomyces mellis*	-	+	+	+	-	+	+	+
T104	*Candida colliculosa*	+	+	+	+	-	-	+	+
T132	*Candida krusei*	+	+	+	+	+	+	+	+
T2	*Kluyveromyces lactis* var. *lactis*	+	+	+	+	-	+	+	+
T138	*Geotrichum candidum*	+	+	+	+	-	+	+	+
T154	*Geotrichum candidum*	+	+	+	+	+	+	+	+
T147	*Geotrichum candidum*	+	+	+	+	+	+	+	+
T90	*Geotrichum candidum*	+	+	+	+	+	+	+	+
T78	*Geotrichum candidum*	+	+	+	+	+	+	+	+
T107	*Candida kefyr*	+	+	+	+	+	+	+	+
T112	*Candida kefyr*	+	+	+	+	+	+	+	+
T120	*Candida kefyr*	+	+	+	+	+	+	+	+
T122	*Candida lipolytica*	+	+	+	+	-	+	+	+
T168	*Candida lipolytica*	+	+	+	+	-	+	+	+
T82	*Candida rugosa*	-	+	+	+	+	+	+	+
T29	*Candida zeylanoides*	+	+	+	-	-	+	+	+
T176	*Candida zeylanoides*	+	+	+	-	-	+	+	+
T60	*Candida zeylanoides*	+	+	+	-	-	+	+	+
T170	*Candida paludigena*	+	+	+	+	-	+	+	+
T51	*Candida paludigena*	+	+	+	+	-	+	+	+
T81	*Candida apicola*	+	+	+	+	-	+	+	+
T166	*Candida japonica*	-	+	+	+	+	+	+	+
T127	*Candida lambica*	+	+	+	+	-	+	+	+
T103	*Candida lambica*	+	+	+	+	-	+	+	+
T139	*Candida lambica*	+	+	+	+	-	+	+	+
T9	*Pichia fermentans*	+	+	+	+	-	+	+	+
T7	*Candida famata* var. *famata*	+	+	+	+	-	+	+	+
T144	*Candida famata* var. *famata*	+	+	+	+	-	+	+	+
T98	*Candida famata* var. *famata*	+	+	+	+	+	+	+	+
T42	*Candida famata* var. *flareri*	+	+	+	-	-	+	+	+

+: positive growth, -: no growth

investigated in those studies are proteolytic and lipolytic activities, and it is reported that some of the strains do not have these properties.

It was reported in a study that yeast strains isolated from Picante cheese were belonging to *D. hansenii*, *Y. lipolytica* and *Cry. laurentii*[23]. Among the isolates, *D. hansenii* had very weak while others had strong lipolytic activities. Cosentino *et al*.[1] reported that *D. hansenii, K. lactis, K. marxianus* and *G. candidum* in the micoflora of Sardinian ewe's cheese had low proteolyic activities, and a few of them had lipolytic activities.

In the present study, all of the isolates belonging to *C. famata* (telemorph: *D. hansenii*) species were found to have lipolytic activities. This result may be important for the further studies when it is thought that *D. hansenii* is the most frequently used species as adjunct starter in cheeses.

Table 4. Growth of yeast strains at different NaCl concentrations

Isolate No.	Yeast species	NaCl (%)		
		5	10	15
T141	*Saccharomyces cerevisiae*	+	+	-
T50	*Saccharomyces cerevisiae*	+	+	-
T106	*Saccharomyces cerevisiae*	+	+	-
T125	*Saccharomyces cerevisiae*	+	+	-
T75	*Saccharomyces cerevisiae*	+	+	+
T110	*Zygosaccharomyces mellis*	+	-	-
T104	*Candida colliculosa*	+	+	+
T132	*Candida krusei*	+	+	-
T2	*Kluyveromyces lactis* var. *lactis*	+	+	+
T138	*Geotrichum candidum*	+	+	+
T154	*Geotrichum candidum*	+	+	-
T147	*Geotrichum candidum*	+	+	-
T90	*Geotrichum candidum*	+	-	-
T78	*Geotrichum candidum*	+	-	-
T107	*Candida kefyr*	+	+	-
T112	*Candida kefyr*	+	+	-
T120	*Candida kefyr*	+	+	+
T122	*Candida lipolytica*	+	+	-
T168	*Candida lipolytica*	+	+	-
T82	*Candida rugosa*	+	+	-
T29	*Candida zeylanoides*	+	+	-
T176	*Candida zeylanoides*	+	+	-
T60	*Candida zeylanoides*	+	+	-
T170	*Candida paludigena*	+	+	+
T51	*Candida paludigena*	+	+	-
T81	*Candida apicola*	+	+	+
T166	*Candida japonica*	+	+	-
T127	*Candida lambica*	+	+	-
T103	*Candida lambica*	+	+	-
T139	*Candida lambica*	+	-	-
T9	*Pichia fermentans*	+	+	-
T7	*Candida famata* var. *famata*	+	+	+
T144	*Candida famata* var. *famata*	+	+	+
T98	*Candida famata* var. *famata*	+	+	+
T42	*Candida famata* var. *flareri*	+	+	+

+: positive growth, -: no growth

Assimilation and fermentation of some sugars

The results related to assimilation and fermentation of glucose, galactose, and lactose are given in Table 2. All of the isolates assimilated glucose, while 17 (49%) of them could ferment this sugar. It is known that glucose is one of the sugars at trace amounts in cheese. It is reported that lactose is partially hydrolysed to glucose and galactose during cheese production, then glucose is rapidly fermented by starter bacteria and very low amounts of glucose can be found in cheese[8].

