Prevalence of Yeasts in Locally Produced Cheese

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Abstract
To generate a comprehensive profile of viable yeasts on cheese as it is purchased by consumers, 40 samples of locally produced cheese were obtained from four Slovakian farms and sampled. The samples were cultured on Malt extract agar with bromocresol green and cultivated for 5 days at 25 °C. Selected colonies were cultured overnight on TSA agar aerobically and used for identification. Pure cultures were obtained and identified by mass spectrometry, as well as growth characteristics and colony morphology. The yeast *Candida kefyr* was the most abundant yeast, present in 90 % of all cheese samples. From the non-smoked and smoked cheese a total of 10 species of 5 yeast genera were identified with MALDI-TOF Mass Spectrometry.

Keywords: cow milk cheese, yeasts, mass spectrometry

1. Introduction

The environmental conditions can affect the growth of the microorganism. Thus, they can grow in close associations and not individually. The coexistence of well adapted yeasts and lactic bacteria make both of the groups to grow intensively, specific interactions being noticed. The yeasts have positive effects on lactic bacteria, including increase of cells viability by changing the pH value and secretion of bio-active compounds in medium [1-3].
Yeasts represent an important component of the microflora of many dairy products, where they play an active role in production or spoilage. The significance of their presence depends on their counts, on the particular type of product or on the presence of biotypes featuring specific metabolism. Due to their diverse metabolic potential, yeasts can play an important role in the fermentation and ripening of many cheese varieties and make a positive contribution to the development of taste and aroma. On the other hand, they may also act as spoilage organisms causing typical defects such as yeasty off-flavour, loss of texture quality, excessive gas formation, increased acidity [4].
The mechanisms by which yeast growth is thought to influence the final quality of cheeses are those associated to the fermentation of residual lactose, utilisation of lactic acid and their proteolytic and lipolytic activities. However, objective and quantitative. criteria to distinguish between desirable and undesirable yeast species and activities for cheese ripening are still lacking. Moreover, it should be possible to identify, for the different metabolites or flavours, thresholds beyond which the desirable changes also become detrimental to cheese quality or stability [5].
Many studies noticed that accidentally contamination of milk and cheese with yeast and moulds contribute to the ripening of speciality cheeses [6-12]. Over 40 non-starter yeasts and

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molds (NSYM) species have been isolated from milk, brines and cheeses, the most common being *Candida catenulata*, *Candida intermedia*, *Candida lusitaniae*, *Candida parapsilosis*, *Candida rugosa*, *Candida zeylanoides*, *Cryptococcus curvatus*, *Issatchenkia orientalis* (syn. *Pichia kudriavzevii*, anamorph: *Candida krusei*), *Kluyveromyces marxianus*, *Pichia fermentans*, *Pichia guillermondii*, *Saccharomyces cerevisiae*, *Torulaspora delbrueckii* (anamorph: *Candida colliculosa*), and *Yarrowia lipolytica* [6, 9, 13-19].

The aim of our study was isolation and identification of yeast form traditional Slovak cheese.

2. Materials and methods

2.1 Cheese Samples

The study was conducted from February 2018 to December 2018. The cheese samples included non-smoked parenica cheese (n=20) and smoked parenica cheese (n=20). Additionally, 40 milk product samples from the western and middle Slovak producers were collected (Bánovce nad Bebravou, Liptovský Mikuláš, Červený Kameň, Važec). Samples were collected in sterilized sample containers and brought to laboratory with icebox for microbiological investigation. Samples were kept in a refrigerator (4±1°C) until the testing began. The primary dilution of the milk products was made by adding of 5 g of sample material to 45 ml of 0.89 % sterile saline. Then, the serial dilutions (10^{-2} to 10^{-4}) were done and 100 µl of each dilution was plated out on to agars.

Figure 1. Map of Slovak republic (www.google.sk)

2.2 Isolation of yeasts

Malt extract agar and acid base indicator bromocresol green (0.020 g/L) were used for yeasts identification. Inoculated plates were incubated at 25 °C for 5 days aerobically and then the growth was evaluated.

The colonies from yeasts were selected for further confirmation with MALDI-TOF. Selected colonies were cultured overnight on TSA agar (Tryptone Soya Agar) aerobically and used for identification.

