The potential of using Saccharomyces boulardii yeast in fermented milk products

Bendelja Ljoljić, Darija; Dolenčić Špehar, Iva; Tudor Kalit, Milna; Rajnović, Ivana; Hulak, Nataša; Kos, Ivica; Vnučec, Ivan; Prđun, Saša; Kalit, Samir

Source / Izvornik: **58. hrvatski i 18. međunarodni simpozij agronoma : zbornik radova, 2023, 363 - 367**

Conference paper / Rad u zborniku

Publication status / Verzija rada: **Published version / Objavljena verzija rada (izdavačev PDF)**

Permanent link / Trajna poveznica: https://urn.nsk.hr/urn:nbn:hr:204:574779

The potential of using *Saccharomyces boulardii* yeast in fermented milk products

Darija Bendelja Ljoljić, Iva Dolenčić Špehar, Milna Tudor Kalit, Ivana Rajnović, Nataša Hulak, Ivica Kos, Ivan Vnučec, Saša Prđun, Samir Kalit

University of Zagreb, Faculty of Agriculture, Svetošimunska cesta 25, Zagreb, Hrvatska (ispehar@agr.hr)

Abstract

Probiotic fermented milks are a group of functional food that, in addition to their nutritional value, contain certain components considered beneficial to human health. For their production, besides probiotic bacterial strains, yeasts - most commonly *Saccharomyces boulardii (S. boulardii)*, are used too in the form of an secondary culture. The biotherapeutic effect of this yeast on the human body has been most studied. *S. boulardii* is also considered a potential natural preservative due to its antagonistic effect against other microorganisms. Its survivability and its effects on organoleptic properties have been studied in the production of yogurt from cow's and goat's milk, kefir, and acidophilic fermented milk. Based on all these factors, these products can be considered as new functional dairy products.

Keywords: probiotic microorganisms, functional food, fermented milk, antagonistic action, *Saccharomyces boulardii*

Introduction

The modern way of life, in which stress together with unbalanced and irregular eating habits prevail, has a significant impact on people's health. For this reason, the number of diseases related mainly to the digestive system and increased body weight has increased recently. The food industry was the first to recognize the need to solve and prevent these problems by producing foods that, in addition to nutritional value, have functional value as well. The functional value of food refers to its components that are considered to promote health, general well-being or reduce the risk of disease occurrence by acting on one or more functions in the body (Samaržija, 2015a). To this end, the dairy industry often enriches its products with probiotic microbial cultures that have a positive health effect. Although strains of bacterial species from the genera *Lactobacillus, Bifidobacterium, Streptococcus, Pediococcus*, *and Leuconostoc* are most commonly used as probiotic cultures, recently more and more attention has been paid to the study and application of yeasts with probiotic potential. *Saccharomyces boulardii* is a unique probiotic and biotherapeutic yeast that has been successfully used as an oral substitute for the treatment of various digestive disorders (Lazo-Vélez et al., 2018). However, the use of *S. boulardii* in the food industry has only recently begun, and relatively few data are available on its potential in preventing digestive disorders and the onset of other diseases, and even fewer on its role and use in the manufacture of dairy products. Therefore, the aim of this article is to consolidate, comparatively and critically, the knowledge on the probiotic properties of *S. boulardii* and its use in the production of fermented milk.

Yeasts as probiotic cultures

Yeasts are a large, heterogeneous group of microorganisms and due to their different biological effects they are of great interest to today's food industry. The most common yeasts used in the food industry are those belonging to the *Ascomycota* group, among which the most scientifically studied species are those of the *Saccharomyces* genus. They are used for various fermentations, for the production of proteins and vitamins, and for biological control of pathogens. They are adaptable to changing of aerobic conditions (Deak, 2006; Hatoum et al., 2012). Yeasts can be non-fermentative, facultative fermentative, or obligate fermentative, usually with aerobic metabolism. In case of anaerobic metabolism, alcoholic fermentation occurs. They are also competitive to nutrients, tolerant to high concentrations of ethanol and a wide range of pH values; from 3 to 8 (Czerucka et al., 2007). They have the ability to acidify the medium in which they grow, they form and secrete mycotoxins and antibacterial components (Viljoen, 2006). All metabolic properties of yeasts are considered to be the basis for their probiotic activity. The antagonistic properties of yeasts are important in biotechnology, medicine, veterinary medicine, and other industries. They also contribute to food safety due to their inhibitory effects on pathogenic microorganisms (Niamah et al., 2017). In addition, yeasts' metabolic activity can influence the sensory quality of fermented foods and their shelf life.