It was found that 24 (69%) of the tested yeast strains had the ability to assimilate galactose, while galactose fermentation ability was detected for 16 (46%) of them. In some studies, galactose in cheese has been reported as an important sugar contributing to reactions during cheese ripening. In a study performed by Ferreira and Viljoen[8], *D. hansenii* and *Y. lipolytica* were used as adjunct starters seperately in Cheddar cheese production. It was reported that maximum 0.86-1.5% galactose were detected in cheese samples after 24 hours during ripening. Galactose percentage was then decreased in those samples and no galactose was detected after 1-5 months. However, in control cheese produced without addition of adjunct

Table 5. Assimilation of some organic acids

Isolate no.	Yeast species	DL-lactate	Citric acid	Succinic acid
T141	*Saccharomyces cerevisiae*	+	-	-
T50	*Saccharomyces cerevisiae*	+	-	-
T106	*Saccharomyces cerevisiae*	+	-	-
T125	*Saccharomyces cerevisiae*	+	-	-
T75	*Saccharomyces cerevisiae*	+	-	-
T110	*Zygosaccharomyces mellis*	-	+	+
T104	*Candida colliculosa*	+	+	+
T132	*Candida krusei*	+	+	+
T2	*Kluyveromyces lactis* var. *lactis*	+	+	+
T138	*Geotrichum candidum*	+	-	+
T154	*Geotrichum candidum*	+	-	+
T147	*Geotrichum candidum*	+	-	+
T90	*Geotrichum candidum*	+	-	+
T78	*Geotrichum candidum*	+	-	+
T107	*Candida kefyr*	+	-	-
T112	*Candida kefyr*	+	-	-
T120	*Candida kefyr*	+	-	+
T122	*Candida lipolytica*	+	-	-
T168	*Candida lipolytica*	+	+	+
T82	*Candida rugosa*	-	-	+
T29	*Candida zeylanoides*	-	-	-
T176	*Candida zeylanoides*	-	-	-
T60	*Candida zeylanoides*	-	-	-
T170	*Candida paludigena*	+	-	-
T51	*Candida paludigena*	+	-	-
T81	*Candida apicola*	-	+	+
T166	*Candida japonica*	+	-	+
T127	*Candida lambica*	+	+	+
T103	*Candida lambica*	+	+	+
T139	*Candida lambica*	+	+	+
T9	*Pichia fermentans*	-	-	-
T7	*Candida famata* var. *famata*	-	-	-
T144	*Candida famata* var. *famata*	+	-	+
T98	*Candida famata* var. *famata*	+	-	-
T42	*Candida famata* var. *flareri*	-	-	-

+: assimilation positive, -: assimilation negative

starter, 0.55% galactose was detected even after 6 months, indicating the significance of yeast contribution to galactose metabolism. In another study in which survival of a probiotic yeast *S. boulardii* in some dairy products was investigated, it was reported that this yeast could use galactose, but unable to assimilate or ferment lactose[25]. Glucose and galactose usage of yeast isolates was investigated in another study by Tempel and Jakobsen[26] in which identification and technological characterization of yeasts originated from Danablu cheese were performed. It was reported that all of the yeast isolates belonging to *C. famata, Candida catenulata, C. lipolytica, Zygosaccharomyces* spp. and *Trichosporan cutaneum* could use glucose and galactose. In our study, all of the isolates belonging to *S. cerevisiae, C. colliculosa, C. kefyr, C. japonica* and *K. lactis* var. *lactis* were able to assimilate and ferment both glucose and galactose.

In the present study, lactose assimilation and fermentation was observed for only a few of the strains (Table 2). Lactose assimilation was determined as positive for 10 (29%) of the isolates belonging to *K. lactis* var. *lactis* (T2), *C. kefyr* (T107, T112, T120), *C. paludigena* (T51), *C. apicola* (T81), *C. famata* var. *famata* (T7, T144, T98), and *C. famata* var. *flareri* (T42). Only four of the isolates

Table 6. Bile salt tolerance of the yeast strains

Isolate No.	Yeast species	Bile salt concentration (%, w/v)	
		0.5	1.0
T141	*Saccharomyces cerevisiae*	-	-
T50	*Saccharomyces cerevisiae*	-	-
T106	*Saccharomyces cerevisiae*	-	-
T75	*Saccharomyces cerevisiae*	-	-
T110	*Zygosaccharomyces mellis*	-	-
T104	*Candida colliculosa*	-	-
T132	*Candida krusei*	+	+
T2	*Kluyveromyces lactis* var. *lactis*	-	-
T138	*Geotrichum candidum*	-	-
T154	*Geotrichum candidum*	-	-
T147	*Geotrichum candidum*	-	-
T90	*Geotrichum candidum*	+ (w)	-
T78	*Geotrichum candidum*	-	-
T107	*Candida kefyr*	+	-
T112	*Candida kefyr*	-	-
T120	*Candida kefyr*	-	-
T122	*Candida lipolytica*	+	+
T168	*Candida lipolytica*	+	+ (w)
T82	*Candida rugosa*	+	+
T170	*Candida paludigena*	-	-
T51	*Candida paludigena*	-	-
T81	*Candida apicola*	+ (w)	+ (w)
T166	*Candida japonica*	+	+ (w)
T127	*Candida lambica*	-	-
T103	*Candida lambica*	-	-
T139	*Candida lambica*	+ (w)	+ (w)
T9	*Pichia fermentans*	+ (w)	+ (w)
T7	*Candida famata* var. *famata*	+ (w)	-
T144	*Candida famata* var. *famata*	-	-
T98	*Candida famata* var. *famata*	-	-
Reference yeast	*Saccharomyces boulardii*	+	+

