ISSN 1330-9862
(FTB-1659)

review

Production of Food Grade Yeasts

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Received: January 30, 2006
Accepted: March 20, 2006

Summary

Yeasts have been known to humans for thousands of years as they have been used in traditional fermentation processes like wine, beer and bread making. Today, yeasts are also used as alternative sources of high nutritional value proteins, enzymes and vitamins, and have numerous applications in the health food industry as food additives, conditioners and flavouring agents, for the production of microbiology media and extracts, as well as livestock feeds. Modern scientific advances allow the isolation, construction and industrial production of new yeast strains to satisfy the specific demands of the food industry. Types of commercial food grade yeasts, industrial production processes and raw materials are highlighted. Aspects of yeast metabolism, with respect to carbohydrate utilization, nutritional aspects and recent research advances are also discussed.

Key words: food grade yeasts, single cell proteins (SCP), raw materials, propagation, baker's yeast, brewer's yeast, distiller's yeast, *Torula*, whey, kefir, probiotics

Introduction

Yeasts are a group of unicellular microorganisms most of which belong to the fungi division of Ascomycota and Fungi imperfecti. Yeasts have been known to humans for thousands of years as they have been used in fermentation processes like the production of alcoholic beverages and bread leavening. The industrial production and commercial use of yeasts started at the end of the 19th century after their identification and isolation by Pasteur. Today, the scientific knowledge and technology allow the isolation, construction and industrial production of yeast strains with specific properties to satisfy the demands of the baking and fermentation industry (beer, wine, cider and distillates). Food grade yeasts are also used as sources of high nutritional value proteins, enzymes and vitamins, with applications in the health food industry as nutritional supplements, as food additives, conditioners and flavouring agents, for the production of microbiology media, as well as livestock feeds. Yeasts are included in starter cultures, for the production of specific types of fermented foods like cheese, bread, sourdoughs, fermented meat and vegetable products, vinegar, *etc*.

The significance of yeasts in food technology as well as in human nutrition, as alternative sources of protein to cover the demands in a world of low agricultural production and rapidly increasing population, makes the production of food grade yeasts extremely important. A large part of the earth's population is malnourished, due to poverty and inadequate distribution of food. Scientists are concerned whether the food supply can keep up with the pace of the world population increase, with the increasing demands for energy, the ratio of land area required for global food supply or production of bioenergy, the availability of raw materials, as well as the maintenance of wild biodiversity (*1–4*). Therefore, the production of microbial biomass for food consumption is a main concern for the industry and the scientific community.

The impressive advantages of microorganisms for single cell protein (SCP) production compared with conventional sources of protein (soybeans or meat) are well known. Microorganisms have high protein content and short growth times, leading to rapid biomass production, which can be continuous and is independent from the environmental conditions. The use of fungi, especially

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yeasts, for SCP production is more convenient, as they can be easily propagated using cheap raw materials and easily harvested due to their bigger cell sizes and flocculation abilities. Moreover, they contain lower amounts of nucleic acids than bacteria (*5–7*).

Yeast Metabolism

Yeasts are facultative anaerobes, and can grow with or without oxygen. In the presence of oxygen, they convert sugars to CO_2, energy and biomass. In anaerobic conditions, as in alcoholic fermentation, yeasts do not grow efficiently, and sugars are converted to intermediate by-products such as ethanol, glycerol and CO_2. Therefore, in yeast propagation, the supply of air is necessary for optimum biomass production. The main carbon and energy source for most yeasts is glucose, which is converted *via* the glycolytic pathway to pyruvate and by the Krebs cycle to anabolites and energy in the form of ATP. Yeasts are classified according to their modes of further energy production from pyruvate: respiration and fermentation. These processes are regulated by environmental factors, mainly glucose and oxygen concentrations. In respiration, pyruvate is decarboxylated in the mitochondrion to acetyl-CoA which is completely oxidized in the citric acid cycle to CO_2, energy and intermediates to promote yeast growth. In anaerobic conditions, glucose is slowly utilized to produce the energy required just to keep the yeast cell alive. This process is called fermentation, in which the sugars are not completely oxidized to CO_2 and ethanol. When the yeast cell is exposed to high glucose concentrations, catabolite repression occurs, during which gene expression and synthesis of respiratory enzymes are repressed, and fermentation prevails over respiration. In industrial practice, catabolite repression by glucose and sucrose, also known as Crabtree effect, may lead to several problems, such as incomplete fermentation, development of off-flavours and undesirable by-products as well as loss of biomass yield and yeast vitality (*8–10*).

