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II.11. UP-TO-DATE KNOWLEDGE ON YEASTS FOR FOOD INDUSTRY

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Abstract

Yeasts have been used for food processing since ancient times due to their special fermentative characteristics. In present, they represent a major group of microorganisms used in food industry as well in related domains of biotechnology. Yeasts are a rich source of proteins, vitamins (especially those from B complex) and are main producers of biocatalysts (lipases, esterases, phospholipases) used to improve aromas and flavours and of different natural additives such as food colorants. Yeasts isolated from traditional dairy products produce inulinases and β- galactosidases important for production of foods intended for weight loss or for people with lactose intolerance. Inulinases exhibit also prebiotic potential, catalysing the growth of probiotic microorganisms. Certain yeast species are used as probiotics since they not participate in horizontal antibiotic resistance genes exchanges. Recently, the yeasts with antioxidant activity are considered as attractive for development of biopreservatives, an economic alternative for classical food preservatives of chemical origin. With a great history developed in the food field, the yeasts continue to surprise the scientific world even in the 21st century through their special metabolic abilities.

Key words*: yeasts, natural food additives, prebiotics/ probiotics, biocatalysts, biopreservatives.*

INTRODUCTION

Yeasts have a rich history in food industry but their potential exceeds their use in baking, beer and wine production. Although they play an important role in obtaining fermented foods and beverages, during past decades, yeasts proved to have huge potential in functional foods development. Functional food is a generic term referring to different products which, in addition to the nutritional value of the ingredients from which they are prepared, offers also great support for maintaining or improving human health. Yeasts are an interesting research model, some of the species are considered GRAS (Generally Regarded as Safe), they do not participate in horizontal antibiotic genes exchanges and they have special metabolic properties that make them suitable for a variety of industrial processes.

Food industry is a constantly changing field. Developing products with improved quality or properties is a hard challenge. Due to their metabolic versatility, yeasts represent a potential solution for many of these challenges. For example, various yeast species have been used as probiotics, while different yeasts cell components proved to be useful for nutraceuticals development. Also, yeasts are able to secret different bioactive metabolites, such as antioxidants, vitamins or enzymes that are both ecological and highly valuable from nutritional point of view (Rai et al., 2018). Also, yeasts showed great potential for producing new flavours and aroma food ingredients. Using yeasts as bioflavouring agents represent a step forward meant to replace the synthetic compounds used in present (Willaert et al., 2005).

The aim of this review is to highlight some important discoveries regarding the biotechnological potential of yeasts in food industry.

YEASTS AS NATURAL ANTIOXIDANTS PRODUCERS

Recently, the interest for developing natural antioxidants has increased significantly since their synthetic alternatives proved to be sometimes harmful for human health. Synthetic antioxidants that are still frequently used as food additives, such as butylated hydroxyanisole (BHA), butylated hydroxytoluen (BHT) and tertbutylhydroquinone (TBHO), have been an intensely debated topic, since their use in high

concentrations can cause cancer in some animal species (Shahidi & Zhong, 2010). In food industry, antioxidants are used as food preservatives that protect fat-based foods (meat, dairy products) against oxidative rancidity (Shahidi, 2015). Oxidation may occur during different stages of food processing and determine the development of different off-flavours, loss of essential nutrient such as fatty acids and fat-soluble vitamins, and last, but not least, the appearance of toxic compounds (Shahidi &. Zhong, 2005). Based on their mode of action antioxidants are grouped into 2 main classes: (1) primary antioxidants or chain-breaking antioxidants, that donate protons or electrons to a free radical thus transforming them to their more stable form and (2) secondary antioxidants or hydroperoxide decomposers, that convert hydroperoxides into nonradical/nonreactive products (Hermund, 2018).

