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REVIEW ARTICLE

SPENT YEASTS AS NATURAL SOURCE OF FUNCTIONAL FOOD ADDITIVES

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ABSTRACT

Spent yeasts are by-products arising from beer and wine production which over many years have been chiefly used as feed additives for livestock. They contain many valuable and bioactive substances which has thereby generated much interest in their exploitation. Up till now, the main products obtained from beer-brewing yeasts are β-glucans and yeast extracts. Other like foodstuffs include dried brewer's yeast, where this is dried and the bitterness removed to be fit for human consumption as well as mannan-oligosaccharides hitherto used in the feed industry. β-glucans constitute the building blocks of yeast cell walls and can thus be used in human nutrition as dietary supplements or serving as food additives in functional foods. β-glucans products obtained via post-fermentation of beer also exhibit a high and multi-faceted biological activity where they improve the blood's lipid profile, enhance immunological status and have both prebiotic and anti-oxidant properties. Yeast extracts are currently being used more and more to enhance flavour in foodstuffs, particularly for meat and its products. Depending on how autolysis is carried out, it is possible to design extracts of various meat flavours characteristic of specific meats. Many different flavour profiles can be created which may be additionally increased in combination with vegetable extracts. Within the food market, yeast extracts can appear in various guises such as liquids, pastes or powders. They all contain significant amounts of glutamic acid, 5'-GMP and 5'-IMP nucleotides together with various amino acids and peptides that act synergistically for enhancing the flavour of foodstuff products. Recent studies have demonstrated additional benefits of yeast extracts as valuable sources of amino acids and peptides which can be used in functional foods and dietary supplements. These products possess GRAS status (*Generally Recognised As Safe*) which thereby also adds further as to why they should be used as natural food additives that are functional.

Key words: *spent yeasts, β-glucans, yeast extracts, functional food additives, food additives, foodstuffs.*

STRESZCZENIE

Drożdże pofermentacyjne są produktem ubocznym przy produkcji piwa i wina stosowanym przez wiele lat głównie jako dodatek paszowy. Zawierają one w swym składzie wiele cennych składników bioaktywnych stąd też obserwuje się duże zainteresowanie ich wykorzystaniem. Głównymi produktami otrzymywanymi od niedawna z pofermentacyjnych drożdży piwowarskich są β-glukany i ekstrakty drożdżowe. Produkowane są również spożywcze preparowane suszone drożdże piwowarskie, które na drodze odgoryczenia i suszenia zostają przygotowane do bezpośredniego spożycia oraz mannooligosacharydy stosowane dotychczas w przemyśle paszowym. b-glukany będące elementem budulcowym ścian komórkowych drożdży, mogą być stosowane w żywieniu człowieka jako suplementy diety lub jako dodatki do rożnego rodzaju produktów spożywczych z grupy żywności funkcjonalnej. Preparaty β-glukanów otrzymane z pofermentacyjnych drożdży piwowarskich wykazują wysoką, wielokierunkową aktywność biologiczną, związaną przede wszystkim z poprawą profilu lipidowego krwi, statusu immunologicznego organizmu oraz z oddziaływaniem prebiotycznym i antyoksydacyjnym.
Ekstrakty drożdżowe znajdują coraz szersze zastosowanie jako substancje polepszające smak rożnego rodzaju produktów głównie o profilu mięsnym. W zależności od sposobu przeprowadzenia procesu autolizy możemy otrzymać ekstrakty o specyficznym smaku mięsa rożnego pochodzenia. Ilość profilów smakowych jest bardzo duża i może być dodatkowo zwiększona poprzez kombinacje z ekstraktami z warzyw. Ekstrakty drożdżowe mogą występować w rożnych postaciach handlowych takich jak płyn, pasta czy proszek. Zawierają one w swym składzie znaczne ilości kwasu glutaminowego, nukleotydów 5'GMP i 5'IMP, oraz różne aminokwasy i peptydy o synergistycznym oddziaływaniu polepszającym smak produktu. Ostatnio wykonane badania wskazują na możliwości dodatkowego wykorzystania ekstraktów jako cennego źródła aminokwasów i peptydów mogących znaleźć zastosowanie w żywności funkcjonalnej i suplementach diety. Preparaty drożdżowe posiadają status GRAS (*Generally Recognised As Safe*), co intensyfikuje możliwości ich zastosowania jako naturalnych dodatków funkcjonalnych.

