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Extraction of crude Mannan oligosaccharides from yeast and their uses

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This review aims to emphasize the importance of mannan oligosaccharides, obtained from various resources especially from yeasts. Attractive features of mannan polysaccharides have had influenced the decisions of food scientists to pursue research on this element, and that may expand their uses and applications in medicine, food industry and animals feed. Food researchers are increasingly focusing on yeast cell wall (YCW) as a promising source of mannan extraction, as the yeast extracts sector generates a huge amount of YCW as a by-product. Extraction is an important method used to obtain the water-soluble mannan oligosaccharides from the wall cells of yeast and in this review was showed the optimal conditions for the extraction of mannan from cell yeast according to results some of the scientific studies along with the deproteinization methods. In the food industry, mannan has various applications such as viscosity modifiers, stabilizers, and increasing the thermo-stability of anthocyanins. Mannan has many health benefits, which have expanded its uses in the medical and pharmaceutical fields. In the animal feed sector, several studies of farm animals have shown significant positive effects of weight gain, reduced bird mortality, and improved fish growth. In pigs, the addition of mannan to pig diets led to obvious economic benefits.

Keywords: mannan, oligosaccharides, yeast cell wall, extraction, prebiotic, food industry, feed additives.

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Экстракция олигосахаридов маннана из дрожжей и их применение

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Рассмотрена роль олигосахаридов маннана, полученных из различных источников, в частности из дрожжей, поскольку незаменимые свойства полисахаридов маннана дают основание для продолжения исследования этих веществ, позволяющее расширить их применение в медицине, в пищевой промышленности и в сельском хозяйстве. Исследователи продуктов питания все больше внимания уделяют клеточной стенке дрожжей (YCW), как перспективному источнику маннана, т. к. при производстве дрожжевых экстрактов огромное количество YCW является побочным продуктом. Экстракция является важным методом, используемым для получения водорастворимых олигосахаридов маннана из клеточных стенок дрожжей. В обзоре, наряду с депротенизацией, приведены результаты научных исследований по оптимальным условиям экстракции маннана из клеточных стенок дрожжей. В современной промышленности маннан применяется в качестве модификатора вязкости, стабилизатора, а также для повышения термостабильности антоцианов. Благодаря своим свойствам маннан широко используется в медицине и фармацевтической промышленности. Исследования в области питания сельскохозяйственных животных, при добавлении маннана в рацион питания, показали существенный прирост их веса, снижение смертности, что привело к очевидным экономическим выгодам.

Ключевые слова: маннан, олигосахариды, клеточная стенка дрожжей, пребиотик, пищевая промышленность, пищевые добавки.

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Introduction

Undeniably, there has been a growing attention on mannan oligosaccharides (MOS), especially in the fields of nutrition and medicine. Indeed, the polysaccharides isolated from yeast, algae, bacteria, and higher plants have viable features that have influenced the decisions of food scientists to pursue research on these elements. According to Korolenko, Bgatova, and Vetvicka (2019), such attractive features of the various polysaccharides include rare adverse side effects, low toxicity, and relatively low price, among other therapeutic actions [1]. Glucans and mannan remain the most studied polysaccharides in the contemporary research.

The importance of mannan oligosaccharides is undoubtedly colossal and goes beyond uses and applications as both human and animal feed. According to Patel and Goyal (2011), oligosaccharides have been utilised as pharmacological supplements and food ingredients [2]. The authors further emphasize on the effectiveness of functional oligosaccharides in pathogen suppression, immunity enhancement, proliferation of gastrointestinal normal flora, and the prevention of dental caries. Patel and Goyal (2011) also argue that non-digestible oligosaccharides have been useful in such applications like weight control, in bakeries and breweries, as a humectant in confectionaries, and as a sweetener and dietary fiber. Moreover, oligosaccharides have been used in cosmetics, drug delivery, as well as in agriculture, including fishery and animal feed.

Various food companies have used mannan extracts. Montes et al. (2010) emphasized the importance of mannan in ensuring the integrity of the cell wall, as well as adhesion to both cells and tissues [3]. They are also crucial in establishing the immune response of cells, especially using mannan-degrading enzymes. Scientists have successfully obtained mannan oligosaccharides during degradation, as emphasized by Rodriguez-Gacio et al. (2012) [4]. *b*-mannanases, *endo-b1* and 4-mannanases were used to achieve the complete degradation process of mannan.