Yeasts can metabolize various carbon substrates but mainly utilize sugars such as glucose, sucrose and maltose. Sucrose is metabolized after hydrolysis into glucose and fructose by the extracellular enzyme invertase. Maltose is transferred in the cell by maltose permease, and metabolized after hydrolysis into two molecules of glucose by maltase. Some yeasts can utilize a number of unconventional carbon sources, such as biopolymers, pentoses, alcohols, polyols, hydrocarbons, fatty acids and organic acids, which is of particular interest to food and environmental biotechnologists. For example, lactose can be utilized by yeasts that have the enzyme β-galactosidase. The yeasts of genera *Kluyveromyces* and *Candida* can grow *e.g.* in whey, yielding biomass under certain conditions, with applications in food production. Biopolymers like starch, cellulose, hemicellulose and pectin can be metabolized by some yeasts directly, or after hydrolysis by non-yeast enzymes. Some yeast species of *Hansenula, Pichia, Candida* and *Torulopsis* are also able to grow on methanol as sole energy and carbon source. The inability of yeasts to ferment certain sugars can be overcome by r-DNA technology, introducing genes of the corresponding enzymes from other species (*8,11*). Finally, elements like N, P, S, Fe, Cu, Zn and Mn are essential to all yeasts and are usually added to the growth media. Most yeasts are capable of assimilating directly ammonium ions and urea, while very few species have the ability to utilize nitrates as nitrogen source. Phosphorus and sulphur are usually assimilated in the form of inorganic phosphates and sulphates, respectively.

Food Grade Yeasts

Various microorganisms are used for human consumption worldwide as SCP or as components of traditional food starters, including algae (*Spirulina, Chlorella, Laminaria, Rhodymenia, etc.*), bacteria (*Lactobacillus, Cellulomonas, Alcaligenes, etc.*), fungi (*Aspergillus, Penicillium, etc.*) and yeasts (*Saccharomyces, Candida, Kluyveromyces, Pichia* and *Torulopsis*) (*6,7*). Among the yeast species, *Saccharomyces cerevisiae* and *Candida utilis* are fully accepted for human consumption, but very few species of yeast are commercially available.

The most common food grade yeast is *Saccharomyces cerevisiae*, also known as baker's yeast, which is used worldwide for the production of bread and baking products. *S. cerevisiae* is the most widely used yeast species, whose selected strains are used in breweries, wineries and distilleries for the production of beer, wine, distillates and ethanol. Baker's yeast is produced utilizing molasses from sugar industry by-products as a raw material. Commercial *S. cerevisiae* and other yeast products available to cover the needs of the baking and alcoholic fermentation industries or for use as nutritional supplements for humans and/or animals are described in the following paragraphs.

Baker's yeast

Fresh baker's yeast consists of approximately 30–33 % of dry materials, 6.5–9.3 % of nitrogen, 40.6–58.0 % of proteins, 35.0–45.0 % of carbohydrates, 4.0–6.0 % of lipids, 5.0–7.5 % of minerals and various amounts of vitamins, depending on its type and growth conditions. Commercial fresh baker's yeast includes products in liquid, creamy or compressed forms and active dry yeast. Compressed baker's yeast is the most commonly used product, consisting of only one yeast species, *S. cerevisiae.* Special strains of *S. cerevisiae* can be used for the production of dry yeast products, like active dry yeast or instant dry yeast. Active dry yeast consists of grains or beads of live dried yeast cells with leavening power, while instant dry yeast usually comes in the form of fine particles that do not require rehydration before use. Unlike active dry yeast, inactive dry yeast is a product without leavening properties, used for the conditioning of dough properties in baking or the development of characteristic flavour.