Słowa kluczowe: *drożdże pofermentacyjne, β-glukany, ekstrakty drożdżowe, dodatki funkcjonalne, dodatki do żywności, żywność*

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INTRODUCTION

Along with the scientific and technological advances made in human nutrition, the food industry is increasingly turning towards natural food additives that complement foodstuff products with nutrients, improve flavour, stabilise texture and increase shelf life. Over many years the use of yeasts, obtained as by-products from the brewing industry, have been investigated as being natural sources of nutritionally bioactive components [27, 20, 44]. Up till now, most of the spent brewer's yeast has been considered an inconvenient waste by the brewing industry and had been used in livestock feed production; being a source of protein, vitamins and minerals [27, 48, 50, 51].

At present however, increasingly more food companies process waste yeast slurries into intermediates that are further used in foodstuff production. More and more published studies demonstrate the pro-health effects of spent yeasts and isolated β-glucans thereof, as well as their application to human nutrition [7, 11, 22, 41]. Another large and rapidly growing area of interest is focused on yeast extracts, particularly those derived from autolysis using the endogenous cellular enzymes present, or when further augmented by adding proteolytic preparations [12, 16, 17]. A drawback of other methods for yeast extraction, such plasmolysis or acid hydrolysis, is the high salt content, which in the latter cases also bears the risk of hazardous chemicals being formed, as is the case in the widely used protein hydrolysates for imparting 'meat flavours' to foodstuffs.

In the manufacture of protein hydrolysates through acid hydrolysis, chlorinated compounds can become generated such as 3-chloro-propanediol, which indeed has been detected in vegetable hydrolysates produced by similar methods [27]. Thus for flavour additives, it is recommended that natural products be used in the form of yeast autolysates. It is certain that in the next few years they will replace such previously and widely used protein hydrolysates obtained from acid hydrolysis.

SPENT YEASTS AS A SOURCE FOR BIOACTIVE COMPOUNDS

The nutritional value and basic components of yeasts may vary somewhat depending on the conditions set for growing and the technologies used in their production. The overall content of nitrogenous compounds in dried yeast counted as protein ranges 45 to 55%, of which 80% is protein nitrogen, 10-12% is nucleic acid nitrogen whereas the rest is made up of glutathione, glucosamine, lecithin, and others [25, 27, 36, 37, 38]. Brewer's and baking yeast are characterised by a high β-glucan content; averaging 7.7% [5]. The cell wall constitutes 15-30% of the dry weight and is a complex high molecular weight structure composed of 50-60% β-glucans and 40% mannanoprotein. Yeast also contains around 6% fat, 7% ash and 32% carbohydrate as well as being a good source of B-group vitamins and minerals such as phosphorus, calcium, magnesium and iron. [4] Brewer's yeast is recognised as being a beneficial dietary ingredient making up healthy and nutritious feed for farmed fish as well as being an immunostimulant [25, 45, 51]. Literature studies investigating the possible uses of dried yeast have mainly focused on baker's yeast, where they are chiefly regarded as a dietary supplement; being a source of B-group vitamins, fibre and protein [42, 51]. The beneficial health effects of dietary yeast seen in domestic animals are principally due to the presence of glucan and mannan [22, 23, 41], along with chitin [8].

Interest in yeasts has also risen from the time that dietary nucleic acids were found to be beneficial to farmed animal health in terms of increased immunity and decreased morbidity rates [3, 18, 38, 39]. Nevertheless in human nutrition, the high consumption levels of yeasts observed may be the cause of certain diseases due to their high nucleic acid content [27, 36, 37, 38]. The borderline dose of nucleic acids that has no impact on uric acid levels in blood is 2 g / day, corresponding to an average consumption of 30-50 g of dried yeast. This intake dose is in fact exceeded many times over that recommended for dietary supplements manufactured with yeast [2].

BETA-GLUCANS FROM SPENT YEAST AS PRO-HEALTH ADDITIVES

Polysaccharides isolated from yeast cell walls consist of linearly linked forms of glucan and mannan between β (1-3) / (1-6) positions, where almost 85% of the β-glucan found in yeast cell walls are made up of β (1-3) linear linkages (ie. around 50% of the cell wall by weight). The remaining 12% β-glucan are branched chains linked by β (1-6) bonds [19]. β-glucans from baker's yeast produce similar health effects to those when β-glucans from cereals or fungi are consumed.