+ (w): weak growth, +: positive growth, -: no growth

had the ability to ferment lactose which were in the species of *K. lactis* var. *lactis* (T2), *C. kefyr* (T107), *C. lambica* (T103) and *C. famata* var. *famata* (T7). It is known that most of the lactose in milk remains in the whey as lactose or lactate during cheese production. However, low levels of lactose (0.8-1.0%) remain in the curd at the end of manufacture. Residual lactose is metabolized quickly to L-lactate during the early stages of ripening at a rate largely determined by temperature and salt-in-moisture levels of curd by the action of starter bacteria. It is reported that as salt levels in Cheddar and other dry-salted varieties increase rapidly on salting, starter activity is stopped very quickly at the end of manufacture. Lactose that remains unfermented by the starter is probably metabolized by secondary flora[17]. Lactose fermentation of yeasts is given as an important technological characteristic directly

Table 7. Inhibition effects of the yeast strains on some pathogenic bacteria

Isolate No.	Yeast species	Inhibition effect (zone formation)		
		E.coli	*S. aureus*	*L. monocytogenes*
T141	*Saccharomyces cerevisiae*	-	-	+ (w)
T50	*Saccharomyces cerevisiae*	-	-	-
T106	*Saccharomyces cerevisiae*	-	-	+ (w)
T125	*Saccharomyces cerevisiae*	-	-	-
T75	*Saccharomyces cerevisiae*	-	-	-
T110	*Zygosaccharomyces mellis*	-	-	-
T104	*Candida colliculosa*	-	-	-
T132	*Candida krusei*	+ (w)	+	-
T2	*Kluyveromyces lactis* var. *lactis*	-	+ (w)	-
T138	*Geotrichum candidum*	-	+ (w)	-
T154	*Geotrichum candidum*	+ (w)	-	+ (w)
T147	*Geotrichum candidum*	+ (w)	-	-
T90	*Geotrichum candidum*	+ (w)	+ (w)	-
T78	*Geotrichum candidum*	-	-	-
T107	*Candida kefyr*	-	+ (w)	+ (w)
T112	*Candida kefyr*	-	-	-
T120	*Candida kefyr*	-	-	-
T122	*Candida lipolytica*	-	-	-
T168	*Candida lipolytica*	-	-	-
T82	*Candida rugosa*	-	+ (w)	-
T29	*Candida zeylanoides*	-	-	+ (w)
T176	*Candida zeylanoides*	-	-	-
T60	*Candida zeylanoides*	-	-	+ (w)
T170	*Candida paludigena*	-	-	+ (w)
T51	*Candida paludigena*	-	-	+ (w)
T81	*Candida apicola*	-	-	+ (w)
T166	*Candida japonica*	-	-	+ (w)
T127	*Candida lambica*	-	-	-
T103	*Candida lambica*	-	-	-
T139	*Candida lambica*	-	-	-
T9	*Pichia fermentans*	+ (w)	-	-
T7	*Candida famata* var. *famata*	-	-	-
T144	*Candida famata* var. *famata*	-	-	-
T98	*Candida famata* var. *famata*	-	+ (w)	-
T42	*Candida famata* var. *flareri*	-	-	-
Reference yeast	*Saccharomyces boulardii*	-	-	-

+: inhibition zone positive, w: weak inhibition zone (<1 mm), -: inhibition zone negative

affecting cheese flavour. It is reported that the fermentation of lactose by yeasts influences the formation of aroma by formation of ethanol and acetaldehyde by limiting acidification by lactic acid and thus affecting the texture of cheese, and by the formation of CO_2[27]. It is also reported that there is a risk of too much openness and the development of a yeast flavour if the number of yeasts at the center exceed counts of 10^6 cfu/g. Welthagen and Viljoen[27] reported that lactose concentration was determined as 2.55% at the beginning of ripening during Cheddar cheese production, while it was detected as 0.262% after 51 days. In the same study, yeast count considerably increased between 24-37 days of ripening, indicating that decrease in lactose concentration was due to the ability of yeasts growing at low temperatures. In another study by Pereira-Dias *et al*.[28], technological characterization of dominant yeast flora of Portuguese ewes' cheese was performed. It was reported that all of the strains belonging to *Candida curvata*, *C. intermedia* and *D. hansenii*, and 10% of *Rhodotorula* strains were able to assimilate lactose, while non of the *C. zeylanoides* strains had this property. In another study, *K. lactis*, *K. marxianus* and *Dekkera anomala* were reported to ferment and assimilate lactose which were originated from Sardinian ewes' cheese[1]. In the present study, although lactose assimilation and fermentation was not a common characteristic among the strains, all of the strains belonging to *C. kefyr* and *C. famata* var. *famata* were able to assimilate and ferment lactose.

Since cheeses can contain glucose, galactose and lactose at variable amounts, metabolization of those sugars may serve as an important technological characteristic for the yeast isolates originated from cheeses. In this study, satisfactory results were obtained especially for galactose assimilation properties. In addition, two strains belonging to *K. lactis* var. *lactis* (T2) and *C. kefyr* (T107) were found to assimilate and ferment all three of the tested sugars.

Growth at different temperatures and pH

Growth abilities of the yeast strains at different temperature and pH values are presented in Table 3. All of the isolates were able to grow at 10 and 28°C. Additionally, growth capabilities of the yeast strains were very good at low temperature such as 4 °C. Only six of the isolates belonging to *S. cerevisiae* (T50, T106, T125), *Z. mellis* (T110), *C. japonica* (T166) and *C. rugosa* (T82) were found unable to grow at 4°C. Most of the strains grew well at 37°C except five of them belonging to *S. cerevisiae* (T125), *C. zeylanoides* (T29, T176, T60) and *C. famata* var. *flareri* (T42). Growth of the yeast strains were generally adversely affected at 45°C. Twelve of the isolates belonging to *S. cerevisiae* (T75), *C. krusei* (T132), *G. candidum* (T154, T147, T90, T78), *C. kefyr* (T107, T112, T120), *C. rugosa* (T82), *C. japonica* (T166) and *C. famata* var. *famata* (T98) were able to grow at 45°C. Ten of the isolates which were in the species of *S. cerevisiae* (T75), *C. krusei* (T132), *G. candidum* (T154, T147, T90, T78), *C. kefyr* (T107, T112, T120) and *C. famata* var. *famata* (T98) were able to grow at all temperatures in the studied range.