Brewer's yeasts

Pure brewer's yeast cultures are produced industrially to supply the brewing industry. Usually two *Saccharomyces* species are used: *S. uvarum*, formerly known as *S. carlsbergensis*, which is used for the production of several types of beer with bottom fermentation (lager yeasts), and *S. cerevisiae*, which conducts top fermentation (ale

yeasts). Due to recent reclassification both ale and lager yeast strains are considered *S. cerevisiae* species. Top-fermenting yeasts are used for the production of ales, porters, stouts, wheat beers, *etc.*, and bottom-fermenting yeasts are used for lager beers like Pilsners, Bocks, American malt liquors, *etc.* (*12*). Inactive brewer's yeast preparations, made from inactive yeast and other special ingredients, are produced commercially to be used as nutrients to reinitiate or avoid sluggish and stuck fermentations.

Nutritional brewer's yeast

Commercial, nutritional brewer's yeast is inactive yeast (dead yeast cells with no leavening power), remaining after the brewing process. Brewer's yeast is produced by cultivation of *S. cerevisiae* on malted barley, separated after the wort fermentation, debittered and dried. It is an excellent source of protein and it is used as a nutrient supplement rich in B vitamins. Brewer's yeast products are usually found in the form of powders, flakes or tablets, or in liquid form. Liquid yeast contains enzymatically digested yeast for better digestion, absorption and utilization. Brewer's yeast should not be confused with »brewer's type yeasts«, which are pure yeasts usually grown on enriched cane or beet molasses under controlled production conditions, and are not by-products of the brewing process.

Brewer's yeast is an excellent source of B vitamins, Ca, P, K, Mg, Cu, Fe, Zn, Mn and Cr and has been studied extensively for its medicinal properties. It is often used for the treatment of diabetes (regulation of insulin levels), loss of appetite, chronic acne, diarrhoea, *etc*. (*13–15*). It is also recommended as a dietary supplement for healthy hair and nails. Nevertheless, according to some, brewer's yeast is suspected of causing various problems, like chronic fatigue, memory disorders, immunodeficiency, irritable bowel syndrome, allergies, *etc.*, mainly due to the presence of yeast antigens and high amounts of Cr and salicylates (*16*,*17*).

Wine yeasts

A wide variety of pure yeast cultures, mainly *Saccharomyces* (*S. cerevisiae, S. bayanus, S. uvarum, S. oviformis, S. carlsbergensis, S. chevalieri, S. diastaticus, S. fructuum, S. pasteurianus, S. sake, S. vini, etc.*) are produced industrially for the use in induced wine fermentations, according to the industrial demands for fermentation efficiency and productivity. The suitable type of yeast is selected with respect to the geographical area, climate, type of grapes and desirable organoleptic quality of the product (taste, aroma, colour, tannin and glycerol content, *etc.*). Pure yeast cultures are also used to conduct specific types of fermentations, like bottle fermentation of Champagne and sparkling wines, or to treat stuck and sluggish fermentations.

Distiller's yeasts

Distiller's yeasts are used for the industrial production of alcohol and spirits (brandy, whiskey, rum, tequila, *etc.*). They are usually isolated from industrial fermentations of fruit pulps and beet or sugar cane molasses. Their selection depends on the desired product properties, including flavour, alcohol yield, productivity, and other technological features. Generally, distiller's yeasts must exhibit low foam formation, high stress-tolerance and high alcohol yields. They must also form controlled amounts of ethyl esters, aldehydes, fatty acids and higher alcohols, which is an important prerequisite for the production of fine quality distillation products. Distiller's yeasts must be able to ferment various substrates, such as corn, barley, wheat, potato, *etc.* after hydrolysation in fermentable sugars. They must be able to conduct fast fermentations with high productivities and low production costs, and tolerate high temperatures, osmotic pressures and alcohol concentrations (*18–20*).