Many natural antioxidants have already been identified and introduced into the food industry among which: tocopherols, ascorbic and erythrobic acids and their salts, different plants extracts and short chain peptides. Natural antioxidants of microbial origin are of great economic importance since the microorganisms require rather simple and economic growth substrates and are able to produce large quantities of products in a short period of time. Yeasts are already known as a great source of antioxidants, being able to synthesize citric acid, carotenoids (torulene and torularhodine), glutathione, ubiquinone, riboflavin and hydroxymethyl/hydroxyethyl furanone (Abass, 2006).

Glutathione (GSH) is a non-proteic thiol compound with great potential both in biomedical field (being used for pancreatic inflammations and liver cirrhosis treatment) and food industry. Glutathione has great importance in oenology being involved in the control of oxidative spoilage of wine. White wine, in particular, is very sensitive to oxygen exposure which determines the development of atypical aging aroma and colour changing (Kritzinger et al., 2012; De Vero et al., 2017). In present, glutathione is produced via two main methods. The first method is based on using glutathione-generating enzymes and the amino acids precursors in a highly controlled environment with ATP consumption (Forman et al., 2009). This method is expensive since using ATP increases the production cost. The second method is a direct fermentative method based on using different microorganisms with natural ability to accumulate glutathione in their cells (Tahmasebi et al., 2016). *Saccharomyces cerevisiae* and *Candida utilis* are recognized as having high ability to produce large amounts of glutathione in low-cost growth media with sugar cane molasses and glycerol as carbon sources (Anschau et al., 2013; Rollini et al., 2010). The biosynthesis of glutathione in yeasts dependents on sulphate and nitrogen uptake. Sulphate intake is mediated through specific membrane permeases (Sul1p and Sul2p). After internalisation, the sulphate enters the metabolic pathway for homocysteine synthesis followed by interconversion of homocysteine to cysteine. Cytoplasmic biosynthesis of glutathione is catalysed by γ-glutamylcysteine synthetase that forms γ-glutamylcysteine as intermediate and GSH synthetase which adds glycine- the third amino-acid from glutathione chemical structure (Suzuki et al., 2011).

Apart from cytoplasmic biosynthesis of glutathione, yeasts are able to assimilate this compound by direct internalisation via Opt1p/Hgt1p transporter. Different studies showed that nitrogen deprivation decreases the amount of intracellular glutathione because it induces the gene expression of an enzyme (γ-glutamyltranspeptidase) that hydrolyses glutathione into L-glutamate and cysteinylglycine (De Vero et al., 2017). Although many yeasts were characterised as being able to produce large amounts of glutathione, winemakers are using different strategies in order to enhance glutathione production by yeasts. Random mutagenesis is one of the main methods used and is based on exposing yeast cells to chemical (ethyl methanesulphonate - EMS, nitrous acid, intercalating agents) or physical mutagens (UV radiation, γ-raddiation) (Li et al., 2004). Another technique frequently used is sexual hybridization, the most efficient way to increase yeast diversity and to improve industrially relevant traits such as flavour profile, stress tolerance and fermentative performance

(Steensels et al., 2014). The most advanced method is evolutionary engineering, in which the yeast strains are exposed to different mutagens, then exposed to specific selective pressure followed by the final selection of best glutathione producing strains (Perez-Torrado et al., 2015).

Ubiquinone (coenzyme Q) is a redox active lipid involved in the electron transfer system. This compound is synthesized *via* a complex metabolic pathway that uses chorismate and polyprenyl diphosphate as precursors. Ubiquinone is highly studied due to its high biomedical potential. This compound proved to be of particular importance in the prevention of cardiovascular disease, neurodegenerative and mitochondrial conditions, diabetes and periodontal disease. Since 1999 when functional foods term was briefly defined in European Union as food products fortified with different active compounds that can improve health and well-being, ubiquinone has gained notoriety among researchers. Ubiquinone can be produced by chemical synthesis, obtained from plants/ animals by tissue extraction or by microbial fermentation. Since chemical synthesis involves using solvents or chemicals during the process, microbial fermentation becomes more attractive both from economic and ecological point of view (Berekova et al., 2008). Different yeast species belonging to *Candida*, *Sporidiobolus* and *Rhodotorula* genera proved to be highly valuable for ubiquinone production. Moreover, this alternative implies low production costs which rends it very attractive for industrial production (Tokdar et al., 2014; Dixson et al., 2011).

