Mannans and mannan oligosaccharides (MOS) from *Saccharomyces cerevisiae* – a sustainable source of functional ingredients

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Abstract

Sustainable industry practices and circular economy concepts encourage the transformation of production waste into by-products. *Saccharomyces cerevisiae* is widely used in fermentation industry worldwide, generating large amounts of spent yeast which is mainly directed to animal feed or discarded as waste. Instead of becoming and environmental problem, spent yeast can be directed to the extraction of valuable compounds such as mannans and mannan oligosaccharides (MOS). This review presents a compilation of the studies up to date regarding the different chemical, enzymatic, mechanical or physical processes addressed for mannans extraction and MOS production. Additionally, the existing studies on the chemical modification of mannans aimed to improve specific characteristics are also discussed. Finally, the more relevant bioactivities and potential applications of mannans, MOS and mannose are presented, together with products on the market containing these compounds.

Keywords: Yeast, Extraction methods, carboxymethyl mannans, Chemical modification, by-products valorisation, bioactivities

Contents:

1. Introduction

Annual worldwide production of beer accounted for 193 billion liters in 2017, enough to fill 77,000 Olympic swimming pools. This production generated 46.26 billion tons of by-products, among which spent yeast corresponds to 12.7%. It is estimated that for every 100 liters of beer produced, 1.5 – 3 kg of spent yeast is disposed (*Brewers By-products Market Outlook, Share, Size, Forecast, Trends, Report*, 2020; *World beer production | Statista*, 2020; Nanyang Techological University, 2017) Indeed, Brewer's spent yeast (*Saccharomyces cerevisiae*, *S. pastorianus* and *S. carlsbergensis* species) is the second major by-product from the brewing and wine industry (Vieira et al., 2016).

The valorization and reuse of spent yeast to extract functional compounds and develop innovative products is an excellent circularity of the industry, in the perspective of the food-health relationship, as well as in the protection of the environment and waste management. *S. cerevisiae* (Brewer's yeast) is described as a *Generally Recognized as Safe (GRAS)* microorganism (Rakowska et al., 2017) and, besides its nutritional value, it also presents some interesting bioactive properties.

Such nutritional and bioactive composition has been increasingly attracting the cosmetic, pharmaceutical, and food industries. Indeed, yeast residue is currently an important ingredient for the food (Butylina et al., 2007) and cosmetic industries; in the latter it is used, for instance, to integrate hydration products formulations (Gaspar et al., 2008).

Mannans are polysaccharides widely present in nature, such as in plants, bacteria, yeasts and other organisms (Olaniyi et al., 2013). They have several commercial applications,

derived from their physicochemical properties (water solubility, viscosity, and stability); typical applications are as a hardener ingredient and as an emulsion stabilizer (Singh et al., 2018).

Additionally, mannans also exhibit relevant health benefits, such as inhibition of pathogen adherence, modulation of bacterial growth (Smith et al., 2020) and improvement of the immune response (Lee & Dugoua, 2011; Onitake et al., 2015). In general, polysaccharides with immunostimulant properties interact (indi)directly with various parts of the immune system, subsequently stimulating different immunological mechanisms (Yin et al., 2019). Due to the abovementioned properties, yeast mannans are commonly used in animal feed as antibiotic replacers (Smith et al., 2020; Spring et al., 2015).

As above described, mannans have been increasingly reported as presenting very promising activities, which tailor their use in various fields of application. The present study intends to overview the current knowledge on yeast mannans, focusing on extraction processes, mannans hydrolysis to produce MOS, and its chemical modification to enhance bioactive properties, a field of research still in its infancy. Furthermore, described bioactivities and potential applications of mannans, MOS and mannose are also revised, as well as an overview on the main products in the market.

2. Mannans

2.1. Sources and Composition

Mannans are long-chain carbohydrates mainly composed of mannose residues (Tester & Al-Ghazzewi, 2013), which can be found in the most diverse sources, such as vegetables, microorganisms and seeds (Figure 1).

Structurally, mannans and heteromannans (composed of two or more different monomers) are polysaccharides distributed in nature as part of hemicelluloses in plant tissue (Capek et al., 2000; Scheller & Ulvskov, 2010) or as a constituent of glycoproteins in yeast (e.g., *Saccharomyces cerevisiae*) or bacteria cell walls (e.g., *Helicobacter pylori*) (Ballou, 1974, 1976; Singh et al., 2018).