Saccharomyces boulardii

Although bacteria are most commonly used, *Saccharomyces boulardii* is a yeast species that has been extensively studied in numerous clinical trials for its probiotic activity. The yeast *Saccharomyces boulardii* was isolated from the tropical litchi fruit (*Litchi chinensis*) in Indochina in 1923 by the French scientist Henri Boulard. Namely, he observed that the local population treated diarrheal diseases with litchi and mangosteen (*Garcinia mangostana*) (Liu et al., 2016). The probiotic properties of the yeast *S. boulardii* as a separate species or variety of the species *S. cerevisiae* are still the subject of numerous scientific investigations. In the literature, the name *S. boulardii* or *S. cerevisiae* var. *boulardii* is often used equally for this yeast species.

S. boulardii is able to assimilate and ferment carbohydrates and is naturally resistant to antibiotics. The optimal growth temperature of *S. boulardii* is 37°C, which is also the physiological temperature of the human body. It also tolerates low pH values of 2 to 4 and is resistant to bile salts and pancreatic juices. For this reason, *S. boulardii* has great potential for use as a probiotic. Moreover, it has no pathogenic properties and the products of its own metabolism show antipathogenic effects (Czerucka et al., 2007; Yerlikaya, 2014; Liu et al., 2016). Since the 1960s, many experimental and preclinical studies have demonstrated its anti-inflammatory, antimicrobial, enzymatic, metabolic, and antitoxic effects (Czerucka et al., 2007, Yerlikaya, 2014; Liu et al., 2016). *S. boulardii* is the only probiotic yeast whose effect has been scientifically demonstrated in numerous studies, and it is now considered one of the most effective probiotic microorganisms (Liu et al., 2016). There are numerous reliable clinical studies describing its therapeutic effect on various digestive disorders in humans such as diarrhea (Rolfe, 2000; Kotowska et al. 2005; Hatoum et al., 2012) and other disorders of the gastrointestinal system (Guslandi et al., 2003; Garcia et al., 2008; Kelesidis and Pothoulakis, 2011).

Application of S. boulardii in the production of fermented milk

The most common form of probiotic availability in the human diet are dairy products, especially fermented milk, and encapsulated probiotic bacterial strains available in dietary supplements. Compared to the rest of the food industry, the dairy industry is the fastest to bring new functional foods to market. Probiotic bacterial strains, which are used in the form of cultures in the production of fermented milk, are most commonly used for this purpose. Yeasts are rarely used as part of the starter culture compared to lactic acid bacteria. The main reason *S. boulardii* is part of the secondary microbial culture is that it cannot ferment lactose. Indeed, yeasts can only develop in a dairy product if lactic acid bacteria break down lactose into glucose and galactose or if sugar is intentionally added to the product (Parrella et al., 2012).

Among other things, it is known that the yeast *S. boulardii* has the ability to be highly proteolytic due to the production of several different peptidases that can also degrade milk proteins (Niamah, 2017). In addition, this yeast species has the ability to produce high concentrations of vitamin B complex. Based on these capabilities, it is believed that the use of *S. boulardii* yeast in the dairy industry may serve to enhance the growth of starter cultures bacteria in milk (Niamah, 2017). However, many very complex studies are conducted on its use as a probiotic culture (Yerlikaya, 2014). As a probiotic yeast, *S. boulardii* has been tested in the production of yogurt, kefir and acidophilic fermented milk, as well as UHT milk and ice cream.

Yogurt

Yogurt is recognized worldwide as a healthy food with beneficial health effects and, along with other types of fermented milk, is the most popular carrier of probiotics in foods (Oliveira et al., 2009; Allgeyer et al., 2010; Cruz et al., 2010a; Mortazavian et al., 2010; Marafon et al., 2011; Oliveira et al., 2011). The global yogurt market is constantly growing, as many consumers associate it with a positive effect on their own health. Yogurts are often fortified with probiotic bacteria, so they could be considered functional foods. However, some studies show that most probiotic products do not retain their probiotic properties until the end of their shelf life (Cruz et al., 2010b). Therefore, an alternative solution was found in the form of probiotic yeasts such as *Saccharomyces boulardii* as follows.

