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The production and antimicrobial activity of Horc cheese, a natural heritage of Erdemli Yoruks

Elif Ayşe Erdoğan Eliuz

Mersin University, Technical Sciences Vocational School, Department of Food Technology, TR-33343, Mersin, Turkey
Corresponding author: eliferdogan81@gmail.com

Abstract
Horc cheese is a traditional whey product that has been produced by nomads (Yoruks) living in Anatolia for centuries. Recently, it is produced in the villages of Erdemli district of Mersin, albeit rarely. In this study, different producers were reached and the Horc production technique was researched and applied with a manufacturer. In the study, 2.5 kg of Horc was obtained from 30 kg of whey, and the production stages of cheese were photographed. In addition, the antimicrobial effect of ethanol and water extract of Horc were researched on *E. coli*, *K. pneumoniae*, *B. subtilis*, *S. aureus*, *C albicans*, *C. tropicalis*, *C. parapsilosis and C. glabrata* by Well Diffusion Method. The highest inhibition zone (IZ) of the cheese were found as 2 mm on *C. parapsilosis*. As a result, with this study, Horc cheese was produced in a natural environment and it was shown to have an antimicrobial effect.
Keywords: Horc cheese, Whey, Production, Antimicrobial

Introduction

In our country where agriculture and animal husbandry are abundant, there are hundreds of types of local cheese produced naturally from dairy products. Although we have an important place in the world in the cheese industry, a significant portion of local cheeses still do not have a national standard. In our country, many cheeses such as Dil cheese (TS 3002), Kashar cheese (TS 3272), Tulum cheese (TS 3001), and Urfa cheese (TS 13129) have been standardized. This, however, does not apply to cheeses obtained from whey, as even in our country, there are only standardized cheese curds (TS 13358) (TSE, 2020).

Whey cheeses are of particular importance in terms of the utilization of cheese wastewater, the production of new probiotic cheeses with high fermentative value, and the introduction of the rich protein and mineral sources in the food sector. Whey is one of the by-products of cheese factories waiting to be treated from among tons of waste. According to the 2020 data from Turkey Statistical Institute (TSI), Turkey is committed to the production of 13.544 million tons of raw milk per year, 20% of which consists of cheese. Thus, corresponding to around 2.302 million tons of cheese, the amount of whey obtained from that highlights the importance of evaluating this waste product (Okur, 2010; Dincoglu and Ardıc, 2012). The remarkable substances found in whey are as follows: 43.5% lactose, 6.6% whey proteins, 1.85% fat and 1.30% casein. In addition, it contains 99.9% potassium, 87.5% sodium, and 70% magnesium elements (Akpinar *et al.*, 2018). Because of its high protein content and macroproteins of beta-lactoglobulin, alpha-lactalbumin, glaucoma-peptides, transferrin, blood serum albumin, immunoglobins, enzymes and phospholipoproteins, it is categorized in protein-rich functional food. Oligosaccharides and whey oils (sphingolipids) selectively support the growth of friendly

intestinal bacteria such as Bifidobacteria. (Smithers *et al.*, 1996; Karagozlu and Bayarer, 2004). Thanks to the bioactive molecules it contains, it prevents cancer tumor formation (De wit, 1998), and shows a protective effect against hypertension (Abubakar *et al.*, 1998). It has also been determined that it prevents obesity (Marshal, 1992, Jakubowicz and Froy, 2013), and shows antimicrobial effects against pathogenic bacteria and viruses (Uniacke-Lowe *et al.*, 2010, Brumini *et al.*, 2016). Moreover, it has been named as an important dietary protein liquid in that it contains beta-lactoglobulin (β-lg), bovine serum albumin (BSA), alpha-lactalbumin, lactoferrin (Lf), lactoperoxidase (Lp) enzymes, and immunoglobulins (Igs) (Gupta and Prakash, 2017) . Also, the lactic acid bacteria found naturally in cheeses are considered to be beneficial for health and reliable in terms of their use (generally recognized as safe (GRAS)) (Hassan and Bullerman, 2008; Abdellah *et al.*, 2014; Baruah *et al.*, 2016).
Except for a Turkish publication about the production method of Horc cheese by Ucgun and Işık (2018), there is no study recorded. The fact that Horc cheese, has been consumed by the local people for years, despite its production being on a decrease, and that the producers are limited to those who are engaged in animal husbandry, make the cheese both valuable and at risk. Therefore, with this study, Horc production will once again be brought to the literature.