2.3 Identification of bacteria with MALDI-TOF MS Biotyper

A sample for MALDI-TOF MS analysis was prepared in accordance with extraction procedure provided by the manufacturer (Bruker Daltonik, Bremen, Germany). Yeast colony was suspended in 300 µL of water and 900 µL of absolute ethanol then mixed and centrifuged at 13000 rpm for 2 min. After removal of supernatant, the pellet was mixed with 10 µL of 70% formic acid (v/v) and an equal volume of acetonitrile. The mixture was

repeatedly centrifuged and 1 μL of the supernatant was spotted onto a polished steel target plate and air dried at room temperature. Each sample was overlaid with 1 μL of MALDI matrix (a saturated solution of α-cyano-4-hydroxycinnamic acid, HCCA, Bruker Daltonik, Germany) in 50% acetonitrile and 2.5% trifluoroacetic acid. Mass spectra were automatically generated using the microflex LT MALDI-TOF mass spectrometer (Bruker Daltonik, Germany) operated in the linear positive mode within a mass range of 2000-20000 Da. The instrument was calibrated using the Bruker bacterial test standard. Results of mass spectra were processed with the MALDI Biotyper 3.0 software (Bruker Daltonik, Germany). The identification criteria used were: a score of 2.300 to 3.000 indicated highly probable identification on species level; a score of 2.000 to 2.299 secure genus identification with probable species identification; a score of 1.700 to 1.999 probable identification to the genus level; <1,700 was considered as unreliable identification.

3. Results and discussion

Dairy products represent a specific environment for the growth and selection of different yeast species [20]. Yeasts are usually detected in high numbers in dairy products reflecting a good adaptation to a substrate rich on proteins, lipids, sugars and organic acids. Wide distribution is a consequence of proteolytic and lipolytic activity, as well as the ability to ferment/assimilate lactose and to utilize citric, lactic and succinic acids. In addition, yeasts are able to grow in substrates with high salt concentration, low temperatures, low pH and water activity. Due to their inherent trait of adapting to the complex substrates, yeasts may play either a beneficial (e.g. in ripening processes) or detrimental role (spoilage organisms, inhibitors of the growth of starter cultures) in the dairy production [21]. As part of a microbial community, together with bacteria, yeasts may contribute to the sensory characteristics of kefyr, koumiss and different cheese varieties influencing the biosynthesis of aromatic compounds [22].

The isolated family, genera and species of yeasts show figure 2. Together 447 isolates were isolate from smoked and non-smoked traditional cow cheese (tab. 1). The most isolated species of yeast was *Candida kefyr*. Mass spectra of *C. kefyr* and order show figure 3.

In study of Lopandic et al. [17] a predominant part of the analysed yeast microflora was represented by ascomycetous yeasts (460 isolates), while only 53 isolates were of basidiomycetous origin. On the basis of the applied approach, 92% of the isolates were identified at the species level and the remaining yeasts were characterized at the genus level. Within 472 (92%) yeast isolates 27 species could be distinguished. *Candida zeylanoides, Debaryomyces hansenii, Geotrichum candidum, Kluyveromyces marxianus and Yarrowia lipolytica* are the most frequently isolated species. The remaining isolates were assigned to *Clavispora lusitaniae, Debaryomyces fabryi, Issatchenkia orientalis, Kazachstania unispora, Kluyveromyces lactis, Pichia fermentans, P. guilliermondii, Saccharomyces cerevisiae, Torulaspora delbrueckii* and different Candida species. Basidiomycetous yeasts were represented by *Cryptococcus curvatus, Rhodotorula mucilaginosa, Trichosporon cutaneum* and *T. ovoides*.

