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Identification of some yeast species in traditional Turkish fermented sausage with Vitek 2 compact system

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ABSTRACT

Objective: Fermented sausage, produced by traditional methods, matures by fermentation of microbial flora originating from the raw materials and the place of production. The source of contamination of the meat industry with yeasts, which are widespread in the environment, are the surfaces of the tools and equipment used in processing. Although the presence of yeasts in meat products contributes to the formation of flavor and aroma, some yeast species can cause undesirable flavors, discoloration, and the formation of a soft texture.

Material and Method: Therefore, our study aimed to determine the yeast profile of fermented sausages using the Vitek2 Compact System, in which various biochemical tests were performed.

Results: In the sausage samples was detected *Candida zeylanoides* in 56.25%, *Candida sake* in 52.10%, *Pichia farinosa* in 25%, *Cryptococcus laurentii* in 10.42%, *Candida glabrata* in 4.17%, and *Rhodotorula glutinis* in 10.42% yeast species.

Conclusion: The difference in yeast species in fermented sausages varied depending on the microbial load of the raw material and compliance with hygiene regulations during processing and fermentation conditions.

Keywords: *Fermented sausage, Vitek2 Compact System, Yeast*

INTRODUCTION

Dry-cured meat products form a wide group of products, ranging from hams to sausages, and have been consumed since ancient times. Among them, dried fermented sausages are widely used worldwide due to their characteristic taste (Flores et al., 2015). Fermented dry sausages are products made by grinding raw meat, adding spices, and stuffing into casings, drying, and fermenting with natural microflora or commercial starter cultures (Mikami et al. 2020). During the maturation of dry-cured meat products, enzymatic and chemical reactions contribute to the development of flavor. Turkish sausage, known as "Soudjouck", a traditional Turkish sausage, is a fermented meat product. Traditionally produced sausage matures by fermentation of raw materials and microorganisms from the flora of the place of production. During production, the product has a flora that includes various lactic acid bacteria, coagulase-negative staphylococci and yeasts. (Siriken et al. 2006; Lorenzo et al., 2007; Loranjo, 2017; Loranjo, 2019). Dry-fermented sausages are considered safe meat products due to their low water activity and low pH, which inhibits the growth of pathogenic microflora during processing and storage (Meftah et al. 2018). Yeast can reach up to 106 cfu/g in dry-cured meat products such as

fermented sausages (Encinas et al., 2000; Cocolin et al. 2006; Simoncini et al., 2007). The most commonly isolated yeast species are Candida, Cryptococus, Debaryomyces, Kluyveromyces, Pichia, Rhodotorula, Saccharomyces, Trichosporon, and Yarrowia spp. (Cano-Garcíaz et al. 2014, Murgia, Marongiu et al. 2019). Yeasts with aerobic or facultative anaerobic properties as starter cultures, Debaryomyces spp. and Candida spp. are commonly used in meat products (Laranjo et al., 2017; Loranjo, 2019). Recent studies have shown that yeasts contribute to the formation of the characteristic flavors of dry-cured meat products through their proteolytic and lipolytic activities (Sørensen, 1997; Martín et al., 2004; Simoncini et al., 2007; Bosse et al., 2018). Yeast growth plays an important role in controlling pathogenic microorganisms in meat products (Metaxopoulos et al., 1996; Purriños et al., 2013). Some of the yeast strains C. catenulata, C. guilliermondii, C. edax, and Cryptococcus and Wingea isolated from Italian ham show lipolytic properties (Simoncini et al., 2007). Yeasts develop and colonize the surface of dry-cured meat products during maturation and have an antagonistic effect on mold growth. In addition, yeasts have always been recognized as safe starter cultures in foods, including meat products and beverages (Jakobsen and Narvhus, 1996; Adesulu-Dahunsi et al., 2020). For this reason, it is important to determine the natural fermentation flora in terms of safety and sensory quality of the product. The aim of this study was to determine the yeast flora of fermented sausages with the Vitek2 Compact System, using YST ID cards with different sugars, enzymes and substrates in their wells.

MATERIALS and METHODS

Material: Forty-eight fermented sausage samples sold in different markets in Erzurum in Turkey were taken in 2013, and these samples were brought to the laboratory under cold chain.

Yeast Isolation: For yeast isolation, appropriate dilutions were cultured on Rose bengal chloramphenicol agar (Merck 106867) using the smear method. Typical yeast colonies formed after 5 days of incubation at 25 °C under aerobic conditions were classified based on colony morphology and examined microscopically (Erdem büyükkiraz et al., 2020).

Yeast Identification: To identify the yeast isolates, biochemical tests were performed on the fresh cultures obtained after incubation at 25 °C for 5 days under aerobic conditions by seeding on RBC agar in a draw method using the YST ID Card in the Vitek2 Compact System. It contains various sugars, enzymes and substrates such as L-lysine arylamidase, L-malate, leucine arylamidase, arginine- GP, erythritol, glycerol, tyrosine arylamidase, beta-N-acetyl glucosaminidase, Arbutin, amygdalin, D-gentibialactose, D-glucose, lactose, methyl-A-D-glucopyranoside, D-cellobiose, gamma-glutamyl transferase, D-maltose, D-refinose, PNP-N-acetyl- BD -galactosaminidase-1, D-mannose, D-melibiose, D-melizitose, L-sorbose, L-rhamnose, xylitol, D-sorbitol, sucrose/sucrose, urease, alpha-glucosidase, D-turanose, D-trehalose, nitrate, L-arabinose, D-galacturonate, esculin, L-glutamate, D-xylose, DL -lactate, acetate, citrate (sodium), glucuronate, L-proline, 2-keto-D-gluconate, N-acetyl-glucosamine, and D-gluconate for biochemical tests (Pincus, 2006).