Lourens-Hattingh and Viljoen (2001), for example, used the probiotic strain *S. boulardii* ATCC 74012 to produce yogurt (standard, UHT, and with fruit) in addition to yogurt culture. During 29 days of storage, an unchanged number of yeasts (7.6 log10 cfu/ml) was detected in standard and UHT yogurt, while in fruit yogurt the initial yeast population increased to 8.1 log10 cfu/ml. The authors attributed the increase in the number of *S. boulardii* yeasts in fruit yoghurt to the greater availability of fermentable sugars, as well as sucrose and fructose derivatives present in the fruit supplement. Notwithstanding the fact that *S. boulardii* does not metabolize lactose, it is able to utilize available organic acids, galactose and glucose derived from bacterial metabolism of lactose by yogurt culture composition in milk. The above study confirmed the survivability of the probiotic strain *S. boulardii* ATCC 74012 in yogurt during 29 days of storage in a number greater than 10^6 cfu/ml, which confirmed the potential of using the above yeast in the production of functional yogurt. A similar study was conducted for standard yogurt where milk was inoculated with the preparation of yogurt culture and *S. boulardii* in concentrations of 1%, 2% and 3%, respectively (experimental group), and by the use of the yogurt culture solely (control group). The rate of milk acidification was lower in the experimental groups of yogurts than in the control group, and the experimental yogurts had higher proteolytic activity due to the production of biopeptides than the yogurts of the control group (Niamah, 2017). Also, the addition of *S. boulardii* in yogurt production has a significant effect on the water retention capacity of the final product. Thus, the water retention capacity of yogurt with the addition of 3% of the S. boulardii preparation was 50% and that of the control yogurt was 40%. In addition, the positive effect of *S. boulardii* yeast on the rheological properties of yogurt was also noted. The determined population of live yeasts of more than 10^6 CFU/g in the yogurt during 21 days of storage met the criteria for its inclusion in the group of probiotic yogurts (Niamah, 2017).

Compared to yogurt made from cow's milk, in which the *S. boulardii* count of 10^7 cfu/g remains unchanged for up to 29 days of storage, the suitability of goat's milk for the production of probiotic yogurt with *S. boulardii* yeast is significantly lower (Karaolis et al., 2013). However, in the above study, the incubation temperature was higher than the optimal temperature for *S. boulardii*, which could be the reason for the weaker growth. However, *S. boulardii* was found to improve the survival of bacteria of standard yogurt culture for 28 days in yogurt prepared from goat milk. In addition, a much milder "goat" taste and odor were noted in yogurt samples with the addition of yeast. During storage, the sensory characteristics of the goat yogurts, especially those with added yeast, also changed as the taste of ethanol was strongly perceived. Although ethanol is the expected metabolite of yeast, its excessive concentration proved to be off-putting to consumers.

Recent research shows that *S. boulardii* in encapsulated form has a better survival rate during the shelf life of yogurt, as well as resistance to gastric pH, bile and salts, and certain digestive enzymes, and the ability to survive in the colon (Rodriguez et al., 2017).

Kefir

Kefir is a traditional fermented milk produced in Eastern Europe. It is considered a natural probiotic because the benefits it brings to human health have been known since ancient times. Like other probiotic fermented milks, kefir contains live microorganisms capable of competing with pathogenic microorganisms and participating in the establishment of the balance of the gut's desirable microbiota. Since kefir cultures do not normally contain *S. boulardii* yeast, a study of their effect and functionality under kefir production conditions was conducted by Ivanova et al., 2012. In the aforementioned study, 7 batches of kefir were produced, and in 6 of them, *S. boulardii* yeast (0.08 mg) was added in addition to the kefir culture (6 g kefir grains). Overall, the results showed that experimental kefir with the addition of *S. boulardii* did not differ from classically prepared kefir. In addition, an improvement in taste was observed, leading the authors to conclude that there is nothing stands in the way of the industrial use of such cultures in the production of kefir (Ivanova et al., 2012).