Probiotic yeasts

»Probiotics are live microbial feed supplements which beneficially affect the host animal by improving its intestinal microbial balance«, or by a wider definition »probiotics are microbial cell preparations or components of microbial cells that have a beneficial effect on the health and well-being of the host« (*21*). Probiotic properties of yeasts, like *S. cerevisiae*, have been reported and displayed as the ability to survive through the gastrointestinal (GI) tract and interact antagonistically with GI pathogens such as *Esherichia coli*, *Shigella* and *Salmonella*. Specifically, *S. boulardii*, a thermophylic, non-pathogenic yeast, has been used for more than 50 years as a livestock feed probiotic supplement as well as therapeutic agent for the treatment of a variety of gut disorders like diarrhoea. This yeast is safe, it is resistant to antibiotics, achieves high cell numbers in the intestine in short time, does not permanently colonize the intestine and is quickly cleared after the cease of administration. Its probiotic effects are also enhanced by its ability to produce polyamines, which are compounds that strongly affect cell growth and differentiation (*22–24*). *S. boulardii* is widely used and is available in various commercial formulations. Other yeasts allowed and commonly used in animal feeds as probiotic additives are *Candida pintolopesii*, *C. saitoana* and *S. cerevisiae* (*25*,*26*).

Yeast extract

Yeast extract is the product of enzymatic digestion of the yeast cell constituents by endogenous and exogenous yeast enzymes. It is rich in peptides, amino acids, nucleotides and vitamins, therefore it is good for use as supplement in culture media. It is also used in pharmaceuticals, as well as flavour and taste enhancer (replacing glutamates and nucleotides) in many canned foods. Although brewer's yeasts contain residual beer flavour compounds (mainly constituents of hops), they are commonly used for commercial food grade yeast extract production, which is destined for use as supplement in both human and animal foods, and as flavour enhancer (*27*).

Torula yeast

Torula or *Candida* yeast refers to products containing *Candida utilis*, which have been used commercially for more than 60 years as nutritional supplements in animal feeds. Food grade *Torula* yeast is cultivated in mixtures of sugars and minerals, usually containing molasses, cellulosic wastes (*e.g.* spruce wood) or brewing by-products. After cultivation the yeast is harvested, washed,

thermolyzed and dried. Thermolysis renders the yeast cells inactive, losing their fermentation ability. The yeast is then usually spray-dried into a fine powder with slight yeasty and meaty flavours. It is a highly digestible and nutritious food, containing more than 50 % of protein (rich in lysine, threonine, valine and glutamic acid), minerals and vitamins (mainly niacine, pantothenic acid and B vitamins). *Torula* yeast can be used as a meat substitute or food additive in many processed foods, in seasonings, spices, sauces, soups, dips, *etc.* It is also used in vegetarian and diet food, in baby food, meat products, doughs, *etc*. (*28–31*).

Whey yeasts

A variety of microorganisms, especially those present in milk microflora, are able to utilize whey, the main by-product of the dairy industry, but only a few are approved as GRAS by the USFDA for use in food industry (*32*). The yeasts most widely studied and used at industrial scale for the production of yeast biomass from whey are the lactose fermenting *Kluyveromyces* yeasts *K. lactis* and *K. marxianus* (formerly classified as *K. fragilis*). *Kluyveromyces* yeasts can efficiently grow on lactose as sole carbon source, although it has been reported that under aerobic conditions (like those used in biomass production) some *K. marxianus* strains present a mixed type metabolism, with intermediate metabolite production (alcohol, aldehydes, esters, *etc*.) and low yields of biomass (*32,33*).