Citric acid is the most common organic acid commercialised in large quantities around the world since 1930. In 2007 global production of citric acid was estimated around 1.7 million tons with an annual increase of almost 4% (Tong et al., 2019). This compound is widely used in food industry as an acidifier/antioxidant to preserve and to improve the flavour of different foods and beverages (fruit juices, ice cream, marmelades etc.). Also, it is frequently used for detergents (as phosphate substitute), pharmaceuticals (for vitamins preservation, as pH corrector, blood preservative) and cosmetic products (Soccol et al., 2006). More than 90% of the required citric acid is obtained from microbial surfaces or submerged cultures. Many microorganisms are already known as being able to produce citric acid among which yeasts occupy a special position. *Candida tropicalis*, *Candida oleophila*, *Pichia guilliermondii*, *Candida citroformans*, *Pichia anomala* and *Yarrowia lipolytica* are known as being able to synthetize citric acid when cultivated under special conditions. Since the citric acid is part of energy metabolism, some of the microbial strains are not suitable for industrial purposes. Therefore, there is a growing interest for improvement of citric acid producing strains by mutagenesis of characterised strains or by isolation and selection of new yeasts with natural producing potential (Ridrigues et al., 2006). Many studies reported high citric acid production using as substrate different raw materials such as: starch, molasses, coffee husk, wheat bran, pineapple waste, citrus waste etc. (Max et al., 2010).

Carotenoids are a group of compounds ubiquitous in nature with yellow, orange or red colour. In general, carotenoids have a polyene backbone form by conjugated C=C bonds that is involved in pigmentation and also in their antioxidant activity assuring the interaction of these compounds with free radicals (Young and Lowe, 2018). Carotenoids are a great source of vitamin A and are recognized as being able to reinforce immune system. Also, these compounds proved to be very efficient for the treatment of eye diseases such as cataract and macular degeneration and for skin protection against ultraviolet radiation (Stahl & Sies, 2007). Since the human body cannot produce carotenoids, it is recommended their use as food additives. Apart from lutein, astaxanthin, zeaxanthin which are already used at industrial scale as natural antioxidants, more natural carotenoids were described lately. Among them, torulene and torularhodin have gained a growing attention. These two compounds are a group of carotenoids mainly produced by yeasts including *Rhodotorula*, *Rhodosporidium* and *Sporobolomyces* species and by filamentous fungi. Torulene and torularhodine present a β-ionone ring connected to a polyene chain (Herz et al., 2007) and their colour varies directly in proportion to their concentration from pale pink to red. The carotenoids act mainly in microbial

cell protection against the reactive form of oxygen and radiation. Different studies proved that torularhodin is even more efficient than β-carotene and α-tocopherol in terms of antioxidant activity (Sakaki et al., 2002; Sakaki et al., 2001). As a consequence, they have huge biotechnological potential, a special attention being granted to the genetic background involved. Therefore, a number of genes were characterised as being involved in carotenoid synthesis in yeasts, coding different enzymes such as: phytoene synthase, phytoene dehydrogenase, lycopene cyclase, pytoene desaturase etc. Many of these genes were overexpressed in order to enhance carotenoids production in yeast cells (Wang et al., 2008). Torulene and torularhodine biosynthesis is strongly influenced by cultivation conditions, the carbon and nitrogen source being extremely important. Some of the most frequently used substrates for enhancing carotenoids production in yeasts are the grape must as carbon source (Buzzini and Martini, 2007) and ammonium sulphate as nitrogen source (El Banna et al., 2012) in adequate aeration conditions (Simova et al., 2003).