Growth ability at low temperatures is usually attributed to occurence of yeasts in cheese. Additionally, it is known that ripening temperature of some cheese varieties are also low. Ripening temperatures are given as 10-12°C for Rokpol cheese[19], 6-8°C for Cheddar cheese, and 10-15°C for smear-ripened cheeses[29]. Emmental cheese is ripened initially for 2-3 weeks at 12°C, after which the temperature is increased to 20-24°C for 2-4 weeks to promote the growth of propionic acid bacteria for the fermentation of lactate to propionate and acetate; the temperature is then reduced to 4°C[29]. Ripening temperature of Erzincan tulum cheese is known as 4-6°C[30]. Thus, the results concerning the ability of yeasts to grow at low temperatures seems expected. It is also thought that those yeasts have potential as adjunct starters in production of the cheeses they originated from. Similar results were obtained in some other studies about technological characteristics of yeasts originating from various cheese varieties. In a study, growth of *D. hansenii* isolated from Danablu was found as positive between 10-30°C, and optimum growth temperature was indicated as 20°C[26]. In another research, growth of yeast isolates originating from Sardinian ewes' cheese was investigated between 6-40°C, and most of the strains were found to grow between 6-10°C[1].

Growth ability of yeasts at 37-45°C gives idea about their survival during cheese manufacture. During cheese production, curd is cooked at the temperatures from 32°C to 54°C after cutting to small pieces. Cooking temperature is

given as 32°C for Cheshire cheese, while it is 40°C for Cheddar cheese and 35°C for Gouda and Edam cheeses. However, for some hard cheeses such as Emmental and Parmigiano in production of which thermophilic starters are used, cooking temperature is used as 54°C[31]. In the present study, approximately 27% of the tested strains were found to have ability to grow at 45°C. In manufacture of Erzincan tulum cheese, coagulation temperature is 35°C, after which curd is cut into pieces and incubated at 20°C for 24 hours[32]. Those strains able to grow at 37-45°C may have potential to be used in productions of cheeses especially which have high cooking temperatures.

In this research, all of the yeast strains originating from Erzincan tulum cheese were able to grow at pH 4.0 and 5.4 (Table 3). Most of the strains had also ability to grow at pH 2.5 except one *C. colliculosa* (T 104) strain. It is known that pH of cheese is usually between 4.5-5.3 at the end of production[29,33]. Yeasts increase pH by using lactic acid and also due to production of some metabolites. In a study performed by Wyder *et al*.[34], when yeasts isolated from traditional cheeses and belonging to *Galactomyces geotrichum*, *Pichia jadinii*, *Y. lipolytica* and *D. hansenii* were used as adjunct starters in production of Raclette cheese, pH was recorded as 5.42 after 4 hours of ripening, while it was 5.07 after 24 hours and 5.34 after 90 hours. The pH of Rokpol cheese was reported as 6.0-6.3 at the end of ripening[19]. In another study, it was reported that *D. hansenii* strains which were predominantly isolated from semi-dry and surface ripened cheeses, were all able to grow between pH 5.0-6.0[35]. It was reported in a study that three *C. famata* strains which were isolated from Danablu cheese were able to grow between pH 4.0-6.0, and optimum growth was observed at pH 6.0[26].

In the present study, pH of Erzincan tulum cheese samples were found to change between 3.33-4.82 (data not shown), which may be varied during stages of ripening. pH of Erzincan tulum cheese during ripening was reported to change between 4.8-5.2 which can be sometimes 4.3[32]. It is thought that the strains isolated from Erzincan tulum cheese in this study grew very well at pH values similar to cheese pH such as 4.0 and 5.4 due to their adaptation to medium during cheese ripening. Resistance to low pH values has importance for survival of yeast strains when they are used in the production of various cheeses. This characteristic is also attributed to probiotic potential of the yeast strains, which is important for their survival in gastrointestinal tract. Gotcheva *et al*.[36] investigated probiotic characteristics of some yeast strains isolated from a fermented drink which were able to grow between pH 2.0-3.0.

Growth at different NaCl concentrations

In order to determine growth ability of the tested strains at different NaCl concentrations, water activity (a_w) values were detected for the media containing 5%, 10% and 15% (w/v) NaCl. When medium without NaCl had a_w value of 0.999, a_w values of media containing 5%, 10% and 15% NaCl were found as 0.969, 0.934 and 0.887, respectively. Results concerning growth of the yeasts at different salt concentrations are presented in Table 4. All of the isolates had the ability to grow in the medium with 5% NaCl. Four of the strains could not grow at 10% NaCl concentration, which were belonging to *Z. mellis* (T110), *G. candidum* (T78, T90), and *C. lambica* (T139). It was found that only 11 (31%) of the yeast strains could grow in medium with 15% NaCl.