Acidophilic fermented milk

In general, acidophilic-fermented milk is fermented milk that, in addition to *Lactobacillus acidophilus* bacteria, also contains lactose-fermenting yeasts that have a strong antibacterial effect against some mycobacteria (Samaržija, 2015b). There is very little literature data on the use of *S. boulardii* yeast in the production of fermented milk, especially acidophilic-fermented milk. Kalpana (2008) experimentally prepared acidophilic-fermented milk from sterilized skim milk to which 2% *S. boulardii* culture (5 different isolates) was added before incubation. At the end of incubation, *L. acidophilus* (NDC 15) was added at a concentration of 1% and the sample was incubated for additional 12 hours. It was found that *S. boulardii* utilized the lactic acid well for its growth and multiplication, maintaining its population above 10^6 cfu/ml. Moreover, a synergistic behavior was observed between the yeasts and the *L. acidophilus* culture, whose growth was enhanced, while no negative sensory changes were observed.

Conclusion

Saccharomyces boulardii yeast is a unique probiotic and biotherapeutic yeast that has been successfully used as an oral substitute to treat various digestive disorders in humans. There is relatively little research on the potential of *S. boulardii* yeast in the production of fermented milk. However, previous scientific research suggests that *S. boulardii* yeast has a positive effect on the sensory characteristics and growth of bacterial species commonly used in the production of fermented milk. Moreover, *S. boulardii* has a good survivability of more than 10^6 cfu/ml in yogurt and kefir made from cow's milk, which is sufficient to confirm its probiotic effect. Considering the potential of this yeast species, it would certainly be useful to carry out further research, focusing on finding strains that have a higher survival rate in fermented dairy products, produce less ethanol, with less proteolitic activity, and contribute to the creation of desirable sensory characteristics.

Literature

Allgeyer L.C., Miller M.J., Lee S.Y. (2010). Sensory and microbiological quality of yogurt drinks with prebiotics and probiotics. Journal of Dairy Science. 93: 4471-4479.

Cruz A.G., Faria J.A.F., Walter E.H.M., Andrade R.R., Cavalcanti R.N., Oliveira C.A.F., Granato D. (2010a). Optimization of the processing of probiotic yoghurt added with glucose oxidase using the response surface methodology. Journal of Dairy Science. 93: 1058-1069.

Cruz A.G., Walter E.H.M., Cadena R.S., Faria J.A.F., Bolini H.M.A., Pinheiro H.P., Sant'Ana A.S. (2010b). Survival analysis methodology to predict the shelf-life of probiotic flavored yogurt. Food Research International. 43: 1444-1448.

Czerucka D., Piche T., Rampal P. (2007). Review article: yeast as probiotics- *Saccharomyces boulardii*. Alimentary Pharmacology and Therapeutics. 26: 767-778.

Deak T. (2006). „Enviromental factors influencing yeasts", Biodeversity and Ecophysiology of Yeasts, In: The Yeast Handbook, Péter, G., Rosa, C. (eds). 155-174. Berlin, Njemačka, Springer.

Garcia V.E., De Lourdes De Abreu F.M., Oswaldo Da Gama T.H., Guerra P.A., Carolina Carneiro A.A., Paiva M.F., Marcos Andrade Goulart E., Sales Da Cunha A. (2008). Influence of *Saccharomyces boulardii* on the intestinal permeability of patients with Chron's disease in remission. Scandinavian Journal of Gastroenterology. 43: 842-848.

Guslandi M., Giollo P., Testoni P.A. (2003). A pilot trial of *Saccharomyces boulardii* in ulcerative colitis. European Journal of Gastroenterology and Hepatology. 15: 697-698.

Hatoum R., Labrie S., Fliss I. (2012). Antimicrobial and probiotic properties of yeasts: from fundamental to novel applications. Frontiers in Microbiology. 3: 421.

Ivanova G., Momchilova M., Rumyan N., Atanasova A., Georgieva N. (2012). Effect of *Saccharomyces boulardii* yeasts addition on the taste and aromatic propeties of Kefir. Journal of the University of Chemical Technology and Metallurgy. 47: 59-62.

Kalpana D. (2008). Biotherapeutic properties of probiotic *Saccharomyces* species and its survival in acidophilus milk. Karnal, Haryana, India: National Dairy Research Institute, doktorska disertacija.

Karaolis C., Botsaris G., Pantelides I., Tsaltas D. (2013). Potential application of Saccharomyces boulardii as a probiotic in goat's yoghurt: survival and organoleptic effects. International Journal of Food Science and technology. 48: 1445-1452.