In plants, mannans and heteromannans are an essential component of the hemicellulose family and can be classified into four subfamilies, according to their monosaccharide composition: pure mannans (containing only mannose); glucomannans; galactomannans and galactoglucomannans (Libjaková et al., 2007; Schröder et al., 2009; Singh et al., 2018). Some of the plant-derived mannans are well known and widely used as thickeners (Millane & Hendrixson, 1994), stabilizers (Mikkonen et al., 2009), and gelling agents (Maekaji, 1974) in the food industry, e.g., locust bean gum galactomannan or konjac glucomannan.

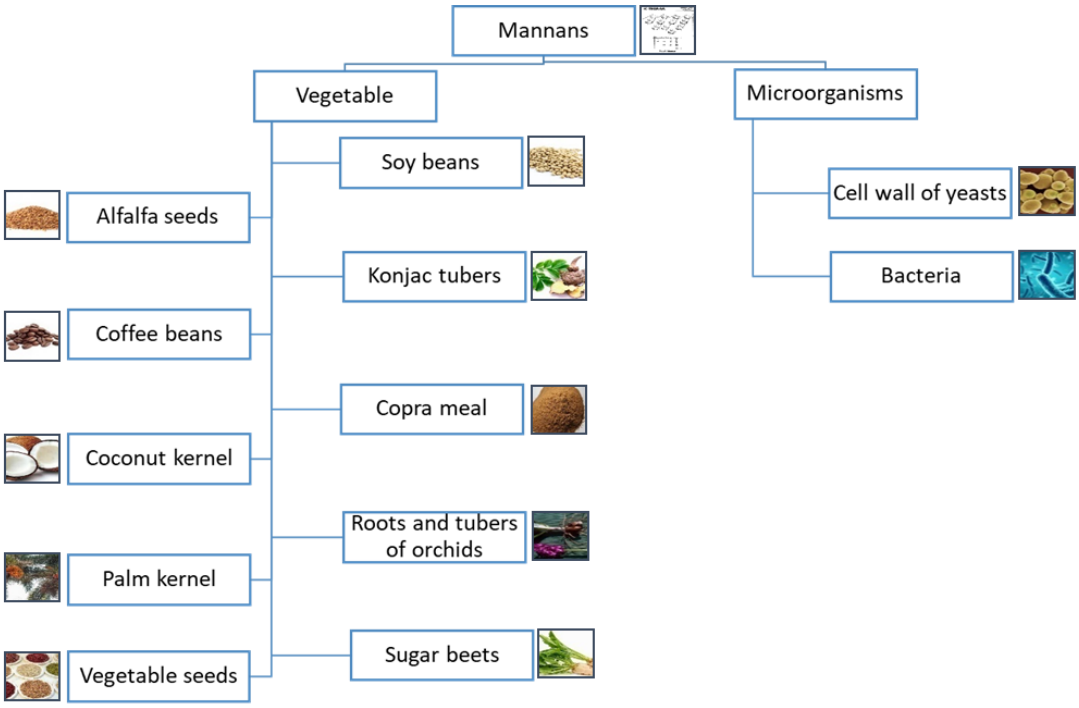

Figure 1: Sources of mannans(Olaniyi et al., 2013; Singh et al., 2018).

Mannans can also be found in the cell wall of various fungi, as summarized in **Table 1**.

Table 1 - Sources of mannans from Fungi, main species and monosaccharide composition (type).

Source	Species	Type	Reference
Fungi	*Aspergillus fumigatus*	Galactomannans	(Stynen et al., 1992)
	Penicillium oxalicum		(Kurakake et al., 2006)
	Trichophyton fermentans		(Gorin et al., 1969; Ikuta et al., 1997)
	Torulopsis gropengiesseri		(Gorin et al., 1969)
	Candida albicans		(Vazquez-Reyna et al., 1999)
	Candida lipolytica		(Ikuta et al., 1997)
	Saccharomyces cerevisiae	Mannans*	(Orlean, 2012)
	Candida utilis	Glucomannans	(Miadoková et al., 2006)

*Mannans from *Saccharomyces cerevisiae* also contain a residual fraction of glucose which is part of the linkage between the polysaccharide and the protein.