Salt concentration has a primary role in variation of cheese microflora. Salt concentration of brine is usually given as 21% and surface of brine-salted cheeses contain high salt concentrations. Especially for those cheeses, secondary flora growing on the surface should be resistant to high salt concentrations[29]. In a study in which chemical changes during ripening process of Turkish white cheese was investigated, brine salt concentration was changed between 12-18%[37]. In another study, all of the yeast strains which were isolated from semi-hard and surface ripened cheeses in Denmark, were reported to grow between 4-10% NaCl concentrations[35]. Cosentino *et al*.[1] reported that only some *D. hansenii* and *K. lactis* strains could grow at 10% NaCl concentrations among the isolates originated from Sardinian ewes' cheese. In another study performed by Hansen and Jakobsen[38], technological properties of *Saccharomyces* spp. isolated from blue veined cheese and fermented Maize and also commercial strains were investigated. Tolerance to NaCl were determined between 0-6% and it was reported that cheese isolates were the most resistant strains and all of

them could grow at 6% NaCl concentration. Most of the yeast strains isolated from Erzincan tulum cheese in this study were able to grow between 5-10% salt concentration, and considerable amount of them at 15% salt concentration. Besides all of the *C. famata* strains, the isolates belonging to *S. cerevisiae* (T75), *C. colliculosa* (T104), *K. lactis* var. *lactis* (T2), *G. candidum* (T138), *C. kefyr* (T120), *C. paludigena* (T170) and *C. apicola* (T81) were able to grow at 15% salt concentration. There are already some reports about resistance of *C. famata* (telemorph: *D. hansenii*) and *K.lactis* to high salt concentrations. In our study, resistance to 15% salt concentration was demonstrated for the first time for *S. cerevisiae* and also *C. kefyr, C. colliculosa, C. paludigena* and *C. apicola* originated from Erzincan tulum cheese.

Assimilation of some organic acids

The abilities of the yeast strains to assimilate lactic, citric and succinic acids were investigated and the results are given in Table 5. It was found that 26 (74%) of the yeast strains were able to assimilate DL-lactate. Eight of the isolates belonging to *Z. mellis, C. rugosa, C. zeylanoides, C. apicola, P. fermentans, C. famata* var. *famata* (T7) and *C. famata* var. *flareri* were found negative in lactate assimilation. Among the organic acids in cheese, lactic acid exists at higher concentrations than the others[33]. Degredation of lactate is given as one of the most important reactions occuring during cheese ripening[17]. Yeasts increase the pH of cheese by oxidation of lactate to CO_2 and H_2O[29]. Ability of lactate assimilation for yeasts has a major importance for testing their potential as adjunct starters. In a study in which four yeast species were used as adjunct starters in Raclette cheese, all of them were reported to assimilate lactic acid. However, lactic acid concentration increased with time in cheese samples inoculated with *P. jadinii* and *D. hansenii* in the same study. This was explained by growth stimulation of lactic acid bacteria by yeasts[34]. In the present study, satisfactory results were obtained for lactate utilization of the strains isolated from Erzincan tulum cheese. Since lactate is utilized mainly by the secondary flora, it is thought that an important technological property was demonstrated for the yeast isolates.

Assimilation of citric acid was observed positive for 9 (26%) of the yeast isolates (Table 5). Those strains were belonging to: *Z. mellis* (T110), *C. colliculosa* (T104), *C. krusei* (T132), *K. lactis* var. *lactis* (T2), *C. lipolytica* (T 168), *C. apicola* (T 81), and *C. lambica* (T 127, T103, T139). Metabolization of citrate is one of the reactions occured during cheese ripening. Citrate can be converted into some aroma compounds such as diacetyl, acetoin and acetate by starter or non-starter lactic acid bacteria[17]. Yeast strains that have ability to use citrate can also contribute to citrate metabolism during cheese ripening. Citrate assimilation is given as a reason for survival of some yeast strains during cheese ripening[8]. In a study in which technological properties of *Saccharomyces* spp. isolated from blue-veined cheese were investigated, it was reported that the yeast strains could assimilate lactate but could not assimilate citrate[38]. These data were in agreement with our results concerning *S. cerevisiae.* In a study by Tempel and Jakobsen[26], it was reported that most of the yeast strains isolated from Danablu cheese were able to assimilate citrate. In another study by Tempel and Jakobsen[22], *Y. lipolytica* and *D. hansenii* strains isolated from blue-veined cheese were used as adjunct starters in Danablu cheese and five of the six strains were reported as citrate positive. Cosentino *et al.*[1] reported that *D. hansenii, Y. lipolytica* and *Rhodotorula rubra* strains isolated from Sardinian ewes' cheese had ability to metabolize citrate. In a study about African fermented milks, it was reported that *Saccharomyces* and *Candida* ganera were frequently isolated from these products. Most of those isolates could not use lactose, but could metabolize galactose, citrate and lactate. Since concentration of citrate in those products is only about 0.2%, growth of yeasts could not be achieved from this substrate alone. But, it was indicated that flavour compunds could be produced from citrate by yeasts in those products, although it has not been fully investigated yet[39]. In the present study, all of the isolates metabolizing citrate are belonging to *Candida* genus, except one *K. lactis* var. *lactis* and one *Z. mellis* strains. It is thought that effects of these yeasts on flavour compound formation in cheese as a result of citrate metabolism may be investigated in the furher studies.

It was determined that 18 (51%) of the yeast strains isolated from Erzincan tulum cheese had ability to assimilate succinic acid. All of the

strains belonging to *G. candidum* and *C. lambica* were able to use succinic acid. However, *P. fermentans*, which is telemorph form of *C. lambica*, gave negative result in succinic acid assimilation test. Succinic acid assimilation is taken into account when technological properties of isolates originating from cheese or other dairy products are investigated. In a study performed with a probiotic yeast *S. boulardii*, survival of this yeast in some dairy products was investigated and the yeast was found to assimilate only lactic acid, but not citric and succinic acids[25]. Occurence of organic acids such as lactic, citric, propionic and succinic acid was reported by Mullin and Emmons[40]. Organic acid profiles of fifty various cheese samples as well as milk, whey and cheese collected from Cheddar production line were investigated. It was reported that Emmental cheese contained lactic, citric, formic, acetic, succinic and propionic acids, and concentrations of succinic and propionic acids were high in that cheese. In the same study, blue-veined cheese was reported to contain succinic acid at trace amounts.

In this study, it was determined that seven of the isolates had the ability to assimilate all three of the organic acids. Those strains were belonging to *C. colliculosa* (T104), *C. krusei* (T132), *K. lactis* var. *lactis* (T2), *C. lipolytica* (T168) and *C. lambica* (T127, T103 ve T139) which may have positive effects in cheese ripening as a result of organic acid metabolism.