Mannan is present in seminal vacuoles and endosperms, together with non-starch reserve polysaccharides in various plants (Bzducha-Wróbel et al., 2013 [5]). There is interest in mannan extracted from copra flour, palm kernel and coffee beans (Scheller & Ulvskov, 2010 [6]). However, the isolation of polysaccharides from the cell wall of microorganisms is more favorable due to their rapid growth and the accumulation of significant amounts of biomass. It should be noted that the production of cellular polysaccharides from waste from fermentation plants is promising. Therefore, interest arose in the isolation, study and application of non-starch polysaccharides of the cell wall of microorganisms in various industries and medicine (Šandula, J. et al., 1999 [7]) in particular yeast of various genera, for example *Saccharomyces*, *Kluyveromyces*, *Candida* (Machova, E. et al., 2015, [8]), *Schizosaccharomyces* (Varona R., Perez P. & Duran A., 1983 [9]). Interesting data obtained by Machova, E.; Fiacanova, L.; Cizova, A.; Korcova, J. [8]. The authors showed that the mannanoproteins from yeast *Candida albicans* consist of comprised 41–46% of mannan and 47–53% of protein, while *Hyphal* mannanoproteins comprised 14% of mannan, 85% of glucan and 3–4.5% of protein.

The prominence of MOS as animal feed gained traction since 1999, primarily after the ban on the growth promoters

of prophylactic antibiotic in animal feed by Europe (Spring et al., 2015 [10]). Spring et al. (2015) highlighted the effectiveness of MOS not only in facilitating digestion and immunity but also providing solution for antibiotic-free diets. MOS's characteristic of limiting or binding the colonisation of pathogens in the gut is the main aspect behind such effectiveness.

Studies have also reiterated the importance of MOS in improving gastrointestinal health (Patel & Goyal, 2011 [2]), thus enhancing the performance (Ganner & Schatzmayr., 2012 [11]), energy levels, as well as the well-being of humans and animals. MOS has demonstrated effectiveness in improving the growth performance and general health of fish (Torrecillas et al., 2007 [12]; Staykov et al., 2007 [13]; Burr et al., 2008 [14]).

There is growing attention among food scientists towards yeast cell wall (YCW) as a dominant source of bioactive molecules (Li & Karboune, 2018 [15]), including mannan oligosaccharides and β -glucan. Undoubtedly, the yeast-extract producing sector is generating a colossal amount of YCW as a by-product. Such production of large amounts of YCW bi-product is attributed to high demand for yeast extract as a flavoring agent, mainly derived from nucleotides, peptides, and amino acids (Chae, Joo, & In., 2001 [16]). Borchani et al. (2014) argue that YCW is being explored for potential use as a source of beta-glucan and has been utilized extensively as an animal ingredient [17]. Indeed, Huang, Yang, and Wang (2010) described YCW as a «*non-specific stimulator of the immune system of both man and animals*» [18]. YCW can bind with undesirable components, thereby preventing and curing stuck fermentations.

In doing so, this characteristic of YCW makes it suitable for use in the wine industry. Nonetheless, there has been a little investigation of the extraction of mannanoproteins, which constitutes approximately 40%, w/w, and are the second-most essential components of the YCW (Quirós et al., 2011 [19]). Similarly, Huang et al. (2005) estimated the contents of mannan oligosaccharides and β -glucan at 30–60% of the YCW [20]. Liu et al. (2018) and Lipke & Ovalle (1998) also reiterated that mannanoproteins are not only glycoproteins but also contain as high polysaccharides as 50–95% [21, 22]. While acknowledging the crucial role of β -glucan in stimulating macrophages and overcoming bacterial infections, Huang, Yang, and Wang (2010) also emphasize the role of mannan oligosaccharides in preventing diarrhea in weaning pigs [18].

By binding itself to the pathogenic bacteria in the gut, mannan oligosaccharides can get rid of the bacteria out of the intestinal tract (Ganner & Schatzmayr, 2012 [11]). In so doing, Mannan oligosaccharides are critical in preventing diseases (Fowler et al., 2015 [23]; Kwiatkowski S. & Kwiatkowski E., 2012 [24]; Van der Werf, 2019 [25]). It is also essential for the growth of the beneficial bacteria in the colon by acting as a nutrient source to the microorganism. Therefore, the current research seeks to explore the techniques of extracting crude mannan oligosaccharides, especially in the wake of the myriad biological functions associated with YCW.