Hydroxymethyl/hydroxyethyl furanones are chemical compounds that present a five-membered heteroaromatic ring containing an oxygen atom. The members of these group are highly valuable for the biomedical field as it has been shown that they can be used as analgesics, antimicrobials, anti-inflammatories, etc. (Husain et al., 2019). Probably the best known furanones is vitamin C (5-(1,2-dihydroxethyl)-3,4-dihydroxy-2(5*H)*-furanone) but there are also other naturally occurring furanones with great biotechnological potential, such as EMHF (4-hydroxy-2 (or 5)-ethyl-5 (or 2)-methyl-3(*2H*)-furanone), a furanone derivate produced by different yeast species. This compound was first identified in soy sauce and miso and it was proven to be responsible for the characteristic flavour. First, the Maillard reactions were thought to be responsible for the particular flavour, but lately it was shown that EMHF occurrence in soy sauce was due to fermentative action of different yeasts such as: *Zygosaccharomyces rouxii*, *S. cerevisiae* and *Y. lipolytica* (Slaughter, 1999). The EMHF was also found in roasted coffee, melons and beer (Uehara et al., 2015). The 3(*2H*)furanones exhibit both anti-oxidative and pro-oxidative activity depending on the availability of oxygen species from the environment (Schwab et al., 2013).

Riboflavin, also known as vitamin B2, is a water-soluble vitamin with great importance for human health. Riboflavin is a precursor of flavin mononucleotide (FMN) and flavin dinucleotide (FAD) coenzymes that act as electron acceptors for various oxidoreductases. Humans are not able to synthetize riboflavin being forced obtain it from their diet. Nevertheless, riboflavin occurs naturally in liver or egg yolk but it is added in many other types of foods such as breakfast cereals or bread. Also, riboflavin is used as food colorant due to its yellowish colour. Chemical production of riboflavin proved to be very expensive since lot of waste is produced and also it requires many organic solvents. Microbial fermentation is much cheaper and has better yield (Kato & Park, 2012). Many yeasts including *Candida famata, P. guilliermondii*, *Candida membraniefaciens* subsp. *flavinogenie*, *Debaryomyces hansenii, Schwanniomyces occidentalis* are already used for industrial production of riboflavin through microbial fermentation (Wang et al., 2008). It seems that overproduction of riboflavin in yeasts is a metabolic response to iron deficiency and is induced by the presence of cobalt ions (Boretsky et al., 2007).

Apart from riboflavin, yeasts are known as an important source of other vitamins such as thiamine, nicotinic acid, pyridoxine, pantothenic acid, cyancobalamin, biotin and folic acid. *Kloeckera apiculata*, *S. cerevisiae* and *Saccharomyces uvarum* accumulate or release during ethanol fermentation very large quantities of thiamine (Abbas, 2005). Ergosterol, a precursor of vitamin D, is an important constituent of cell membrane lipids, many studies reporting *Candida tropicalis* as an important ergosterol producing yeast species (Liu et al., 2019; Abbas, 2005). Also, *Candida guilliermondii*, *C. utilis* and *Saccharomyces fragilis* grown on media containing lard or waste fats as carbon sources and ammonium salts or uree as nitrogen sources, produced high amount of nicotinic acid, pantothenic acid, riboflavin, pyridoxine and cobalamine (Abbas, 2005).

AROMA FORMATION IN YEASTS

The importance of yeasts in obtaining fermented foods (mainly alcoholic and non-alcoholic beverages such as wine, spirits beverages, bear) is also due to the fact that they produce many flavours and aromas. Among them, most known compounds synthetized by yeasts are fusel alcohols, fatty acids and their derived esters.

Fusel alcohols are a group of aroma compounds characteristic mainly to alcoholic beverages such as rums, brandies and whiskeys. Isoamyl alcohol and isoamyl acetate are the main fusel alcohols produced by yeasts (Abe & Horikoshi, 2005). *Torulaspora delbrueckii*, *Pichia fermentas* and *Kluyveromyces maxianus* are known as yeast able to produce satisfactory quantities of fusel alcohols or derived esters (Hernández-Carbajal et al., 2013).