Among the β-glucans identified in *Saccharomyces cerevisiae*, zymozan is one of note which is an insoluble long chain glucose polymer with antibacterial properties that enhances immunity, by *inter alia* activating macrophages and the secretion of cytokines such as IL-1, IL-6 and IL-8. Zymozan also stimulates the release of tumour necrosis factor (TNF-α) and it exhibits antioxidant properties [47]. If present in sufficient amounts, β-glucans enhance the immune system by stimulating skin cells to 'quench' free radicals and provide protection against contamination from the environment as well as delaying the cell aging process [4, 6, 46].

At the Department of Functional Food, Ecological

Food and Commodities from the Warsaw University of Life Sciences (SGGW), β-glucans derived from spent brewer's yeast have been found to possess potent and multi-directional biological activity chiefly associated with improving the blood lipid profile and the ability to mobilise the immune system (ie. immunomodulation), together with having prebiotic and antioxidant actions. As a dietary additive fed to rats, β-glucan favourably affects lymphocytes and macrophage levels in the blood, promotes neutrophil phagocytosis in peripheral blood against *Staphylococcus aureus* and *Candida albicans*, increases lymphocyte blast proliferation and significantly increases interferon gamma (INF-γ) release with moderate TNF-α production. Depending on the physicochemical properties of spent brewer's yeast or of dried yeasts containing β-glucan given in dietary supplements, then lowered blood cholesterol and reduced liver lipid concentrations can be observed coupled with increased amounts of the lactic acid-producing bacteria *Bifidobacterium* and *Lactobacillus* in the intestinal microflora when compared to controls along with limiting growth rates of the unfavourable yeast-like fungi *Candada albicans*. Spent yeasts can adjust impaired blood lipid metabolism resulting from an atherogenic diet, they improve intestinal microflora composition and constitute an effective hypo-cholesterolaemic factor irrespective of β-glucan solubilities, but are dependent on the intake dose [48, 52].

The legal options for using yeast-derived β-glucans as a novel food ingredient come under the Regulation of the European Parliament and Council (EU) 2015/2283 from 25th November 2015 concerning novel foods, amending the Regulation of the European Parliament and of the Council (EU) No 1169/2011 and repealing Regulation (EC) No 258/97 of the European Parliament and Council along with Commission Regulation (EC) No 1852/2001 [34, 35]. Thus accordingly, β-glucan yeasts can be used in dietary supplements, fruit drinks, cereal bars, biscuits, crackers, breakfast cereals, yoghurt, chocolate, soup, protein bars and foodstuffs intended for particular nutritional uses; excepting infant formulae.

Introducing β-glucan into foodstuffs bestows upon them the characteristics of functional foods. A study by *Piotrowska* et al. [26] showed that when β-glucan, obtained post-production of brewer's yeast, is added to yogurt in 0.15 to 0.9% amounts then at 0.3% this does not adversely affect sensory food quality, texture and stability of the liquid product during storage. A dietary intake of 250 g of this product can provide the body with 0.7 g of β-glucan, affording pro-health benefits and meeting the criteria set for functional foods [49]. Appearing on the European market, in tablet form, are dietary supplements containing yeast derived β-glucans from the baker's yeast *S. Cerevisiae* and spent yeasts. These are recommended for preventing diseases of the upper respiratory tract, infections and of being of immunological benefit to patients suffering allergies, together with activating monocytes in cancer patients [40, 43].

SPENT YEAST EXTRACTS AS NATURAL FLAVOURING SUBSTANCES

As a mainly cheap biomass, in the form of spent yeasts obtained from breweries, such yeasts are used for manufacturing yeast extracts and to a lesser extent are made in food processing plants where they are grown in various types of specific substrates. In order to release and digest yeast cell contents, a variety of methods are used; the most common ones being autolysis, hydrolysis and plasmolysis. Autolysis and plasmolysis are used for living cell extraction, whilst acid hydrolysis and aqueous extraction are used for non-living cells [15, 30]. Yeast autolysates are concentrated forms of yeast cell contents and are produced principally from autolysis during which hydrolysis occurs spontaneously by the endogenous enzymes present. These yeast autolysates are universally termed as 'Yeast Extracts' [10, 29].

During autolysis, naturally occurring enzymatic reactions take place, where intracellular enzymes become activated by appropriate conditions such as temperature and reaction duration resulting in partial degradation of cell wall structures. Extraction of valuable intracellular proteins, carbohydrates and vitamins is thus facilitated that maintain their native structure. Yeast extracts can also be obtained through using acids or adding enzymes that degrade cell walls and are then referred to as yeast hydrolysates, whereas yeast plasmolysates are obtained by adding large amounts of salts thereby achieving cell wall collapse by osmotic pressure. Autolysates are the purest products of these types because plasmolysates and acid hydrolysates contain large amounts of salt and sodium that are used during plasmolysis or in neutralising acids used in hydrolysis. Autolysis is frequently supplemented by adding proteolytic enzymes to enhance its effectiveness [17, 28].