The cell wall composition of yeast and filamentous fungi varies between species, although there are structural similarities (Abbott et al., 2015). The three major components of *S.*

cerevisiae cell wall are mannoproteins (mannans bonded to protein), glucans, and chitin (Orlean, 2012; Pinto et al., 2015). These three components assign approximately 90% of the entire cell wall, with proportions varying significantly based on stress, environment, and growth stage (Orlean, 2012). Mannans are described as being present both in the yeast cell wall and periplasmic space, always associated with proteins (Kath & Kulicke, 1999; Orlean, 2012).

2.2. Mannans' relevance in *Saccharomyces cerevisiae* cell wall

Mannans are an important structural component of the yeast cell wall (Kath & Kulicke, 1999), with a composition directly related with the chemical and environmental conditions prevailing during culture growth (Bonaly et al., 1971). The yeast presented a cell with an oval to round shape, normally having an average diameter of approximately 5-13 µm (Kath & Kulicke, 1999). A schematic representation of the cell is shown in **Figure 2**, as described in the literature.

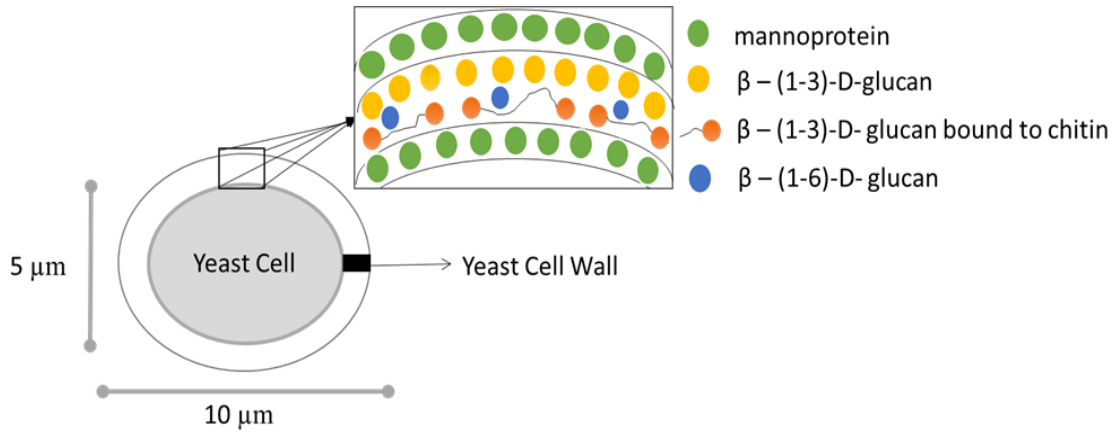

Figure 2: Yeast cell wall structure consisting of two layers: a mannoproteins complex located on the outside, and glucans (chains) as a structural polymer on the inside. Adapted from Kath & Kulicke (1999).

The cell wall is estimated to have ca. 70 nm of thickness with a layered structure, and represents 15-30% of the cell dry weight (Bacon et al., 1969; Kath & Kulicke, 1999; Klis et al., 2006; Latgé, 2007). Its chemical composition consists of 3% ash; 13% protein; 8.5% lipid (mostly neutral fat) and two major polysaccharides, glucans and mannans; the latter are associated with protein present in the wall, forming mannoproteins complexes (Northcote & Horne, 1977). More specifically, mannoproteins range 35%, and glucans are divided into β-(1-3)-D-glucan (25%) - β-(1-3)-D-glucan bound to chitin (35%) – and β-(1-6)-D-glucan (5%) (**Figure 2**) (Ikuta et al., 1997). It is also possible to observe a small amount of chitin (1-2%) (Feuillat, 2003; Orlean, 2012).

While mannans are the second major polysaccharides of the yeast cell wall, glucans are established as the main components responsible for the structural integrity of the cell wall (Eggensperger, 1997; Kath & Kulicke, 1999), playing a key role in the cross-linking of

its components (Kollár et al., 1997). Chitin is a linear polymer of 1-4 linked *N*-acetyl glucosamine units with relatively minor abundance. Still, it is an important constituent in the performance of the role maintaining the rigidity and the morphology of the cell (Matsumoto et al., 1980). The relative quantities and localization of individual wall components vary according to the cell cycle or developmental stage, growth phase, nutritional conditions, and wall stresses imposed by hypo-osmolarity, mutational loss of wall bio- synthetic activities or wall proteins, or drug treatment (Orlean, 2012).