Fatty acids and their esters represent another major group of aroma compounds synthetized by yeasts. Short chain volatile fatty acids (propionic acid, butyric and isobutyric acid, caproic acid, capric acid, caprylic acid, etc.) are probably the best known fatty acids highly valuable for food industry. The presence of the fatty acids with 6 to 10 carbon atoms in alcoholic beverages gives the final product musty and rancid specific aromas. Many yeast species are characterised as aroma producers. *S. cerevisiae* is used for ethyl caproate synthesis in culture conditions characterised by small concentration of inositol. This compound is important for Scotch whisky production conferring its specific aroma (Chen et al., 2014).

Carbonyl, sulphur and phenolic compounds. The carbonyl compounds of great interest for food industry are the aldehydes, such as diacetyl and 2,3-pentanedione that offers beer a specific buttery flavour. These compounds have an undesirable effect on beverages quality since its specific aroma is not well tolerated by individuals (Zhang et al., 2005). Sulphur compounds (hydrogen sulphide, diethyl sulphide), derived mainly from sulphur containing amino-acids (cysteine and methionine), have also undesirable effect on beverage due to their offensive smell. Despite that, these compounds acts as antioxidants preventing thus oxidation that can definitely affect the final product. In this group, the phenolic compounds occupy a central position. Compounds such as 4-ethylphenol,4-ethylguaiacol, 4-methylguaiacol, in small concentration are desirable. Commonly known yeast involved in aroma development based on carbonyl, sulphur and phenolic compounds are *S. cerevisiae*, *Dekkera bruxellensis* and *Dekkera anomala* (Dzialo et al., 2017).

The **lactones** are also an important group among the aromas produced by yeasts. They confer specific aroma of peach, apricot or coconut to various types of foods. Chemically, lactones have a carbon ring with an oxygen atom and are produced by many yeasts especially *Sporobolomyces odorus, Y. lipolytica, Sporidiobolus ruinenii* (Abbas, 2005).

THE YEASTS - SOURCE OF ENZYMES

Yeasts are able to synthesize various enzymes such as lipases, esterases, amylases and glycosidases more stable than plant or animal enzymes, which act as biocatalysists improving food flavour, appearance or processing. For example, yeast proteases, lipases, β-glucosidase and invertase have high impact on the organoleptic characteristics of the bread and, also, influence the dough structure, the crust colour, crumb texture and firmness of the bread. The ***α-amylases*** (1,4-α-D-glucan-glucanohydrolases, E.C. 3.2.1.1) are used in the backing industry not only as flavour enhancers, but also for starch conversion in dextrins, maltose and glucose, representing the carbon substrates for yeast metabolism. The main yeast species producing α-amylases belong to *Schwanniomyces* - *S.* (*Debaryomyces*) *occidentalis*, *S. aluvius*, *Cryptococcus* (*C. flavus*), *Saccharomycopsis* (*S. fibuligera*) and *Candida* - *C. utilis*, *C.* (*Pichia*) *guilliermondii*, *C. famata* (*Debaryomyces hansenii*), *C. antarctica*. The characteristics of yeast α-amylases depend on the yeast species, presenting variable molecular weight (38-75 kDa) and optimal activity at temperatures from 30 to 70°C and pH values from 4 to 6 (Djekrif et al., 2016).

Glycosidases have important role in wine industry, hydrolyzing the sugar-conjugated precursors existent in the grapes, releasing thus the terpens (aglycons) responsible for the