Material and Methods

The production of Horc

Support was received from three different producers (n=3). The production of Horç cheese under natural conditions was practiced in the village of Güzeloluk (1422 m) in the village of Erdemli / Mersin / Turkey on 17.08.2020.

Preparing ethanol and water extract of Horc

Horc ethanol and water extract was performed from 10 g of the fresh Horc in 50 mL solvent (ethanol or water) via shaker incubator for 2 hours at 25ºC. Two extractions were obtained; Horc ethanol extract (EE) and Horc water extract (WE).

Antimicrobial screening using Well Diffusion Method

The pathogens, *Escherichia coli* (ATCC 25293), *Klebsiella pneumoniae* (ATCC 10031), *Bacillus subtilis* (ATCC 6633), *Staphylococcus aureus* (ATCC 25923), *Candida albicans* (ATCC 90028), *C. tropicalis* (ATCC 750), *C. parapsilosis and C. glabrata* were taken from Refik Saydam Hıfzıssıhha Centre (Ankara/Turkey). The inoculums of pathogens were prepared in 4 mL Triptic Soy Broth for bacteria and 4 mL Sabouraud Dextrose Broth for yeast, respectively, and incubated at 37°C, overnight. To determine of inhibition zone of Horc WE and EE on *E. coli, K. pneumoniae, B. subtilis, S. aureus, C albicans, C. tropicalis, C. parapsilosis and C. glabrata,* the well diffusion method was used. For this, the pathogen cultures at stationary phase were spread onto Mueller Hinton Agar plates and 6 mm diameter wells were drilled into the middle of petri dishes. The 50 µL of Horc extract was added to the wells and petri dishes were left for incubation at 37°C for 24 h. After 1 day, inhibition zones formed around the well were measured. Then, the incubation period was increased for 1 more day and the zones were measured at the end of the 48th hours. Accordingly, it was interpreted as susceptible strain (S) if there is an inhibition zone around the well, as a tolerant strain (T) if a re-colony occurs in the inhibition zone. If the sensitivity continued at the end of the 48th hours, it was expressed as a persistent susceptible (SP) strain. The ethanol (Tekkim Laboratory Chemicals) and distilled water were used as negative control The experiment was repeated twice.

Results

The production technique of Horc cheese

Horc cheese was made by nomads settled in Guzeloluk village of Erdemli district (altitude 1422) with 30 liters of goat's milk (Figure 1A). Raw milk was taken to the pot and heated at approximately 30ºC.

Afterwards, 5 mL of commercially purchased rennet was added (MAYASAN) (Figure 1B,C). The pot was covered with a cloth and left to ferment for 2 hours. (Figure 1D,E). The cheese is taken to the stove for cooking. Scratches are removed before cooking the cheese using a spoon. It is mixed while cooking so that all sides are cooked evenly. Adding leavening, the light on it is identified by the yellow water coming out (Figure 1F, G). Boiled cheese is put in a cloth bag and the water is drained (Figure 1 H). The yellow water on the cheese and the whey obtained after the cheese was filtered were brought together and put back on the stove (Figure 1 I,J). It is cooked for about 10 hours until it darkens. The final product formed is "Horc" (Figure 1 K,L).

Figure 1: Place of production (A), heating milk (B), fermentation process (C), Fermentation (D), cheese formation (E), cooking cheese (F), yellow water formed on the surface (G), filtration (H), whey (I), cooking whey (J), and "Horc" (K,L).

In this study, The cheese produced was in caramel-brown color. The 2.5 kg Horc was obtained from 30 kg milk and the yield was 8.3%.

Antimicrobial Activity

The results showed that Horc EE, not Horc WE, was effective against *E. coli, K. pneumoniae, B. subtilis, S. aureus, C. albicans, C. tropicalis, C. parapsilosis and C. glabrata,* by agar well diffusion method. The inhibition zones at the end of the 24 th and 48th hours were 0.8 mm for *E. coli*, 0.2 mm for *K. pneumoniae*, 1.2 mm for *B. subtilis*, 1.3 mm for *S. aureus,* 2.1 mm for *C. albicans*, 0.1 mm for *C. tropicalis*, 2 mm for *C. parapsilosis and* 1.8 mm for *C. glabrata.* All microbes against Horc WE were resistant; inhibition zone of the extract was "0" for all pathogens (Table 1). For negative control, during the first 12 hours of ethanol incubation with pathogens, an opening of 2 mm was seen around the ethanol well at the 12th hours of incubation but the opening was closed during further incubation (24 h and 48 h) and 100% ethanol inhibition calculated as “0” for all microorganisms. Pure water did not inhibite all pathogens.