Food researchers are increasingly focusing on yeast cell wall (YCW) as the dominant source of bioactive molecules (Éder Galinari, Diego Araújo Sabry, et al., 2016 [26])

(Li & Karboune, 2018 [15]), including mannan oligosaccharides and β -glucan. Moreover, this characteristic of YCW makes it suitable for use in the wine industry. Nonetheless, yeast extract is widely used in the food industry and medicine. Undoubtedly, the yeast extract production sector generates an enormous amount of YCW as a by-product. peptides and amino acids (Chae, Joo, & In, 2001 [16]). Borchani et al. (2014) claim that YCW is being studied for potential use as a source of beta-glucan and is widely used as an ingredient in animal origin [17]. A small study was conducted on the recovery of mannoproteins, which make up approximately 40% by weight and are the second most important component of the YCW (Quirós et al., 2011 [19]). Similarly, Huang et al. (2005) estimated the content of mannan and β -glucan oligosaccharides in 30–60% of YCW [20]. Liu et al. (2018) and Lipke and Ovalle (1998) also confirmed that mannoproteins are not only glycoproteins, but also contain polysaccharides in an amount of 50 to 95% [21, 22]. Studies have also confirmed the importance of MOS for improving gastrointestinal health (Patel & Goyal, 2011 [2]).

Thus, using microorganisms can significantly increase the production of mannan, which is necessary for various industries. Primarily, medicine, livestock, and the food industry (Ganner & Schatzmayr, 2012 [11]).

Therefore, current research is aimed at studying methods for the extraction of crude mannan oligosaccharides, especially in light of the many biological functions associated with YCW.

Extraction of mannan

The extraction of mannan oligosaccharides should be studied together with the necessary method of deproteinization, since the majority of mannan in the cell wall exists in the form of a complex mannoprotein.

There are three important methods that can be used to extract pure mannan oligosaccharides and deproteinization, including the Sevage, hydrochloric acid, and trichloroacetic acid methods (Yamabhai et al., 2013 [27]).

The three deproteinization methods operate in different principles. The Sevage method denature dissociative protein using an organic solvent and an insoluble substance. The centrifugation method is applied to pure mannan oligosaccharides (Huang, Yang, & Wang, 2010 [18]). Similarly, the TCA method operates under the principle that the protein cations have the potential to link the TCA, thus forming an insoluble salt at an isoelectric point. Finally, the hydrochloric acid method of deproteinization is used because of the belief that the protein is soluble at low pH, thus ensuring optimum deproteinization and a slightly higher rate of mannan oligonucleotide loss compared to the other methods.

The extraction water-soluble mannan oligosaccharides from 5 g yeast is done with the help of 1% of 50 mL sodium hydroxide subjected to 100 °C for two hours (Huang, Yang, & Wang, 2010 [18]; Yamabhai et al., 2013 [27]). The cooling and neutralizing of the soluble mannan oligosaccharide are done at a pH of 7 using a diluted chlorine solution (Bzducha-Wróbel et al., 2013 [5]). Absolute ethanol (200 mL) is added to the mannan oligosaccharides to precipitate it after filtration. Both diethyl ether and absolute ethanol are used for washing the precipitate.

The use of mannan in medicine

Huang, Yang, and Wang (2010) described YCW as a «non-specific stimulator of the human and animal immune systems» [18]. YCW can bind to undesirable components, thereby preventing and eliminating stuck fermentation. The fact of the adsorption of pathogenic microorganisms by mannan was discovered (Li, J. & Karboune, 2018 [15]).

It has been found that in addition to the action of individual mannan or glucans, glucomannan conjugates contribute to lowering cholesterol and improving immune responses (Onitake, T. et al., 2015 [28]).

The immune-modulating properties of polysaccharides were used to develop new approaches to the prevention and treatment of hyperlipidemia and atherosclerosis. (Korolenko T. A., Johnston TP et al., 2018 [29])

Mannan has probiotic activity and stimulates the growth of selected beneficial intestinal bacteria. These gut bacteria include specific groups such as *Lactobacillus* spp.

Enterococcus spp., *Bifidobacterium* spp, etc. (Roberfroid et al., 2010 [30]).

Ghosh, S, et al. emphasized the importance of mannoproteins as a probiotic During the study, which was carried out on calves. The results showed that a prebiotic (mannan oligosaccharide) can be given to calves for better performance (Ghosh, S et al. 2012 [31]).

Loginova and et al showed that the addition of mannan to the intestinal microbiota reduces plasma cholesterol and the development of atherosclerosis. (Loginova et al., 2013 [32]). That is confirmed in recent works by Hoving, L. R and al. (2018) [33].

The use of mannan in livestock, aviculture, and fisheries

Mannan oligosaccharides are recommended to be used as feed additives. To increase mass gain and improve cattle health, a yeast fraction rich in hydrolyzed mannan and glucan was proposed (Pukrop, J. R. et al., 2018 [34]).

Credence Research (2018) and Spring et al. (2015) found that many feed industries continue to use yeast cells as substitutes for antibiotics [35, 10]. Gene et al. (2018) argue that the immune-modulatory properties of yeast β -glucans improve the innate and associated adaptive immune system. As noted by Piotrowska and Masek (2015), β -glucans increase the resistance of livestock to infections because they can bind mycotoxins [36].