flavour and odor of the wine. Yeast species belonging to *Candida*, *Kluyveromyces*, *Debaryomyces*, *Hanseniaspora*, *Hansenula (Wickerhamomyces)*, *Pichia*, *Metschnikowia*, *Rhodotorula* and *Trichosporon* genera synthesize β-glucosidases (EC 3.3.1.21). These enzymes cleave the non-reducing terminal β-D-glucosyl residues from cellulose and remove the β-D-glucose. For some yeast species, the genes coding the β-glucosidases are regulated by the substrate or the growth conditions. For example, the gene *WaExg2* from *Wickerhamomyces anomalus* is active at low pH (3.5-4.0), high sugar (20% w/v) and ethanol (10-15% v/v) concentrations and presence of sulphites or cations, and inhibited by glucose. In *D. hansenii* the highest activity was observed under aerobic conditions, at pH 4.0-5.0, during the exponential growth phase, the enzyme production being inhibited by high glucose concentrations (Maicas and Mateo, 2015; Claus & Mojsov, 2018). Some species from *Candida*, *Kluyveromyces*, *Debaryomyces* and *Pichia* genera synthesize extracellular glucose-tolerant β-glucosidases, but only a third of them showed high glucose tolerance (Rosi et al., 1994). Yeasts β-glucosidases are also of great interest in table olive processing contributing to oleuropein hydrolysis which allows removing the natural bitterness without using large amounts of water (Anagnostopoulos et al., 2017). On the other hand, *Candida*, *Hanseniaspora* and *Pichia* cells have been used as hosts for cloning β-glucosidase genes from *Aspergillus oryzae* for obtaining terpenols from monoterpenyl glycosides from wort and must (Verstrepen et al., 2006).

Xilanases are a group of enzymes extensively used in food industry due to their ability of cleaving the xylan, a major component of hemicellulose. In backing, the xylanase break down the hemicellulose from the wheat flour and increase the binding of water in the dough, improving thus the bread quality and volume. In beverages, the xylanases hydrolyze the cell wall of barley in beer production and also assure a better quality improving the organoleptic properties of the juices (Raveendran et al., 2018). Yeast β-D-xylosidase (EC 3.2.1.37) is important in wine making. Few yeast species, such as *Pichia anomala*, present extracellular, cell-wall-bound and intracellular β-D-xylosidase acivity, while other yeast species present only one form of activity, *i.e*. *Hanseniaspora uvarum* - cell-wall-bound. The thermostability, pH variations and resistance to stress conditions (glucose and ethanol concentrations) of yeast β-D-xylosidases, represent an important asset for their application in enhancing wine aroma and flavour (Romano et al., 2006; Burlacu et al., 2016).

Lipases (triacylglycerol acylhydrolyses) catalyze the hydrolysis of long-chain triacylglycerides and have wide applications in food industry for processing of meat and dairy products or in baked foods. For example, *Y. lipolytica* synthesize intracellular and extracellular lipases, assuring approximately 60% of the flavours associated with ripening in meat products based on pork fat. The enzymes are able to reduce mainly the content of free fatty acids after a short period of incubation, lowering thus the probability of *rancid* odor and consistency in the products (Romano et al., 2006). The expression of several genes coding for lipases in *Y. lipolytica* seems to be substrate-driven and is induced by the presence of the oleic acid (*LIP2*) or glucose (*LIP11* and *LIP13*). However, lipases were synthesized also in the presence of olive oil, oleic acid, sunflower oil, tributyrin and Tween 80 (Csutak & Sarbu, 2018).

Candida rugosa lipases (CRL) have GRAS status and are used in food industry. The coding genes and lipase structure are well studied, many of the lipases being synthesized as isoforms with different thermal stabilities and substrate specificities. In order to increase the thermostability and activity and to improve the industrial processes, various technologies of enzyme immobilization in celite, nylon fiber, polymethyl methacrylate, glass beads, silicage a.s.o, were developed: covalent methods, the use of photo-cross-linkable resins or adsorption. Free or immobilized *C. rugosa* lipases are successfully used in production of ice-cream, of fermented foods used for storage and as food supplies, or for obtaining specific fragrances in dairy products (Benjamin & Pandey, 1998).

Candida (*Pseudozyma*) *antarctica* synthesize cold-active lipases A and B (CALA and CALB) with optimal activity at 20ºC. The lipase CALB

is used in Novozym®435 (used for the production of human milk fat substitute) where it promotes the replacement of the palmitic acid from tripalmitin with unsaturated free fatty acids allowing thus to obtain of triacylglycerols with similar structure from human milk (Szczęsna-Antczak et al., 2013; Guerrand, 2017).