2.3. Structure

Mannans are generally associated with proteins in the outer and inner membrane. Such structure (mannoprotein complex) can be represented in three different sections: the peptide chain; the core region (central region) and in the external chain, in which the polymeric fraction is located (**Figure 3**). The polymeric region consists of an α-(1-6)-glycosidic basic chain that carries α-(1-2)- and α-(1-3)-glycosidically linked oligomannans as side groups. The peptide region contains oligomeric mannans directly linked to amino acids.

Characteristically, *S. cerevisiae*'s mannans are composed of long D- mannose chains linked by α-(1-6) bonds (backbone), with short side chains in the α-(1-2) and α-(1-3) linkages (Ballou, 1974; Peat et al., 1961); these macromolecules are linked to asparagine residues (Ballou, 1974; Sentandreu & Northcote, 1968) (**Figure 3, a)**. In addition, mannans are also present as small oligomannosidic units (mannan oligosaccharides – MOS) with α-(1-2) and α-(1-3) linkages that are linked to serine or threonine residues (**Figure 3, b**).

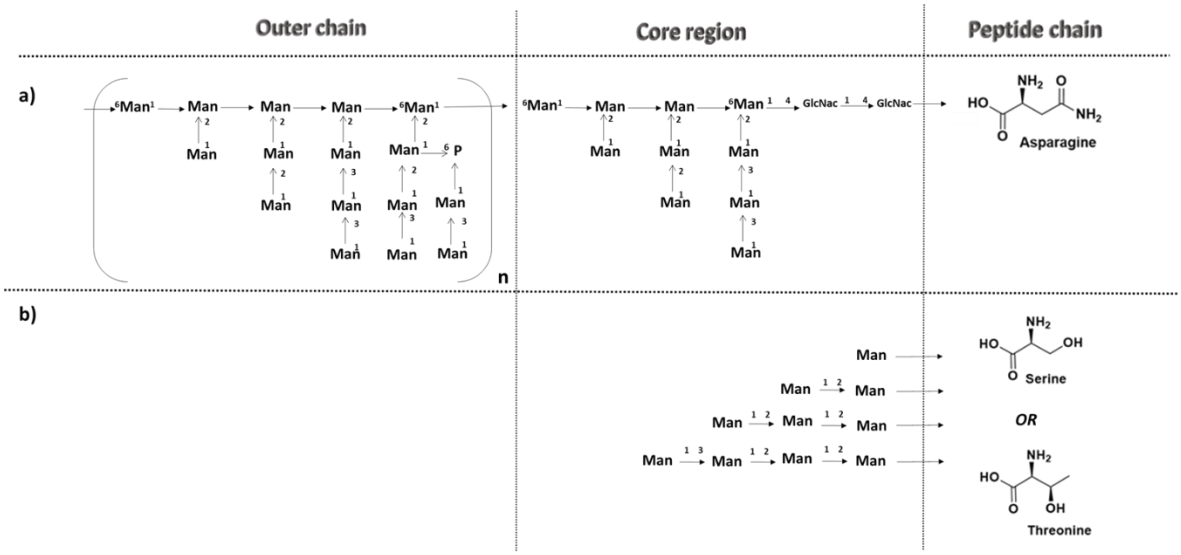

Figure 3: Representation of the mannoproteins complex, adapted from Kath & Kulicke (1999), and Ballou (1976). Man represent Mannose, P represent Phosphorus and GlcNac represent glycosidic linked.

2.4. Extraction Methods

Mannans extraction usually includes several steps, namely: yeast cell lysis; separation of the released mannoproteins from the remaining cell components; fractionation of mannoproteins and purification of the resulting mannans fraction. Depending on the purity requirements of the final extract, some of the abovementioned steps can be circumvented or extended. Cell lysis is the most studied step in the process, as it will dictate the final yield. The yeast cell lysis can be performed through chemical (usually using alkaline reagents or detergents); enzymatic (taking advantage of the existing internal enzymes – autolysis – or by addition of external enzymatic cocktails); physical (relying on temperature and pressure) or mechanical processes (e.g. by grinding cells with glass beads).