Lactose fermenting yeasts are also found in kefir, a natural mixed culture found in the Caucasian milk drink. It contains various microorganisms, sharing symbiotic relationships, including species of lactose-fermenting yeasts such as *Kluyveromyces*, *Candida*, *Saccharomyces*, *Debaryomyces*, *Zygosaccharomyces,* lactic acid bacteria and occasionally acetic acid bacteria (*34*). Kefir yeasts have been used at semi-industrial scale for whey lactose utilization and production of value added products such as ethanol, biomass, lactic acid and alcoholic beverages (*35*). Kefir produced using whey has also been evaluated as starter culture in bread making and maturation of cheeses with good results (*36–38*).

Sourdough starters

Sourdough is a mixture of flour and water, containing yeasts and lactic acid bacteria, used as starter culture to leaven bread. The use of sourdough has a number of important advantages over baker's yeast, such as the development of characteristic flavour (*39*) and texture (*40*), as well as extension of preservation time through the *in situ* production of antimicrobial compounds (*e.g.* bacteriocins) (*41*). Therefore, sourdoughs are produced at commercial level using various combinations of yeasts and bacteria, and are used for the conditioning of dough, improvement of preservation time and the development of breads and baking products with special organoleptic properties (*38*).

Nutritional Aspects

Today yeast SCP are considered a potential protein source for humans as well as animals. Food grade yeasts can provide proteins, carbohydrates, fats, vitamins (mainly the B group), minerals, essential amino acids (mainly lysine) (*6,42*). Generally, the lysine content in yeasts is higher than in bacteria or algae. Moreover, yeasts contain low amounts of nucleic acids (6–12 % on dry mass basis) (*6,7*). The acceptability of a particular microorganism as food or feed depends on its nutritional value and safety (including nucleic acid content, presence of toxins and residual undesirable compounds such as heavy metals). SCP for human consumption should be free from nucleic acids as purine bases are metabolized to uric acid, creating problems to humans that do not possess the enzyme uricase (*6*). Nucleic acid content in SCP can be reduced by chemical treatment and autolytic methods (precipitation, acid or alkaline hydrolysis and/or enzymatic treatment). Generally, the processes involved in SCP production include mechanical disruption of cell walls, removal by centrifugation, precipitation and extrusion of proteins to form the textured products (*6*). Today, the only species fully acceptable as food for humans is *S. cerevisiae* (baker's and brewer's yeasts). Novel SCP sources demand extensive quality controls and should be purified to meet international safety standards.

Yeasts may cause common food intolerances, although in smaller frequency than other foodstuffs such as milk, eggs, nuts, fish, shellfish, meat, chemical additives, *etc*. Salicylates occurring naturally or added in foods as flavouring agents (benzyl, methyl, ethyl, isoamyl, isobutyl and phenethyl salicylates) may be present in yeast and yeast extracts and may be associated with food intolerance symptoms in susceptible people (*43,44*). Also, the foreign protein in yeasts may cause allergic reactions to humans. Finally, digestibility is an important factor that should be considered when SCP is used as food supplement.

Yeast Production: Established Technology and Research

Raw materials

The raw materials used as substrates for industrial yeast biomass production are usually agricultural, forestry and food waste by-products. There are two types of raw materials depending on the grown microorganism: conventional materials like starch, molasses, distiller's wash, whey, fruit and vegetable wastes, wood, straw, *etc*., and unconventional ones like petroleum by-products, natural gas, ethanol and methanol (*6*).

Molasses

The most widely used substrate for baker's yeast production is cane or beet molasses, the main by-product of the sugar industry. Molasses contain 45–55 % fermentable sugars including sucrose, glucose, fructose, raffinose, melibiose and galactose. The use of molasses for the production of food grade yeast is determined by their availability and low cost, their composition and absence of toxic substances and fermentation inhibitors (*45*). The fermentation mixture for optimum yeast biomass production is usually fixed to pH=4.5–5.0 and enriched by the addition of extra nutrients (N, P, Mg, Ca, trace amounts of Fe, Zn, Cu, Mn, and vitamins, usually biotin), depending on the initial composition of molasses. Molasses

contain approx. 40 % (dry mass) of nonfermentable substances that are eventually rejected and constitute a significant cause of pollution and increase of production cost due to required waste treatment operations. The nonfermentable substances are usually collected and used as animal feed or as fertilizers.