Another yeast species, *Pichia pastoris* is recognized as QPS (Qualified Presumption of Safety) by the European Food Safety Authority and can be used for production of enzymes for food industry. *P. pastoris* cells are used as hosts for production of 34.6% of total recombinant lipases mentioned in research studies, due to the presence of the powerful promotor *AOX1*/*MOX1* and to a reduced level of hyperglycosylation. These lipases are mainly used in the synthesis of short-chain flavour esters. Also, the CALB lipase from *C. antarctica* was expressed on the surface of *P. pastoris* cells, acting as a biocatalysis for sugar monoester production (Borrelli & Trono, 2015). Other *Pichia* lipases are also commercialized (*P. roqueforti* - Lipase R, Amano; Lipomod™, Biocatalysts; *P. camemberti* - Lipase G, Amano) and used in the dairy industry for hydrolysis of milk-fat triacylglycerols.

PROBIOTIC/PREBIOTIC YEASTS

Due to their presence in many fermented foods, the yeasts are an important part of human daily diet by providing vitamins of the B group. Therefore, the GRAS yeasts represent a growing interest for obtaining probiotics. In order to become a true candidate as a probiotic, a microorganism must present several attributes including: growth at low pH, a specific degree of cell surface hydrophobicity and the ability to tolerate bile (Rai et al., 2019). Yeasts are generally resistant to antibiotics and proved to be able to resist in low pH environments (for the passage through the gastrointestinal tract). Also, there are no reports regarding yeast implication in the transfer of antibiotic resistance genes. Therefore, some yeast species can be used successfully for development of new probiotics (Rima et al., 2012; Fakruddin et al., 2017). *S. cerevisiae* and *Saccharomyces boulardii* are able to produce biotherapeutic agents very efficient in treatment of different types diarrhea, such as antibiotic associated diarrhea (Duman et al., 2005), *Clostridium difficile* associated diarrhea (McFarland et al., 2006) or traveler`s diarrhea (McFarland, 2007). It seems that these species are able to produce polyamines that increase short fatty acids and disaccharide enzymes activity in order to stimulate the well function of intestinal cells (Ali et al., 2012). Also, some probiotic yeast exhibit hypocholesterolemic activity (Saikia et al., 2017) and present the ability to reduce oxidative stress (Romanin et al., 2015) being thus an emerging tool for improvement of human health. Many yeast species are able to produce lipases that hydrolase tributyrin to glycerol and butyric acid, an important phenomenon since the butyric acid is useful for colonocytes health (Glueck et al., 2018). The butyric acid acts as a fuel source for the colonocytes, regulates the water and electrolyte absorption and provide protection against mucosal inflammation and oxidative stress (Canani et al., 2012). Also, some studies reported butyric acid as a valuable anticarcinogen drug being able to limit the evolution of several types of cancers (Kuefer et al., 2004).

Although the mechanism is not fully understood, *Saccharomyces* probiotic species inhibit growth of pathogens belonging to the *Enterobacteriaceae* (*Escherichia coli, Salmonella typhi, Shigella dysenteriae, Salmonella enteritidis* and *C. difficile*) family and increase the population of *Bifidobacteria,* a beneficial microorganism from the gut microbiota (Ali et al., 2012). Also, *S. boulardii* is able to inactivate the bacterial toxins, to stimulate host immune defenses and to enhance nutrient absorption (Fakruddin et al., 2017). Therefore, yeasts can also be used for prebiotics synthesis (Nascimento et al., 2012).

The *Kluyveromyces* yeasts (*K. lactis*, *K. marxianus, K. fragilis*) are seldom isolated from dairy products and are able to assimilate lactose and to degrade inulin. The synthesis of β-galactosidase (lactase) is an important step in the intracellular metabolism of lactose to glucose and galactose which is subsequently degraded through the Leloir pathway (Csutak, 2014). The yeast β-galactosidase are used in the dairy industry, to improve the sweetness of products

and to degrade the whey, major waste from the dairy industry.