In early studies, mannans were isolated by extracting either whole yeast cells or isolated cell walls with alkali, followed by precipitation of the solubilized mannans, as copper complex, with Fehling's solution (Fritsche, 1971; Haworth et al., 1937; Orlean, 2012; Phaff, 1963). These processes also employed high temperature and pressure, typically by autoclaving whole cells or cell walls in a neutral or alkaline solution (Fleet, 1985).

Mannans can also be extracted by cell wall enzymatic hydrolysis with proteases (Cawley et al., 1972; Kath & Kulicke, 1999; Russell et al., 1973) and glucanases (Fleet & Manners, 1977; Kath & Kulicke, 1999; Shibata et al., 1983; Valentin et al., 1984).

Although the recovery of mannan from solution can be performed by the abovementioned mannan-copper complex, solvent precipitation (e.g. with ethanol, methanol or acetone) has become more conventional. Other commonly used purification methodologies include chromatographic methods (e.g. affinity chromatography, size exclusion chromatography) and ultrafiltration.

More recently, other technologies, such as ultrasounds (US) and pulsed electric field (PEF) have shown some encouraging results (Martínez et al., 2016; Snyman et al., 2021). The use of US for mannoprotein extraction was explored by Snyman et al. (2021); in this study mannoprotein was extracted by US, enzymatic hydrolysis or a combination of both procedures and the yields were compared. Authors suggest that the extraction conditions that result in the best yield would involve subjecting the yeast cells to US, followed by an enzymatic hydrolysis – exact conditions presented in Table 2.

Other comparison studies have also been published analysing different methodologies for the extraction of mannoprotein, such as the publication by Li et al. (2020), in which three approaches under scrutiny were thermal, sodium dodecyl sulfate (SDS) and enzymatic (Zymolase®) treatments. The methodology which resulted in the highest mannoprotein yield was the enzymatic treatment (98% and 78% in Baker's and Brewer's yeast, respectively). Both studies used the same enzymatic treatment (Zymolase®) and both point to better yields resulting from those treatment when compared to other methodologies. However, the use of different analytical techniques for the quantification of mannan and the lack of standardized information does not allow for an unambiguously withdrawal of conclusions.

In an effort to compare results from a myriad of publications, a compilation of the different methodologies, the mannan/mannoprotein purity and a calculation of the yield, whenever possible, is provided in Table 2.

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227 Table 2 – Methods for extraction of yeast mannans
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Yeast Source	Solvent	Ratio (yeast weight; by volume of solvent)	Cell lysis Process	Separation Process I	Separation Process II	Purification Process	Yield of Process (%) *	Extract Purity (%)	References
Baker's yeast	Citrate buffer 0.19 mM; pH 7.0		Autoclave 2 h; 120 °C	Ethanol precipitation	Copper complex with Benedict's solution	Purification resin (to eliminate the residues of copper complexes formed)	0.77%		(Stewart et al., 1968)
Baker's yeast	6% NaOH		Boiling temperature, 8 h	Acidification (acetic acid). Centrifugation and neutralization of supernatant. Ethanol precipitation	Complex with Fehling's solution	Washing with warm water and ethanol 60%	First step 2.1%; second step 65%		(Haworth et al., 1937)
Baker's yeast	Citrate buffer 0.19 mM; pH 7.0		Autoclave 2 h; 140 °C	Centrifugation and washing of solid with acetic acid. Ethanol precipitation	Solubilisation of pellet in water and alkalinisation. Precipitation with Fehling's solution	Washing with warm water, acidification and precipitation with ethanol	First step 0.99%; second step 0.54%		(Peat et al., 1961)
Candida atmospherica *C. diddensii* *C. parapsilosis* *Debaryomyces globosus* *Hansenula angusta* *Pichia bispora* *Saccharomyces lactis (NRRL*	Citrate buffer 0.02 M; pH 7.0	50%	Two cycles of autoclave for 90 min; 125 °C	Centrifugation. Alkalinisation of supernatant.	Complex with Fehling's solution	Purification in cationic resin	After two precipitions (Fehling's solution) 1.7 – 0.42%		(Kocourek & Ballou, 1969)