Yeast extracts have diverse flavours depending on the methods used for their manufacture and are affected by the interactions between amino acids, nucleotides, carbohydrates and peptides present in the extracts. By controlling the manufacturing process, different flavours such as those of chicken soup, meat, cheese, mushrooms and others can be obtained. Meat flavours in extracts are produced, *inter alia,* by the reaction of 5'-nucleotide glutamic acid and cysteine. The sensory properties of the extracts are strongly affected by processes like thickening and drying, during which *Maillard* compounds are formed which are responsible for giving cooked meat flavours. In addition, regulating

the manufacturing process conditions can also impart different colourings to the yeast extracts, from off-white to brown, which can thereby confer a final colour to the finished food product. The presence of glutathione, *Maillard* reaction products and sulphur-containing amino acids also greatly impact on their high antioxidant properties [21].

COMPOSITION AND NUTRITIONAL VALUE OF YEAST EXTRACTS

The chemical composition of yeast extracts depends on the methods and composition of the medium (yeast slurry) used for yeast culturing. The main component of yeast extracts is hydrolysed protein, wherein the average nitrogen values of protein content in the extracts is 73-75% [27, 33].

The free amino acid content may range to 35-40% of the total protein, that includes 10-15 % di-, tri- and tetra-peptides whose molecular weights are below 600 Daltons (Da). Oligo-peptides of 2000 – 3000 Da molecular weight constitute 40-45% of the total protein, whilst the smallest fraction are the larger oligo-peptides at 2-5%. In those yeast extracts obtained by autolysis, the ratio of free amino acids to the di-, tri-, tetra- and oligo-peptides is relatively constant, but may become altered significantly, if exogenous enzymes are added for degrading cell walls. The ratio of total to free amino acids is not constant because at least 85% leucine, alanine, methionine and phenylalanine are present in their free forms whilst for aspartic acid, glycine, and arginine this only ranges at 14-37%. Such outcomes arise from the characteristics of the degradation process through the activity of proteases and peptidases, which during autolysis sever internal chemical bonds within amino acids, whereas carboxy-peptidases and amino-peptidases break the external amino acid bonds. Yeast autolysis products have high contents of B vitamins and trace elements. Vitamin contents can vary depending on the autolysis process, and, so that high amounts are therefore maintained, methodological parameters become vital such as pH, reaction times and sterilisation conditions. Differences in mineral content likewise depend on how the filtration and separation of yeast extracts are performed from their insoluble cellular components such as glucans or mannano-oligosaccharides. A standard yeast extract of 100 g contains about 3 mg of thiamine, 11.9 mg of riboflavin, 68 mg niacin, 3.1 folic acid and 30 mg calcium pantothenate. The mineral content in 100 g of a standard extract contains about 120 mg of calcium, 200 mg magnesium, 3.3 g of potassium and 5 mg of copper; the sodium content obtained when no salts are added is less than 0.5 g [16, 27].

APPLICATIONS OF YEAST EXTRACTS

The legal use of yeast extracts is covered by the Regulation of the European Parliament and Council Regulation (EC) No 1334/2008 of 16th December 2008 on flavourings and certain food ingredients with flavouring properties for use in and on foods, where extracts are classified as being natural flavourings [7, 35]. Yeast extracts have a GRAS status (Generally Recognised As Safe), which thereby promotes their likely use as natural additives. Yeast autolysates and extracts are used as additives to intensify the flavour and aroma of foodstuffs, especially those enhancing meat characteristics that contain more than 20% of the amino acids glutamic and aspartic acid, (being flavour enhancers), as well as the nucleotides 5'-GMP and 5'-IMP which act synergistically with these amino acids to impart the 'umami' (savoury) taste to foods. The flavour enhancement afforded by such yeast extract additives, through natural means, in the production of ready-to-eat food has permitted a marked reduction in the use of glutamate and ribonucleotides. These yeast formulations are used in the manufacture of many food products, including conventional and organic food: eg. in soups and sauces for reheating ready-to-eat dinner dishes, meat and mushroom fillings, cold meats, pate, savoury snacks and a range of food concentrates. In dietetic and 'light' foodstuffs, with reduced fat or carbohydrates, yeast extracts improve their sensory qualities [14, 24, 27].