Whey

Whey is the main waste of the dairy industry. It is produced worldwide in large amounts and its disposal causes serious environmental problems due to its high organic load (COD 35 000–68 000), which makes its full treatment impossible (*5*). On the other hand, whey has a significant nutritional value since it contains respectable amounts of proteins, lactose, organic acids, fat, vitamins and minerals. Therefore, its conversion to products of added value is a major concern for science and industry. The composition (high salt concentrations) and temperature of whey at the moment of its production in the factory do not allow easy microbial utilization. Lactose, the main sugar constituent in whey, can be metabolised only by a few species of the *Kluyveromyces* and *Candida* yeasts. The yeast *S. cerevisiae* cannot utilize lactose because it lacks the enzyme β-galactosidase and lactose permease. *K. marxianus* is the only strain used for biomass production from whey on a commercial scale.

Starch

S. cerevisiae can utilize starch, only after its conversion to fermentable sugars, glucose and maltose. Hydrolysis of starch to glucose can be done either by treatment with acid or non-yeast enzymes. Enzymatic treatment includes three different processes: gelatinisation by heating, liquefaction by thermostable α-amylases, and saccharification by mixed enzyme activities (*46*). Nevertheless, processes like these imply considerable costs, which is the main limiting factor in industrial utilization of starch for yeast biomass production. Starch can be utilized by mixed cultures of yeasts and amylolytic fungi like *Aspergillus* species for SCP or ethanol production (*6*,*46*).

Residues of forestry and agriculture

Wastes of agriculture and forestry are rich in cellulose, hemicellulose and lignin. Their enzymatic conversion to fermentable sugars requires chemical pretreatment that leads to various polymer fragments. *S. cerevisiae* does not have the variety of enzymes required to hydrolyse these polymers. As a result, yeast biomass production on lignocellulosic wastes implies a high economic cost. A solution to this problem could be the use of mixed cultures of *S. cerevisiae* and cellulolytic microorganisms, but this process is today applied for ethanol production in pilot plants only (*47*).

Propagation processes

Industrial propagation of yeast is done on abundantly available and cheap agricultural and industrial wastes, mainly molasses, by successive submerged fermentations. After fermentation, the yeast biomass is harvested and may be subjected to downstream processing steps like washing, cell disruption, protein extraction and purification.

Industrial yeast production generally involves the following stages as described below: propagation, involving a number of fermentation processes, harvesting, concentration and/or drying, packaging and shipment. Fig. 1 presents a commercial baker's yeast propagation scheme (*48*).