Y1140 and NRRL Y1250) Saccharomycodes ludwigii Lodderomyces elongasporus									
Saccharomyces cerevisiae	1% NaOH	10%	2 h; 100 °C	Centrifugation and filtration of the supernatant	Ethanol precipitation	Isoelectric point (to eliminate protein)	18%	96 %	(Liu & Huang, 2018)
Baker's yeast	50 mM phosphate buffer (pH 5,7,9)	5%	Autoclave 4 h; 120 °C	Centrifugation and collection of the supernatant		Enzymatic isolation using Zymolase	12%		(Li & Karboune, 2018)
Baker's yeast	water	1-20%	90 °C-100 °C; 1-3 h	Centrifugation and collection of supernatant		α-mannanase at 3 -4% for 8-12 h at 50-70 °C, pH 4.0-6.0; Proteases at 0.5-3% for 6-10 h at 50-70 °C, pH 7.0-8.0		40-44%	(Yu et al., 2008)
Baker's yeast	0.1M potassium buffer solution	20%	Autoclave for 3 h; 120-121 °C	Centrifugation and collection of supernatant	Precipitation with 3 volumes of 95% ethanol containing 1% (v/v) acetic acid	Ultrafiltration	18%		(Cameron et al., 1988)
Baker's yeast	Tris buffer; pH 7.5	4%	Mechanical disruption with glass beads diameter (0.25-1.5 mm) for 12 min	Enzyme cocktails (mixture of proteases and glucanases) EC1- an enzyme cocktail from *Helix pomatia* Conditions: pH=5.0, 37 °C EC2- an enzyme cocktail from *Cytophaga* sp. Conditions: pH=7.5, 37 °C EC3- lyticase		Insoluble glucan was separated by centrifugation and mannans were purified by dialysis			(Kath & Kulicke, 1999)

Brewer's yeast			Autolysis 16 h; 55 °C	Protease (Protex 6L) at pH=9.5, 55°C during 16 h		Ultrafiltration (10 kDa)			(Sedmark, 2014)[a]
Brewer's yeast			Autolysis 14 h; 55 °C	Protease, Amylase and/or Lipase (Protex 6L and Glucoamylase); pH=9.5, 55°C, 14 h + 4 h with Glucoamylase		Ultrafiltration (10 kDa)			(Sedmark, 2014)[a]
Baker's yeast and brewer's yeast	Sodium phosphate buffer (50 mM, pH 7.5)	5%		Enzymatic hydrolysis (167U/g DC;35ºC;4h)		Affinity chromatography	27%-31% (Mannan Recovery Yield: 98%-75%)	37%-38%	(Li et al., 2020)
Saccharomyces boulardii SB62	0.1 M phosphate buffer, pH 6.5		Ultrasounds (4min; 80% amplitude; 50% duty cycle)	Enzymatic hydrolysis with a glucanase (4000U lyticase/g DC;37ºC;20h)	Precipitation with 3 volumes of 100% Acetone				(Snyman et al., 2021)

229 * The yield was obtained from the weight of extract divided by the weight of original yeast (wet or dried) and multiplied by 100

230 [a] Patent US8753668B2

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From the analysis of Table 2 it can be observed that, from the several methods available for extracting/isolating yeast mannans, final. Yields are usually inversely related with purity, *i.e.* the higher the purity of the final extract, the lower yield will be obtained, as a consequence of the increased number of steps required for purification. Nevertheless, there is no "perfect" extraction method since the targeted application of the extract will drive the choice of the method. The highest yields are usually obtained when using enzymes, but this may also increase costs, depending on its specificity and enzymatic hydrolysis conditions, such as temperature or time, when compared to other extraction methods. The high temperature procedures may present the additional advantage of decontamination from a possible microbial presence.

One important issue noticed from literature search is that many studies prefer to focus on glucans extraction from yeast cell wall, or the simultaneous recovery of mannans and glucans, which makes sense from a commercial point of view, since both molecules have shown biological activity. Patents that describe extraction methods to simultaneously obtain mannans and glucans (e.g. Patent US8753668B2 (Sedmark, 2014), Patent US9320291B2 (Juhani Saarinen et al., 2016), Patent EP0950716B1 (Lazzari, 1999), Patent US20110045545A1 (Yu et al., 2011)) involve the methodologies above described, such as autolysis, enzymatic hydrolysis, alkaline and acid hydrolysis. The fractionation and purification of these extracts involves a myriad of methodologies such as solvent precipitation, chromatographic methods, and ultrafiltration, depending on the characteristics and purity expectations of the final extract.