Yeast extracts can also mask sour and bitter tastes, so enhancing food flavour, and simultaneously serving as food colourings and antioxidants. In those foodstuffs rich in 5'- GMP (guanosine monophosphate) and 5'-IMP (inosine monophosphate) sweet and salty flavours may be slightly reinforced but at the same time bitter and sour tastes will become significantly lessened. Improving foodstuff flavour by adding yeast extracts arises from the interaction between different amino acids (including glutamic acid), and when 5'-nucleotides are coupled to peptides and their reaction products. The combined action of these components causes a constant stimulation of taste bud receptors creating a greater sensory potential for such flavouring substances. Glutamate is the most important flavour enhancing substance and its impact threshold lies at a concentration of 100-300 ppm, whereas for 5'-GMP and 5'-IMP this threshold stands respectively at 35 and 120 ppm (concentrations in aqueous solution). Glutamate's impact on flavour may however be 10-15 times greater when used in conjunction with the 5'-nucleotides. By having appropriate control over autolysis *via* regulating temperature, pH, and reaction duration this allows manufacturers to change the sensory profile of the produced extracts, thus enabling flavours such as roast meat, bouillon (broth), meat and even cereal to be achieved [28, 29, 53, 14, 32].

Yeast extracts in the form of pastes are valuable components of both vegetarian and conventional diets, where they are used for toast spreads and as an ingredient in soups and ready-to-eat meals [1]. Using pastes or extracts in powder form for vegetarian diets is entirely justifiable as they contain all the essential amino acids, especially large amounts of lysine, valine and isoleucine along with Group B vitamins. Due to their composition, they can be used in those foodstuffs requiring enrichment of amino acids and B vitamins, for example in vegetarian and cereal products made from flour with low wholemeal content or also in vegetable juices [31]. Studies at the Department of Functional Food, Ecological Food and Commodities from the SGGW conducted by *Podpora* et al. [27] demonstrated that autolysate protein can be obtained from spent yeasts possessing specific functional features ie. with an appropriately designed content of free amino acids and amino acids in the form of polypeptides of specific molecular weight, and at the same time having high contents of essential amino acids. Such autolysates could be useful in manufacturing functional foods and dietary supplements similar to current methods for making enzymatic protein hydrolysates derived from milk protein. The study used *Saccharomyces cerevisiae*, a spent brewing yeast from Polish breweries, with a protein content above 42%. According to the conditions applied for autolysis, the products so obtained can have free amino acids ranging from 11.2% to 77.5%, and peptides are suitably designed to range from molecular weights of 1000 Da to 6000 Da. These types of autolysates can thus extend the range of protein hydrolysates found on the market that are used in food supplements and nutritional additives. They can also be particularly useful in vegetarian diets [27].

CONCLUSIONS

As by-products in the manufacture of beer and wine, spent yeasts are increasingly used not only as animal feed additives but as valued and fairly inexpensive nutrition products from which natural food additives are derived thereof such as: β-glucans and yeast extracts. β-glucans obtained from spent foodstuff yeasts, like β-glucans from oats and barley, have several health-promoting effects and can be widely used in food supplements and functional foods.

β-glucans derived from spent yeasts and baker's yeast exhibit high and multidirectional biological activity which has been demonstrated in laboratory animals; mostly consisting of improved lipid profiles in the blood and liver, stimulating immunomodulation as well as producing prebiotic and antioxidant effects. They are safe to use and possess GRAS status (Generally Recognised As Safe). Indeed, they are used in the United States; having been authorised by the Food and Drug Administration (FDA). In Europe, β-glucan and yeast fall under the Regulation of the European Parliament and Council Regulation (EC) No 1334/2008 [35] and the Regulation of the European Parliament and of the Council (EU) No 2015/2283 [34]. Yeast extracts are valuable and natural flavouring additives that reinforce meat flavours in many foodstuffs and spice mixtures, replacing the commonly used protein hydrolysates obtained by acid hydrolysis. Latest studies indicate that they can also be used in functional foodstuffs and dietary supplements as sources of amino acids and peptides. They can thus fulfil the dual function of being a pro-healthy functional additive and simultaneously being an important flavouring additive. The commonly used additive glutamic acid, and its salts, for giving meat profile flavours to foodstuffs is now increasingly being replaced by yeast extracts and spices due to the unfavourable opinions of consumers regarding the former.

Conflict of interest

The authors declare no conflict of interest.

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