The *K. lactis* enzyme shows optimal activity as high pH values (6.0-7.0) and 30-35ºC, making it suitable for milk and sweet whey hydrolysis (Saquib et al., 2017). In present, the *K. lactis* β-galactosidase is produced and commercialized at industrial scale as Maxilact (DSM Food Specialties, The Netherlands) and is used for the production of lactose-free dairy products for the benefit of lactose intolerant individuals. The stability of the enzyme produced by *K. lactis* can be increased by immobilization on polyacrylamide beads, using freeze-dried liposomes (Rubio-Texeira, 2005), or by entrapement in cellulose triacetate fibers – the product commercialized by Centrale del Latte of Milan, Italy (Xavier et al., 2017). On the other hand, the enzyme from *K. fragilis* has optimal activity at pH at 4.8 and 50ºC, which recommends it for treatment of acid whey (Raveendran et al., 2018).

An important process that appear during lactose hydrolysis is transgalactosylation wich is associated with β-galactosidase and formation of galactooligosachharides (GOS), important prebiotic compounds. The *K. lactis* enzyme is sucessfully used in this process using skimmed milk permeate fortified with lactose (Xavier et al., 2017), while the company Amano Enzyme, Inc. (Amano, Japan) obtained the GRAS notification for an enzyme derived from *Papiliotrema* (*Cryptococcus*) *terrestris* for use as a processing aid in the production of GOS (Keller & Heckman, 2017).

According to the RDC Resolution 205/2006, besides the β-galactosidases synthesized by *Kluyveromyces* sp., in the dairy industry are also accepted the enzymes produced by *Saccharomyces* sp. or *Candida tropicalis*. Thus, Morioka et al. (2019) obtained a β-galactosidase from permeabilized *S. fragilis* IZ 275 cells, at 44ºC and pH 7.0.

The *Kluyveromyces* yeasts are also able to produce inulinases (β-2,1-D-fructan fructanohydrolase; EC 3.2.1.7), enzymes that degrade the inulin, a polysaccharide with important role of energy storage in plants. The inulin is formed from β-(2,1)-linked glucose and fructose units with a polymerisation degree of 2 to 60. Fructose is considered as GRAS and is extensively used in food industry as glucose replacement and also for improving food flavour and product stability. The inulin is not naturaly decomposed in the human body in the gastrointestinal tract where it acts as a dietary fiber for controling the body weight and as a probiotic compound assuring an enhanced absorbtion of calcium and magnesium. *K. marxianus* produces extracellular and cell wall-bound inulinases at pH = 4.5 and 52-55ºC, allowing the production of fructooligosaccharides (FOS) that act as a growth factor for many *Bifidobacteria* (Jain et al., 2012; Csutak, 2014) and as noncarnicogenic sweeteners with low caloric value for use by diabetic patients. Singh et al., 2007 described an thermostable exoinulinase (β-D-fructan fructohydrolase, EC 3.2.1.80) with high pH stability and significant kinetic properties that hydrolized the raw inulin from *Asparagus racemosus* producing a high-fructose syrup. Also, Stuyf et al. (2018) presented the strain *K. marxianus* CBS6014 able to synthesize inulinase that can be used for production of whole meal breads with low content of Fermentable Oligo-, Di-, Monosaccharides And Polyols (FODMAPs), molecules that are poorly absobted in the small intestine representing thus the main cause of irritable bowel syndrome. An inulinase was also isolated from the marine strain of *Cryptococcus aureus* G7a able to produce high ammount of mono- and oligisaccharides (Sheng et al., 2007).

CONCLUSIONS

Based on their rich history as fermentative microorganisms, the yeasts remain one of the main players in food industry as well in numerous biotechnology domains. Yeasts have shown great potential for processing and improving the quality of fermented foods. Although, in present, many studies are based on using modern technologies to improve the biotechnological potential of yeasts, the interest for exploring the microbiota of traditional fermented foods gains more interest. These type of products continue to surprise the scientific world as they represent a valuable source of numerous new yeasts strains with exceptional metabolic qualities.

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