2.5. Chemical Modification

The biological functionalities of different polysaccharides could be tuned and improved by the introduction of specific chemical moieties in the polysaccharide native structure through derivatization (Li et al., 2016; Xie et al., 2020; Xu et al., 2019). Regarding the chemical modifications of mannans isolated from yeast strains, it is clear that this field is only in its infancy, with the majority of studies being focused on the carboxymethylation of pathogenic yeasts of *Candida* genus (Korcová et al., 2015; Machová, Bystrický, et al., 2014; Machová, Čížová, et al., 2014; Oka et al., 1972).

The carboxymethylated yeast mannans have already demonstrated their higher performance over the underivatized mannans, namely in the antitumor activity, where the carboxymethylation demonstrated to be an effective pathway to remove the toxicity of the original mannans without loss of antitumor activity (Oka et al., 1972), but also in the antioxidant (Korcová et al., 2015; Machová, Čížová, et al., 2014) and thrombolytic activities (Korcová et al., 2015). Furthermore, it is also possible to find reports focused on the preparation of different oxidized forms of mannans from Candida genus used for the preparation of glycoconjugates vaccine precursors (Ďurana et al., 2006), and in the preparation of cationic and amphoteric mannans from *Candida albicans* (Čížová et al., 2016, 2019).

Regarding the chemical modification of *S. cerevisiae,* the first report (dated from 2014) described the efforts in the optimization of the synthetic pathway in order to obtain carboxymethyl derivatives of yeast mannans, and the elucidation of their structure and properties through a myriad of characterization techniques (FTIR-ATR, UV measurements, optical rotation, and potentiometric titration) (Machová, Bystrický, et al., 2014). Moreover, it is important to emphasize that while the carboxymethylation is well studied in the functionalization of insoluble (cellulose, starch) and soluble (dextran, hyaluronan) polysaccharides, in the specific case of mannans functionalization it is evident the demand for more information. The abovementioned study constituted one of the few studies of mannans functionalization by carboxymethylation using three different procedures/media (water/alcohol system; non-aqueous dimethyl sulfoxide system and only in water). The derivatization of mannans in pure water under alkaline medium was described as the most promising procedure after the optimization of the following set of reaction parameters: mannans and sodium monochloroacetate concentrations, reaction temperature and time, which led to the establishment of the best conditions to obtain the carboxymethylated product - 100 mg of mannan in 1 mL of 6 M sodium hydroxide at room temperature for 30 min, followed by addition of 300 mg of sodium monochloroacetate at 70 °C for 5 h, and neutralization by acetic acid to pH 7 followed by dialysis. Under these conditions, the carboxymethyl derivative of *S. cerevisiae* mannans was obtained with a good yield, with a degree of substitution of 0.43 (determined by potentiometric titration) and the strong alkaline conditions revealed to have a negligible effect in mannans' degradation. The successful carboxymethyl derivation of the original mannans was undoubtedly confirmed by FTIR-ATR with the assignment of a new vibration in the spectrum at 1587 cm^{-1} and 1408 cm^{-1}, indicating the substitution of $COO^{-}Na^{+}$ group on mannans chain. The UV measurements also corroborated the successful chemical modification by the UV absorbing carboxylate in the carboxymethyl mannans product. The ^{1}H NMR data showed to be complex due to the non-uniform carboxymethylation, which led to the impossibility in the assignment of all NMR resonance signals, while optical rotation reflected the structural pattern of the different compounds with α-glycosides exhibiting positive rotation (Machová, Bystrický, et al., 2014).

In the same year, the carboxymethyl derivatives from mannans *S. cerevisiae* obtained by the optimized conditions above described (Machová, Bystrický, et al., 2014) were studied as potential antioxidants, along with the carboxymethyl derivatives from β-glucan and dextran (Machová, Čížová, et al., 2014). The antioxidant potential was ascribed by the analysis of hydroxyl radical capture, the DPPH radical-scavenging and by the Fe(II) chelating activity. All carboxymethyl derivatives proved to be stronger antioxidants against the hydroxyl radical and presented a higher Fe(II) chelating activity against the original mannans. Regarding the scavenging activity against DPPH, the carboxymethyl products exhibited a lower performance when compared with the underivatized ones.

The properties and antioxidant performances for the carboxymethyl mannans extracted from *S. cerevisiae* are depicted in Table 3.