Kelesidis T., Pothoulakis C. (2011). Efficacy and saftey of the probiotic *Saccharomyces boulardii* for the prevention and therapy of gastrointestinal disorders. Therapeutic Advances in Gastroneterology. 5: 111-125.

Kotowska M., Albrecht P., Szajewska H. (2005). *Saccharomyces boulardii* in the prevention of antibiotic-associated diarrhea in children: randomized double-blind placebo controlled trial. Alimentary Pharmacology and Therapeutics. 21: 583-590.

Lazo-Velez M.A., Serna-Saldıvar S.O., Rosales-Medina M.F., Tinoco-Alvear M., Briones-Garcıa M. (2018). Application of *Saccharomyces cerevisiae* var. *boulardii* in food processing: a review. Journal of Applied Microbiology. 125: 943-951.

Liu J.J., Kong I.I., Zhang G.C., Jayakody L.N., Kim H., Xia P.F., Kwak S., Sung B.H., Sohn, J.H., Walukiewicz H.E., Rao C.V., Jin Y.S. (2016). Metabolic Engineering of Probiotic *Saccharomyces boulardii*. Applied and Enviromental Microbiology. 82: 2280-2287.

Lourens-Hattingh A.L., Viljoen B.C. (2001). Growth and survival of a probiotic yeast in dairy products. Food Research International. 34: 791-796.

Marafon A.P., Surimi A., Alcântara M.R., Tamime A.Y., Oliveira M.N. (2011). Optimization of the rheological properties of probiotic yoghurts supplemented with milk proteins. LWT - Food Science and Technology. 44: 511-519.

Mortazavian A.M., Khosrokhavar R., Rastegar H., Mortazaei G.R. (2010). Effect of dry matter standardization order on biochemical and microbiological characteristics of freshly made probiotic doogh (Iranian fermented milk drink). Italian Journal of Food Science. 1: 98-104.

Niamah A.K. (2017). Physicochemical and microbial characteristics of yogurt with added *Saccharomyces boulardii*. Current research in Nutrition and Food Science Journal. 5: 300-307.

Oliveira R.P.S., Florence A., Silva R.C., Converti A., Oliveira M.N., Gioielli L.A. (2009). Effect of different prebiotics on the fermentation kinetics, probiotic survival and fatty acids profiles in nonfat symbiotic fermented milk. International Journal of Food Microbiology. 128: 467-472.

Oliveira R.P., Patricia P., Oliveira M.N., Converti A. (2011). Effect of inulin as a prebiotic to improve growth and counts of a probiotic cocktail in fermented skim milk. LWT - Food Science and Technology. 44: 520-523.

Parrella A., Caterino E., Cangiano M., Criscuolo E., Russo C., Lavorgna M., Isidori M. (2012). Antioxidant properties of different milk fermented with lactic acid bacteria and yeast. International Journal of Food Science and Technology. 47: 2493-2502.

Rodrigues F., Ludovico P., Leão C. (2006). Sugar metabolism in yeasts: an overview of aerobic and anaerobic glucose catabolism, In: The Yeast Handbook, Péter, G., Rosa, C. (eds). 101-121, Berlin, Njemačka, Springer, Berlin.

Rolfe R.D. (2000). The role of probiotic cultures in the control of gastrointestinal health. The Journal of Nutrition. 130: 396-402S.

Samaržija D. (2015a). Značenje fermentiranih mlijeka u proizvodnji i prehrani. In: Fermentirana mlijeka. 2-25, Zagreb, Hrvatska, Hrvatska mljekarska udruga.

Samaržija D. (2015b). Probiotička, prebiotička i simbiotička fermentirana mlijeka. In: Fermentirana mlijeka. 297-328. Zagreb, Hrvatska, Hrvatska mljekarska udruga.

Viljoen B. (2006). Yeast ecological interactions. Yeast'yeast, yeast'bacteria, yeast'fungi and yeasts as biocontrol agents, In: Yeasts in Food and Beverages, Querol, A., Fleet, G. (eds.), 83-110, Berlin, Njemačka, Springer.

Yerlikaya O. (2014). Starter cultures used in probiotic dairy product preparation and popular probiotic dairy drinks. Food Science and Technology (Campinas). 34: 221-229.