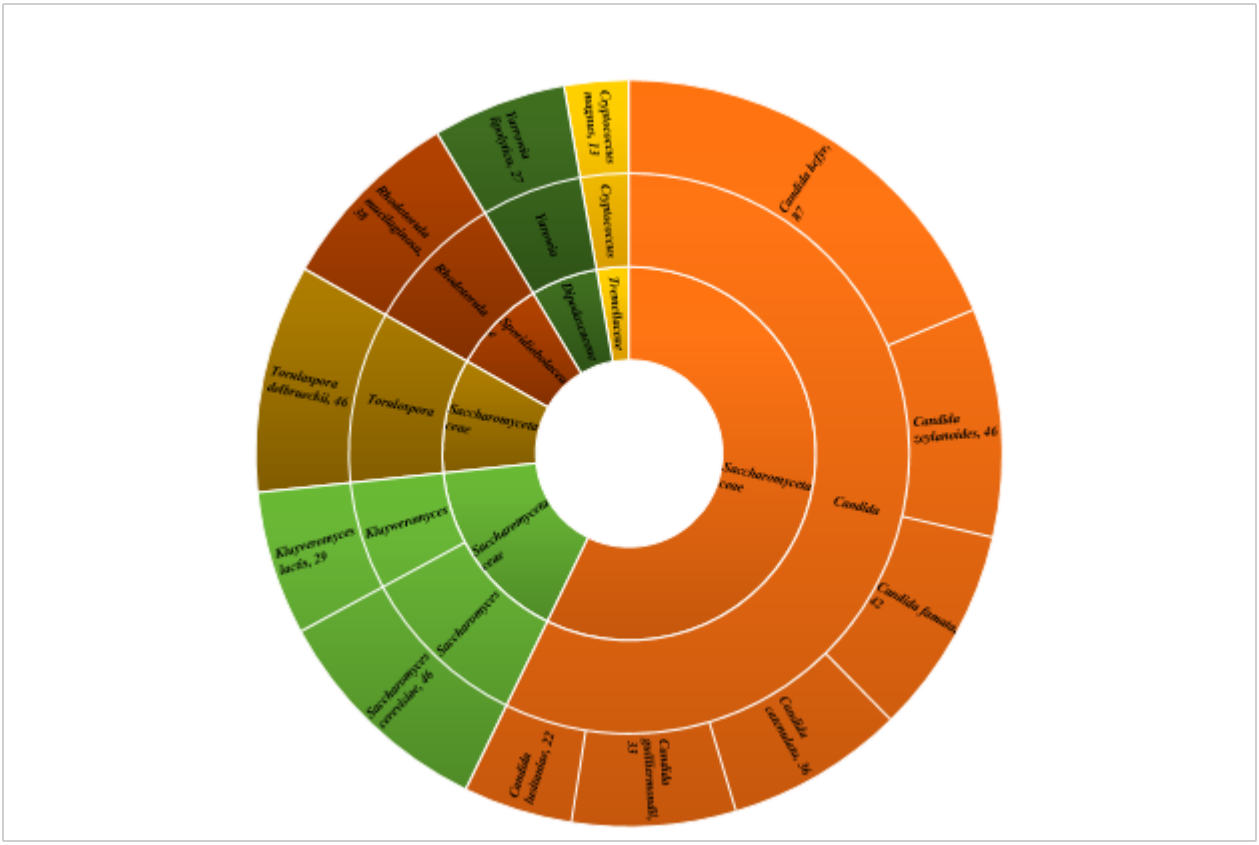

Figure 2. Isolated yeasts from Slovak smoked and non-smoked cheese

Table 1. Isolated number of yeasts from cheese samples

Species	Smoked	Non-smoked	Number of isolates
Candida catenulata	21	15	36
Candida famata	20	22	42
Candida guilliermondii	15	18	33
Candida kefyr	45	42	87
Candida lusitaniae	12	10	22
Candida zeylanoides	32	14	46
Cryptococcus magnus	5	8	13
Kluyveromyces lactis	15	14	29
Saccharomyces cerevisiae	25	21	46
Rhodotorula mucilaginosa	16	22	38
Torulaspora delbrueckii	21	25	46
Yarrowia lipolytica	15	12	27
Total number	**242**	**223**	**447**

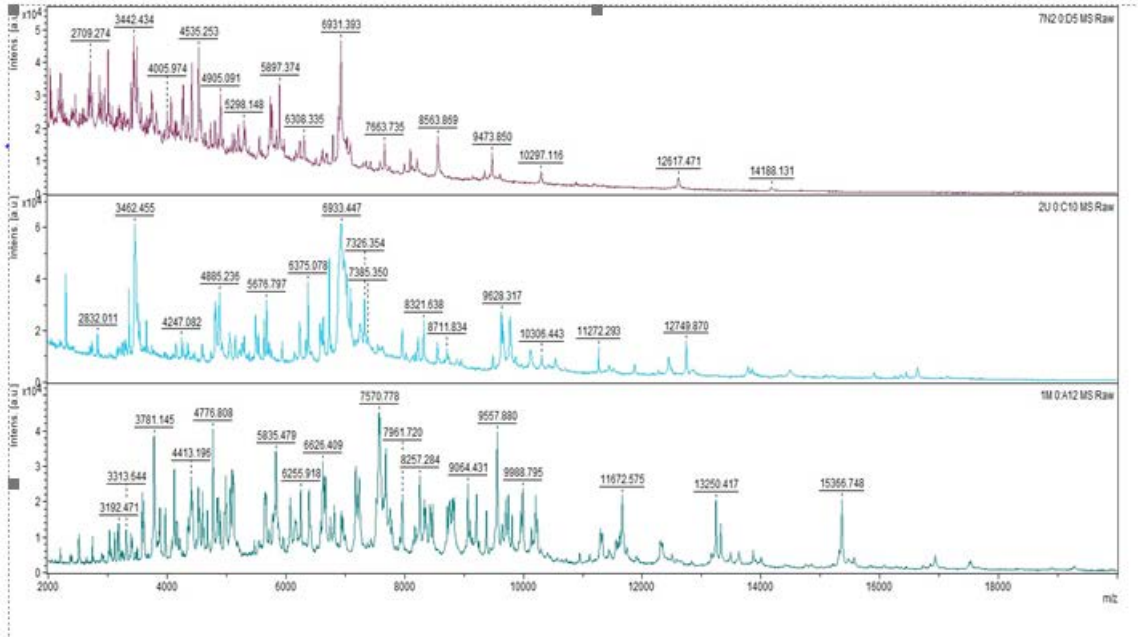

Figure 2 Mass spectrum of *Candida* species (*C. lusitane, C. famata, C. kefyr*)

4. Conclusions

In our study the yeast was identified with MALDI-TOF MS Biotyper from smoked and non-smoked Slovak cheese. Together four families, seven genera and twelve species of yeasts were isolated from cow cheese.

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