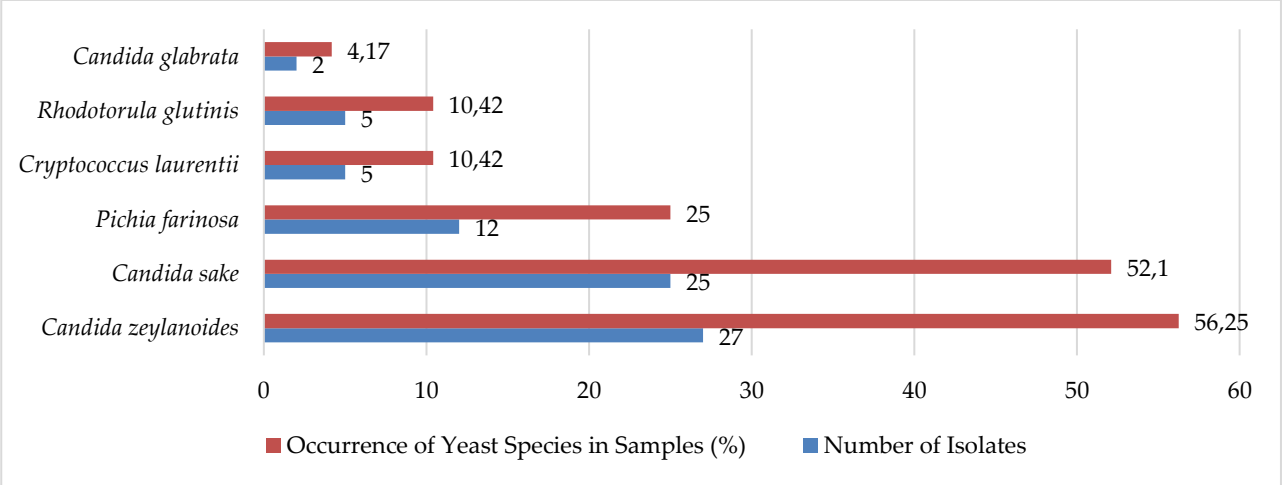

Figure 1. The number of isolates obtained from fermented sausage samples and the rate of presence in the samples

RESULTS

It was detected that *Candida zeylanoides* in 56.25%, *Candida sake* in 52.10%, *Cryptococcus laurentii* in 10.42%, *Candida glabrata* in 4.17%, *Rhodotorula glutinis* in 10.42% and 25% *Pichia farinosa* from the analyzed 48 fermented sausages (Fig 1).

DISCUSSION

The source of contamination in the meat industry by yeasts, which are widely distributed in the environment, are the surfaces of machines that come into contact with meat during processing (Hernández et al., 2018). Although yeast growth in meat products contributes to the formation of flavor and aroma, some yeast strains can cause off-flavors, discoloration, and slime formation (Nielsen et al., 2008). Yeasts can grow and multiply on complex substrates containing large amounts of sugar, salt, low pH, low temperature, and low water activity (Jakobsen and Narvhus, 1996; Lopandic et al., 2006). *Candida sake* type yeasts were also one of the yeast strains isolated from fermented sausages (Nielsen et al., 2008; Franciosa et al., 2021). *Candida zeylanoides*, which is commonly isolated in fermented meat products, is believed to be a pathogenic yeast and may pose a public health problem (Asefa et al. 2009). *Candida zeylanoides*, which is commonly isolated in fermented meat products, is thought to be a pathogenic yeast and could be a public health concern (Asefa et al. 2009). *Pichia farinosa*, a halotolerant yeast, is an important yeast strain in the fermentation industry due to its ability to produce high levels of glycerol and xylitol (Wang et al. 2006). *Cryptococcus laurentii*, a psychrotrophic yeast, may contribute to flavor formation due to its lipolytic property, although it has only been isolated in small numbers (Saldanha-da-Gama, 1997). *Rhodotorula glutinis*, which is widely distributed in the environment, is a unicellular yeast that generally produces nontoxic metabolites such as endogenous proteins, biological pigments (β-carotene, astaxanthin, lycopene), unsaturated fatty acids, and cell walls (Kot et al., 2016). *Rhodotorula glutinis*, isolated in small numbers in fermented sausages, was detected in only 10.42% of samples (Asefa et al.2009).

The difference in yeast species in fermented sausages varies depending on the microbial load of the raw material, the hygiene rules during processing, and the conditions of fermentation. In addition, the decrease in pH and water activity during maturation causes changing the yeast flora. For this reason, it is important to adjust the choice of quality raw materials and ripening conditions to obtain the standard product in fermented sausage production. The importance of using food safety systems such as HACCP and ISO 22000 in food production areas is becoming increasingly clear.

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