Bile salt tolerance

Bile salt tolerance was investigated by using media containing 0.5% and 1% (w/v) bile salt. At this stage, the yeast *S. boulardii* was also used in the experiments as a control strain because of its known probiotic characteristics. The results concerning the growth of the yeast strains in the media containing 0.5% and 1% (w/v) bile salt were represented on Table 6. Eleven of the tested yeast isolates (36%) were able to grow in the medium containing 0.5% bile salt, while 8 of them (26%) were resistant to 1% (w/v) bile salt concentration. The strains resistant to 0.5% (w/v) bile salt concentration were belonging to *C. krusei* (T132), *G. candidum* (T90), *C. kefyr* (T107), *C. lipolytica* (T122, T168), *C. rugosa* (T82), *C. apicola* (T81), *C. japonica* (T166), *C. lambica* (T139), *P. fermentans* (T9) and *C. famata* var. *famata*. None of the *S. cerevisiae* strains could grow at this medium. The strains growing in the media containing 0.5% (w/v) bile salt were also resistant to 1% (w/v) bile salt concentration except three strains in the species of *G. candidum* (T90), *C. kefyr* (T107) and *C. famata* var. *famata* (T7). Weak growth was observed in the medium containing 1% bile salt for most of the strains except *C. krusei* (T132), *C. lipolytica* (T122) and *C. rugosa* (T82). The growth of the control strain *S. boulardii* was well for both concentrations of the bile salt tested.

Growth and survival of the three strains which grew well in the medium containing 1% (w/v) bile salt, were further investigated in a medium containing 0.3% (w/v) Ox-Bile. When cell counts of *C. lipolytica* (T122) at the beginning and after 72 hours were compared, similar results were obtained. For *C. krusei* (T132) and *C. rugosa* (T82), yeast counts increased by 2.5 and 2.8 logarithmic units after 72 hours, respectively (data not shown). Control strain *S. boulardii* also grew well and cell count of this strain increased by approximately 2.5 logarithmic units in this medium which was an expected result. The obtained bile salt tolerance for the isolates *C. lipolytica* (T122) and especially *C. krusei* (T132) and *C. rugosa* (T82) are promising for evaluation of them as potential probiotics.

Bile salt tolerance is an important selection criterion for determination of probiotic characteristics of microorganisms. For application of strains as probiotics, it is important that they are able to survive the bile salt in the intestine, the normal level of which is around 0.3%, but may range up to the extreme 2% during the first hour of digestion[36]. In a study in which some probiotic characteristics of *Trich. cutaneum*, *C. rugosa* and *C. lambica* were investigated, bile salt tolerance was determined between 0.2-2% oxgall. It was reported that all of the strains survived at all tested concentrations, but increase in yeast count was only observed in the media containing 0.2% and 0.3% oxgall[36]. In the present study, tolerance to high bile salt concentrations was a rare property among the tested strains, of which *C. rugosa* (T82) gave the best result for growing in Ox-Bile medium.

Inhibition effects on some pathogenic bacteria

Inhibition effects of the yeast strains on *E. coli*, *S. aureus* and *L. monocytogenes* strains were investigated and results of agar-cup diffusion tests were given on Table 7. For 5 of the strains belonging to *C. krusei* (T132), *G. candidum* (T90,

T154, T147) and *P. fermentans* (T9), weak inhibition effect on *E. coli* was observed. Inhibition effect on *S. aureus* was observed for 8 of them which were *C. krusei* (T132), *K. lactis* var. *lactis* (T2), *G. candidum* (T138, T90), *C. kefyr* (T107), *C. rugosa* (T82) and *C. famata* var. *famata* (T98). Among these 8 strains, inhibition effect of *C. krusei* was strong, determined with an inhibition zone of 5 mm, while those of others were weak. It was found that 10 of the isolates had weak inhibition effect on *L. monocytogenes* which were in the species of *S. cerevisie* (T141, T106), *G. candidum* (T154), *C. kefyr* (T107), *C. paludigena* (T170, T51), *C. apicola* (T81), *C. japonica* (T166) and *C. zeylanoides* (T60, T29).

Antagonistic effect of yeasts on pathogenic bacteria is known as an important characteristic especially for potential probiotics, but it is observed that this property is rarely detected among yeasts in the reported studies. In a study by Addis *et al*.[41], some yeast and bacterial strains were isolated from Cammembert and blue-veined cheeses. It was reported that none of the yeast strains which were belonging to *D. hansenii*, *Y. lipolytica*, *K. marxianus* and *S. cerevisiae* had inhibition effects on 15 *Staphylococcus* spp., 4 *Micrococcus* spp. and also *Enterobacter faecium* and *Enterobacter faecalis*. Similarly, no inhibition effect was reported on *Salmonella* Typhimurim, *E. coli* and *S. aureus*, while some *Y. lipolytica* strains inhibited the growth of *L. monocytogenes* and *B. cereus*. Gotcheva *et al.*,[36] reported that a *Trich. cutaneum* strain had antagonistic activity against some pathogenic bacteria, while *C. rugosa* and *C. lambica* strains inhibited the growth of *Salmonella* spp. and *Pseudomonas aeruginosa*. In another study, anti-listerial potential of 404 foodborne yeast strains, 304 of which were isolated from smear-ripened cheeses, were investigated[42]. It was reported that only 4% of the red smear cheese isolates clearly inhibited growth of *L. monocytogenes*. The yeast strains showing high inhibitory effect were mainly in the species of *C. intermedia* ve *K. marxianus*. In another study by Georges *et al*.[43], 175 yeast strains isolated from different sources were screened for their anti-listerial activities. It was reported that 14% of the yeast strains had anti-listerial activity, and one *Pichia norvegensis* strain inhibited *L. monocytogenes* by 7 log units.