Table 1: IZs (mm) of Horc EE and WE against *E. coli, K. pneumoniae, B. subtilis, S. aureus, C. albicans, C. tropicalis, C. parapsilosis and C. glabrata.*

Microorganism	Horc-Ethanol Extract	Horc-Water Extract	Ethanol 100%	Water
E. coli	0.8 mm S^{α}-P^{ω}	0	2 mm T*+0-$S^{¥}$	0
K. pneumoniae	0.2 mm S^{α}-P^{ω}	0	2 mm T*+0-$S^{¥}$	0
B. subtilis	1.2 mm S^{α}-P^{ω}	0	2 mm T*+0-$S^{¥}$	0
S. aureus	1.3 mm S^{α}-P^{ω}	0	2 mm T*+0-$S^{¥}$	0
C. albicans	2.1 mm S^{α}-P^{ω}	0	2 mm T*+0-$S^{¥}$	0
C. tropicalis	0.1 mm S^{α}-P^{ω}	0	2 mm T*+0-$S^{¥}$	0
C. parapsilosis	2 mm S^{α}-P^{ω}	0	2 mm T*+0-$S^{¥}$	0
C. glabrata	1.8 mm S^{α}-P^{ω}	0	2 mm T*+0-$S^{¥}$	0

*T: tolerant strain, at the first 12th hour, $S^{¥}$ susceptible strain, at the first 24 and 48th hours, S^{α}-P^{ω}: persistent susceptible strain.

Discussion

Cheeses produced as cheese curd in small enterprises in our country have similarities with the production of Horc. In the production of a normal curd, whey is heated at temperatures above 85°C and proteins such as α-lactalbumin and β-lactoglobulin are denatured. These cheeses with lower fat content are highly valuable because they contain essential amino acids, and various vitamins and minerals (Fox *et al.,* 1990; Gonzales-Fandos *et al.,* 2000; Pintado and Malcata, 2000; Samelis *et al.,* 2003). Due to differences such as the fact that Horc cheese is boiled for a much longer time than curds and its color is much darker, it is more like Dolaz cheese, whose production has decreased in Isparta and surrounding regions. With a boiling time of about 14-15 hours, Dolaz cheese is similar to Horc cheese in this respect. However, the raw materials used differ slightly. In Dolaz cheese, the first ingredient is prepared by mixing buttermilk, yoghurt and curd cheese in addition to whey (Okur and Güzel-Seydim, 2011). Lactic acid bacteria (LAB) are the most important actors of the chemical transformations that occur during the production and maturation phase of all cheeses. They are the microorganisms that perform fermentation that transform the lactose in milk to lactic acid and cause flavor changes such as taste, odor and acidity in cheese (Nessler *et al.,* 1994; Khalid, 2011). Lactic acid bacteria were found in Dolaz cheese (Okur and Guzel-Seydim, 2011). Likewise, it is likely to be detected in Horc as well.

With this study, the antimicrobial activity of Horc cheese was examined for the first time. It was determined that ethanol extract, but not the aqueous extract, had an antimicrobial effect. This may be due to the fact that ethanol releases substances that are antimicrobial agents, including aromatic compounds in Horc cheese.

Conclusion

Research and protection of our natural resources gain importance within the scope of "Innovative Production, Stable and High Growth" and "Use of our country's resources in productive areas" included in the 2023 development program of our country (Gun *et al.*, 2018). Studies related to traditional foods began in 1995 and Turkey continues to work within the scope of the “traditional products” project. Dairy products have an extremely important place within the context of these products. Despite the large number of researches on making cheese, it is emphasized that further systematic and comprehensive scientific information is necessary. Because, whey cheeses are similar to each other in their production, but each differs from one another in terms of a few technical differences. (Gursoy *et al.*, 2008).

Since it is faced with the danger of disappearance, there is a need for larger studies to include Horc cheese, among functional foods list as a bioactive food and to evaluate it as an alternative product that will contribute to the economy in terms of preventing waste in the dairy industry.

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