In pigs, especially weaned piglets, mannan oligosaccharides have proven useful. Weaned piglets experienced stressful events that could interfere with their immune and intestinal responses, leading to reduced growth, health and feed intake (Campbell et al., 2013 [37]). According to Spring et al. (2015), piglets fed mannan oligosaccharides had an improved feed intake of 1%, a bodyweight of 3.6%, and a feed conversion ratio of 3% [10]. In addition to weaned piglets, feeding sows with mannan oligosaccharides increased litter size and improved colostrum production and quality (Taylor-Pickard, 2015 [38]). Therefore, adding mannan oligosaccharides to pig diets can lead to huge economic benefits.

The benefits of calf mannan oligosaccharides have become critical since they are used as milk substitutes. Mannan oligosaccharides, added to milk and other substitutes, help

improve feces in these calves because mannan oligosaccharides act as antibiotics (Spring et al., 2015 [10]). As a result, it curbs diarrhea, which is a significant cause of death among young animals. According to Berge et al. (2016) [39], calves treated with mannan oligosaccharides after adding milk to the substitutes reported improved productivity, markedly increased total body weight by 15% and gained daily body weight by 10%. Thus, mannan oligosaccharides have proven to be extremely important for calf productivity and health. Yeast cell wall supplements in poultry feed have been beneficial. Various studies have shown that birds feeding on yeast cell wall supplements gain weight and report high feed conversion rates (Spring et al., 2015 [10]). Similarly, Hooge et al. (2013) reported a decrease in the mortality rate of birds feeding on manna oligosaccharides [40]. For example, in more than 100 studies with broiler chickens Hooge et al. (2013) noted that broilers that fed mannan oligosaccharides reported improved productivity and increased body weight by 2.0–3.4% and improved feed conversion ratio by 2.3%. Similarly, turkeys fed on these yeast cell wall supplements reported improved performance.

MOS has been shown to be effective in improving growth and overall health of fish (Torrecillas et al., 2007 [12]; Staykov et al., 2007 [13]; Burr et al., 2008 [14])

The use of mannan in the food industry

Mannan oligosaccharides have been useful in many sectors, including but not limited to the food industry. Recent studies have reiterated the importance of mannan extracted from YCW. In a survey conducted by Al-Manhel and Niamah (2017) [41], mannan extracts from yeast cells were utilized in the bio-yogurt production. The researchers obtained the mannan extracts from four different sources before mixing them with buffalo milk. In this study, the authors identified the mannan extract using their physiological and morphological properties. The study utilized a probiotic starter culture containing *Streptococcus thermophiles*, *Bifidobacterium sp.*, and *Lactobacillus acidophilus*. The outcome of the study reported a decline in the pH values after fermentation to 4.6–4.8 down, with a total acidity level of 0.89–1% (Al-Manhel & Niamah, 2017 [41]). Importantly, mannan extract was useful in the study as it increased the viability of probiotic bacteria, particularly after the yogurt production process.

Jane Wu, and et al found that mannoprotein increases the thermal stability of anthocyanins, which are food colourants with strong antioxidant activities. The study showed that the addition of mannoproteins decreased the degradation rate constant and increased the half-life by 4 to 5-fold. Thus, mannoproteins can expand the use of anthocyanins as natural colourants (Jane Wu et al., 2015 [42]).

The various properties of mannan, such as water stability, viscosity, and biodegradability may expand its application in the food industry and it can be used as a perfect stiffener and as an emulsion stabilizer, non-toxicity permits their use in different forms for human consumption in food products along with biomedical, and pharmaceutical (Cerqueira, M, A et al., 2011 [43]).

Conclusion

Mannan is a part of the polysaccharides found in plant tissues, especially hemicelluloses (Bzducha-Wrobel et al.,

2013 [5]). Both heteromannan and mannan form part of the yeast cell and make up glycoproteins. Therefore, mannan is responsible for metabolic networks, storage, and structural functions.

The importance of mannan oligosaccharides is undoubtedly enormous and goes beyond the use and application as food for humans and animals. In addition, oligosaccharides were used in cosmetics, drug delivery, and agriculture, including fishing and animal feed.

The optimal conditions for the extraction of mannan from cell yeast were when using a 1% NaOH solution at a temperature 100 °C for 2 hours.

There was more loss in the recovery of mannan oligosaccharides using the TCA method and hydrochloric acid method compared with Sevage method, which exhibited a lower percentage of deproteinization, but a little low percentage of polysaccharide loss. That may be due to more evident damage of mannan oligosaccharides caused by TCA and hydrochloric acid, respectively.

The Sevage method is not widely used to remove proteins, because it contains poisonous chloroform, which is environmentally disadvantageous.

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