In the present study, inhibition on all of the three pathogenic bacteria; *E. coli*, *S. aureus* and *L. monocytogenes* could be achieved by only different *G. candidum* strains. It is reported that *G. candidum* has the ability to excrete D-3-phenyllactic acid which inhibits the growth of *L. monocytogenes*[44]. It is thought that demonstrating inhibitory effects of Erzincan tulum yeast isolates on pathogenic bacteria may be important for their probiotic potential, although most of the inhibition effects were weak. In addition, it is thought that strong inhibition effect of *C. krusei* (T132) on tested *S. aureus* strain may be valuable for further investigations, since there is no similar previous report about this property.

CONCLUSIONS

When all of the technological and probiotic characteristics were evaluated together, four strains; *G. candidum* T138, *C. kefyr* T120, *C. lipolytica* T122 and *C. lambica* T103 gave positive results for most of the tests, which may be selected for further investigations as adjunct starters. For technological characteristics except probiotic characteristics, *C. kefyr* T120 was the best one having proteolytic and lipolytic activity, growing at all investigated pH, temperature and NaCl concentrations, assimilation of all investigated sugars, and also lactic and succinic acid. This strain gave negative result for only lactate fermentation and citrate assimilation. This strain was followed by *C. lambica* T103, which could assimilate all of the organic acids, but galactose and lactose-negative. When tested probiotic characteristics were also taken into account, *G. candidum* T138 which had inhibition effect on *S. aureus* and *C. lipolytica* T122 which had high bile salt tolerance were the best strains among the selected potential adjunct strains.

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REFERENCES

1. Cosentino, S., Fadda, M.E., Deplano, M., Mulargia, A.F., Palmas, F. Yeasts associated with Sardinian ewe's dairy products. *Int. J. Food Microbiol.*, 2001; **69**: 53-58.
2. De Freitas, I., Pinon, N., Maubois, J-L., Lortal, S., Thierry A. The addition of a cocktail of yeast species to Cantalet cheese changes bacterial survival and enhances aroma compound formation. *Int. J. Food Microbiol.*, 2009; **129**: 37-42.
3. Wyder, M.,T., Puhan, Z. Role of selected yeasts in cheese ripening: an evaluation in aseptic cheese curd slurries. *Int. Dairy J.*, 1999; **9**: 117-124.
4. Padilla, B., Manzanares, P., Belloch, C. Molecular identification and enzymatic activity of yeasts isolated from artisanal cheeses made from ewes' milk. International Conference on Food Innovation, 2010; 25-29 October, Valencia, Spain, 224.
5. Viljoen, B.,C., Knox, A.M., Jager, P.H., Laurens-Hattingh, A. Development of yeast populations during processing and ripening of blue veined cheese. *Food Technol. Biotechnol.*, 2003; **41**(4): 291-297.
6. Fröhlich-Wyder, M.T. Yeasts in Dairy Products. In: *Yeasts in Food* (Boekhaut T, Robert V, eds). Hamburg, Germany: B.Behr's Verlag, GmbH & Co. KG, 2003; pp 209-237.
7. Jakobsen M., Narvhus J. Yeasts and their possible beneficial and negative effects on the quality of dairy products. *Int. Dairy J.*, 1996; **6**: 755-768.
8. Ferreira, A.D., Viljoen B.C. Yeasts as adjunct starters in matured Cheddar cheese. *Int. J. Food Microbiol.*, 2003; **86**: 131-140.
9. Yilmaz, G., Ayar, A., Akin, N. The effect of microbial lipase on the lipolysis during the ripening of Tulum cheese. *J. Food Eng.*, 2005; **69**: 269-274.
10. Kamber, U. The traditional cheeses of Turkey: Eastern Anatolia Region. *Food Reviews Int.*, 2008; **24**: 148-174.
11. Harrigan, W.F (ed): Laboratory Methods in Food Microbiology. London: Academic Press, 1998; 532p.
12. Yarrow, D. Methods for isolation, maintenance, and identification of yeasts. In: *The yeasts: A Taxonomic Study* (Kurtzman CP, Fell JW, eds). Amsterdam: Elsevier, 2000; pp 77-100.
13. Deák T., Beuchat L.R (eds): Handbook of Food Spoilage Yeasts. USA: CRC Press, 1996; 210p.
14. Kurtzman, C.P., Boekhout, T., Robert, V., Fell, J.W., Deak, T. Methods to identify yeasts. In: *Yeasts in Food* (Boekhaut T, Robert V, eds). Hamburg, Germany: B.Behr's Verlag, GmbH & Co. KG, 2003; pp. 69-121.
15. Psomas, E., Andrighetto, C., Litopoulou-Tzanetaki, E., Lombardi, A., Tzanetakis, N. Some probiotic properties of yeast isolates from infant faeces and Feta cheese. *Int. J. Food Microbiol.*, 2001; **69**: 125-133.
16. Izgu, F., Altinbay, D., Yucelis, A. Identification and killer activity of a yeast contaminating starter cultures of *Saccharomyces cerevisiae* strains used in the Turkish baking industry. *Food Microbi*ol, 1997; **14**: 125-131.
17. McSweeney, P. Biochemistry of cheese ripening. *Int. J. Dairy Technol.*, 2004; 57(2/3): 127-144.
18. Wit, M.D., Osthoff, G., Viljoen, B.C., Hugo A. A comparative study of lipolysis and proteolysis in Cheddar chese and yeast-inoculated Cheddar cheeses during ripening. *Enzyme Microbial Technol.*, 2005; **37**: 606-616.
19. Wojtatowicz, M., Chrzanowska, J., Juszczyk, P., Skiba, A., Gdula A. Identification and biochemical characteristics of yeast microflora of Rokpol cheese (short communication). *Int. J. Food Microbiol.*, 2001; **69**: 135-140.
20. Suzzi, G., Lanorte, M.T., Galgano, F., Andrighetto, C., Lombardi, A., Lanciotti, R., Guerzoni M.E. Proteolytic, lipolytic and molecular characterization of *Yarrowia lipolytica* isolated from cheese. *Int. J. Food Microbiol.*, 2001; **69**: 69-72.
21. Klein, N., Zourari, A., Lortal S. Peptidase activity of four yeast species frequently encountered in dairy products - comparison with several dairy bacteria. *Int. Dairy J.*, 2002; **12**: 853-861.
22. Tempel, T., Jakobsen, M. The technological characteristics of *Debaryomyces hansenii* and *Yarrowia lipolytica* and their potential as starter cultures for production of Danablu. *Int. Dairy J.*, 2000; **10**: 263-270.
23. Freitas, A.C., Pintado, A.E., Pintado, M.E., Malcata, F.X. Role of dominant microflora of Picante cheese on proteolysis and lipolysis. *Int. Dairy J.*, 1999; **9**: 593-603.
24. Martin, N., Berger, C., Du, C.L., Spinnler, H.E. Aroma compound production in cheese curd by coculturing with selected yeast and bacteria. *J. Dairy Sci.*, 2001; **84**: 2125-2135.
25. Laurens-Hattingh, A., Viljoen, B.C. Growth and survival of a probiotic yeast in dairy products. *Food Res Int.*, 2001; **34**: 791-796.
26. Tempel, T., Jakobsen, M. Yeasts associated with Danablu. *Int. Dairy J.*, 1998; **8**: 25-31.
27. Welthagen, J.J., Viljoen B.C. The isolation and