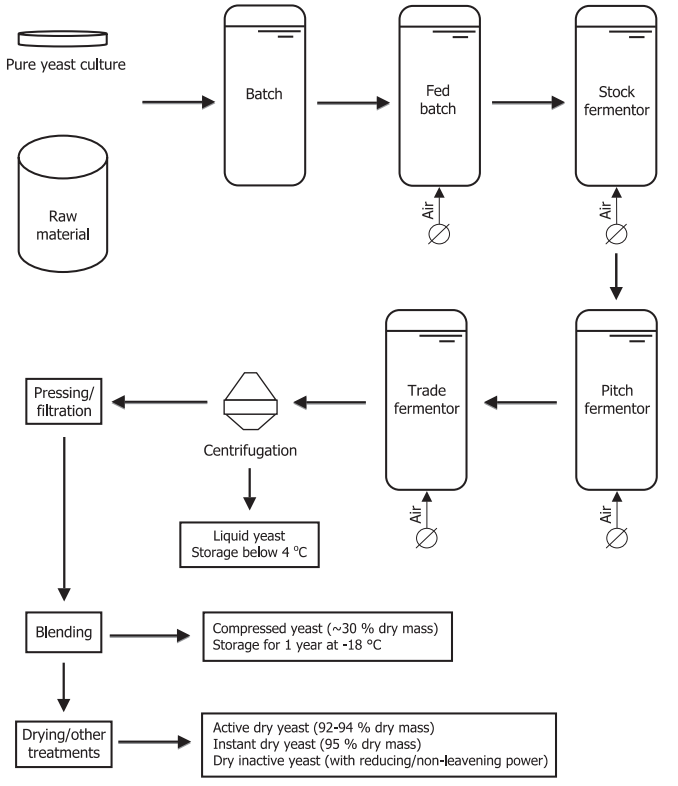

Fig. 1. Description of a propagation scheme for the production of baker's yeast (adapted from Randez-Gil *et al.* (*48*) and industrial data)

Yeast cells are grown in a series of fermentation bioreactors, which are operated under aerobic conditions to promote yeast growth. Initially, cells from a pure yeast culture are grown on a suitably adjusted mixture of molasses in the laboratory and the produced biomass is transferred aseptically into one or more bioreactors, which operate in batch mode without air supply. The next bioreactor usually operates in fed batch mode with air supply, and the produced biomass is used to pitch the stock bioreactor. The biomass produced in this bioreactor is harvested by centrifugation and used in the next stage, the pitch fermentation. Both these stages operate in fed batch mode with vigorous aeration and incremental addition of nutrients. The biomass produced in the pitch bioreactor is used to pitch the final trade fermentations. At the end of the process the content in the trade bioreactors is aerated for an additional time period, and this is the maturation stage. The amount of yeast biomass produced increases from stage to stage, and the sequence and the number of fermentation stages vary among manufacturers. Food grade yeast biomass can also be produced as by-product of industrial ethanol production on molasses (*e.g.* Vogelbusch technology) (*49*).

Treatments and packaging

The yeast in the final trade bioreactor is concentrated by centrifugation and finally harvested by a filter press or a rotary vacuum filter, until it contains 27–33 % of dry cell mass. The yeast cake is blended with suitable amounts of water and emulsifiers and cutting oils (soybean or cottonseed oil) to obtain its extrudable form. The yeast is then packaged and shipped as compressed fresh baker's yeast, or thermolysed and dried to form various types of dry yeast. The dried yeast is packed under vacuum or nitrogen atmosphere. The packaging method varies among manufacturers and depends on the type of yeast product.

Recent advances and research

Various by-products of the food industry and agriculture have been proposed for the production of food grade yeast biomass. Some of these efforts are summarized in Table 1 (*50–77*). Species of *Candida*, *Saccharomyces*, *Kluyveromyces*, *Pichia*, *Rhodotorula*, *etc*., alone or in mixed cultures with other yeasts, have been grown on vegeta-

Table 1. Production of yeasts using alternative, low cost waste by-products of the food and agricultural industries

Microorganism	Raw material	Ref.
Rhodotorula rubra, Candida tropicalis, C. utilis, C. boidinii, Trichosporon cutaneum	salad oil manufacturing wastewater	(*50*)
Candida arborea	rice straw hydrolysate	(*51*)
Candida halophila, Rhodotorula glutinis	glutamate fermentation wastewater	(*52*)
Saccharomyces cerevisiae	extracts of cabbage, watermelon, green salads and tropical fruits	(*53*)
Candida utilis	defatted rice polishings	(*54*)
Candida versatilis, Kluyveromyces lactis, Kluyveromyces marxianus	whey	(*55*)
Candida utilis, Pichia stipitis, Kluyveromyces marxianus, Saccharomyces cerevisiae	waste chinese cabbage	(*56*)
Candida utilis	apple pomace	(*57*)
Saccharomyces cerevisiae	virgin grape marc	(*58*)
Saccharomyces sp., *Pichia* sp., *Rhodotorula* sp., *Candida* sp., *Kluyveromyces* sp. and *Trichospora* sp.	lettuce brine	(*59*)
Candida langeronii	cane bagasse hemicellulosic hydrolyzate	(*60*)
Torulopsis cremoris, Candida utilis, Kluyveromyces fragilis	whey	(*61*)
Pichia guilliermondii	waste brine from kimchi production	(*62*)
Geotrichum candidum	orange peel	(*63*)
Candida, Rhodotorula, Leucosporidium	prawn shell waste	(*64*)
Hansenula sp.	sugar beet stillage	(*65*)
Candida utilis	pineapple cannery effluent	(*66*)
Saccharomyces cerevisiae	waste date products	(*67*)
Saccharomyces cerevisiae	hydrolyzed waste cassava	(*68*)
Saccharomyces cerevisiae, Torula utilis, Candida lipolytica	deproteinized leaf juices of turnip, mustard, radish and cauliflower	(*69*)
Saccharomyces cerevisiae	shrimp shell waste	(*70*)
Candida krusei, Saccharomyces sp.	sorghum hydrolysate	(*71*)
Candida rugosa	sugar beet stillage	(*72*)
Kluyveromyces fragilis	cheese whey	(*73*)
Cellulomonas flavigena, Xanthomonas sp.	sugarcane bagasse pith	(*74*)
Candida spp. (*utilis, tropicalis, parapsilosis* and *solani*)	molasses and sugar beet pulp	(*75*)
Kluyveromyces, Candida, Schizosaccharomyces sp.	jerusalem artichoke	(*76*)
Pichia pinus	mango waste or methanol	(*77*)

ble processing wastewaters, hydrolysates and pulps (rice, cabbage, apple, lettuce, pineapple, radish, cauliflower, turnip, sorghum, *etc*.), on dairy wastes (whey), sugar and ethanol industry by-products (molasses, vinasse, stillages, bagasses, sugar beet pulps), fishery by-products (prawn-shell waste), *etc*. The need to design feasible and financially viable processes and the utilization of low cost industrial wastes as raw materials for edible yeast biomass production is extremely important, as it gives a solution to the management of these wastes and the environmental pollution caused by their disposal. Moreover, apart from providing alternative sources of food for humans or animals and reducing pollution, food grade yeast production using waste materials is attractive to manufacturers as it leads to increased profits from the use of low cost raw materials, production of added value and reduction of waste treatment costs.

Today, modern techniques like DNA recombination, induced mutations, and selection methodologies can also be employed to obtain new specialized yeast strains with improved properties, according to the manufacturer's demands for fermentation efficiency and productivity (*48*, *78–80*). For example, the modern baking industry demands the production of more stable yeast strains, tolerant of pH and temperature variations and high osmotic pressures. Especially, probiotic yeasts must be able to survive food production conditions, the presence of antimicrobial agents and storage.

Industrial strains should be improved to face problems related to glucose repression when mixed carbohydrate substrates are used, to avoid the production of undesirable by-products like ethanol and glycerol under aerobic conditions (*10*). Genetic engineering has made possible the creation of such yeast strains, with new or enhanced enzymatic properties for maximum utilization of various problematic raw materials, like cheese whey, starch, sugar cane bagasses, lignocellulosic materials, *etc*., for bioremediation purposes and optimum biomass yields. Finally, strains have been constructed to increase the nutritional value of foods, such as, for example, amino acid overproducing baker's yeast for more nutritious bread (*42*). Therefore, genetic engineering can lead to the reduction of yeast production costs by increasing the availability of the raw material, and avoiding the traditional chemical reatment methods for their conversion. In the frame of these efforts, new rapid methods for DNA analysis have been introduced for the identification of specific industrial yeast strains, and novel aerobic bioreactor designs have been proposed to enable optimum production of yeast biomass, maximum utilization of the raw material, reduction of cost and simultaneous reduction of environmental pollution. Nevertheless, despite the tremendous progress in the genetic engineering of yeasts achieved at the end of the 20th century (establishment of genetic transformation of yeast in 1978 and determination of the complete genome sequence in 1996), the genetically modified (GM) yeasts have not yet been used commercially. Only two GM yeast strains have been officially approved for commercial use in 1990 (baker's yeast) and 1994 (brewer's yeast), but none has been used commercially (*81*).

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