identification of yeasts obtained during the manufacture and ripening of Cheddar cheese. *Food Microbiol.,* 1999; **16**: 63-73.

28. Pereira-Dias, S., Potes, M.E., Marinho, A., Malfeito-Ferreira, M., Loureira, V. Characterization of yeast flora isolated from an artisanal Portuguese ewes' cheese. *Int. J. Food Microbiol.,* 2000; **60**: 55-63.
29. Cogan, T.M. Microbiology of cheese. In: *Encyclopedia of Food Microbiology* (Robinson RK, Patt CA, Patel PD, eds). London: Academic Press, 2000; pp 306-313.
30. Hayaloglu, A.A., Ozer, B.H., Fox, P.F. Cheeses of Turkey: 2. Varieties ripened under brine. *Dairy Sci. Technol.,* 2008; **88**: 225-244.
31. Cogan, T.M., Beresford, T.P. Microbiology of hard cheese. In: *Dairy Microbiology Handbook* (Robinson RK, ed). New York, USA: John Wiley & Sons, Inc., 2002; pp 515-560.
32. Hayaloglu, A.A., Fox, P.F., Guven, M., Cakmakci, S. Cheeses of Turkey: 1. Varieties ripened in goat-skin bags. *Dairy Sci. Technol.,* 2007; **87**(2): 79-95.
33. Beresford, T.P., Fitzsimons, N.A., Brennan, N.L., Cogan, T.M. Recent advances in cheese microbiology. *Int. Dairy J.,* 2001; **11**: 259-274.
34. Wyder, M.T., Bachmann, H.P., Puhan, Z. Role of the selected yeasts in cheese ripening: an evaluation in foil wrapped raclette cheese. *Lebens. Wiss. Technol.,* 1999; **32**: 333-343.
35. Petersen, K.M., Westall, S., Jespersen, L. Microbial succession of *Debaryomyces hansenii* strains during the production of Danish surface-ripened cheeses. *J. Dairy Sci.* 2002; 85: 478-486.
36. Gotcheva, V., Hristozova, E., Hristozova, T., Guo, M., Roshkova, Z., Angelov, A. Assessment of potential probiotic properties of lactic acid bacteria and yeast strains. *Food Biotechnol.,* 2002; **16**(3): 211-225.
37. Güven, M., Yerlikaya, S., Hayaloglu, A.A. Influence of salt concentration on the characteristics of Beyaz cheese, a Turkish white-brined cheese. *Dairy Sci. Technol.* 2006; **86**(1): 73-81.
38. Hansen, T.K., Jakobsen, M. Taxonomical and technological characteristics of *Saccharomyces* spp. associated with blue veined cheese, *Int. J. Food Microbiol*., 2001; **69**: 59-68
39. Narvhus, J.A., Gadaga, T.H. The role of interaction between yeasts and lactic acid bacteria in African fermented milks: a review. *Int. J. Food Microbiol.* 2003; **86**: 51-60.
40. Mullin, W.J., Emmons, D.B. Determination of organic acids and sugars in cheese, milk and whey by high performance liquid chromotography. *Food Res. Int.,* 1997; **30**(2): 147-151.
41. Addis, E., Fleet, G.H., Cox, J.M., Kolak, D., Leung, T. The growth, properties and interactions of yeasts and bacteria associated with the maturation of Camembert and blue-veined cheeses. *Int. J. Food Microbiol.,* 2001; **69**: 25-36.
42. Georges, S., Aigner, U., Silakowski, B., Scherer, S. Inhibition of *Listeria monocytogenes* by foodborne yeasts. *Appl. Environ. Microbiol.,* 2006; **72**(1): 313-318.
43. Georges, S., Koslowsky, M., Velagic, S., Borst, N., Bockelmann, W., Heller, K.J., Scherer, S. Anti-listerial potential of food-borne yeasts in red smear cheese. *Int. Dairy J.,* 2011; 21: 83-89.
44. Wouters, J.T.M., Ayad, E.H.E., Hugenholtz, J., Smit, G. Microbes from raw milk for fermented dairy products. *Int. Dairy J.,* 2002; **12**: 91-109.