Table 3 – Compilation of properties and antioxidant activities of *S. cerevisiae* mannans and functionalized mannans (Machová, Bystrický, et al., 2014; Machová, Čížová, et al., 2014).

Properties	Mannans	Carboxymethyl mannans	References
Molecular Weight (M_{peak} (kDa))	67	183	(Machová, Bystrický, et al., 2014)
UV spectrophotometric (A_{210nm})	0.06	0.39	
Optical rotation $[\alpha]_D^{20}$	+77.97°	+55.49°	
HO• (%)	11.8 ± 1.1	45.1 ± 1.4	(Machová, Čížová, et al., 2014)
DPPH• (%)	5.0 ± 0.6	4.1 ± 0.4	
Fe(II) chelating activity	10.9 ± 1.7	14.5 ± 1.5	

In the field of chemical modification of polysaccharides that are demanded to improve a variety of properties, such as solubility, robustness, and a myriad of biological activities, there is a clear contrast between what happens with mannans, where the information is very scarce, and other polysaccharides present in the yeast cell wall, namely glucans. In the last case, it is possible to find in the literature several studies reporting different chemical modifications (carboxymethylation, sulfonylation, phosphorylation and acetylation) and their impact in the solubility and in the biological properties, namely the antioxidant, anticoagulant and antitumor activities (Kagimura et al., 2014; Theis et al., 2019).

The clear potential of the chemical modification of mannans in order to improve their properties demands deep studies in this niche that certainly will prompt new and exciting discoveries that may contribute to the prevention and treatment of countless diseases or even interesting properties to be used in nutraceutical and food industries.

3. Mannan Oligosaccharides (MOS)

Mannan oligosaccharides (MOS) are non-digestible oligosaccharides derived *via* partial hydrolysis of the mannans polysaccharide (Tester & Al-Ghazzewi, 2013), which can be generally divided into two main groups: α- and β-MOS. While α-MOS are obtained by cleavage of α-(1-6) bonds from yeast cell wall mannans, β-MOS are commonly obtained from mannans-rich plants through cleavage of β-(1-4)-glycosidic bonds (Jana et al., 2020; Yamabhai et al., 2016).

MOS have gained significant interest as a prebiotic (Gibson et al., 2004). According to Gibson et al., (2017) the prebiotic concept has expanded, because of advances in tools for microbiome research, leading to the proposal of a new definition where a prebiotic is

"a substrate that is selectively utilized by host microorganisms conferring a health benefit". According to this new definition fructooligosaccharides (FOS), galactooligosaccharides (GOS) (Gibson et al., 2017), MOS (Al-Khalaifa et al., 2019; Gibson et al., 2017), xylo-oligosaccharides (XOS) (Charalampopoulos & Rastall, 2012; Gibson et al., 2017) and inulin (Gibson et al., 2017; Van Loo, 2004) are classified as prebiotics, apart from other non-carbohydrate matrices.

MOS are widely used as prebiotics in the animal industry with the aim of selectively stimulate the growth or metabolic activity of a limited number of internal microorganisms, suppressing enteric pathogens and improving the integrity of the intestinal mucosa (Baurhoo et al., 2007; Ghasemian & Jahanian, 2016; Jana et al., 2020; Spring et al., 2000, 2015).

3.1. Production Methods

The different methods for obtaining MOS may be roughly divided into those using chemicals and those that apply enzymes. Among the former, selective acetolysis (Lee & Ballou, 1965; Stewart et al., 1968; Young et al., 1998), acid or alkaline hydrolysis, combined or not with high temperatures, can be cited (Nakajima & Ballou, 1974; Ogawa et al., 1994; Peat et al., 1961).

3.1.1. Chemical Methods

3.1.1.1. Selective Acetolysis

The bibliographic search conducted revealed a notable scarcity of information on large-scale processes to obtain MOS from *S. cerevisiae*'s mannans with chemical methods. One of the few exceptions is perhaps selective acetolysis, although this is a reaction only used in yeast mannans structural elucidation, due to the toxicity of some chemicals involved (Kocourek & Ballou, 1969). Nevertheless, this procedure has the ability to selectively cleave the backbone α-(1-6) linkages between mannose units, yielding the relatively stable α-(1-2) and α-(1-3) linked oligosaccharides (Figure 4) (Lee & Ballou, 1965).

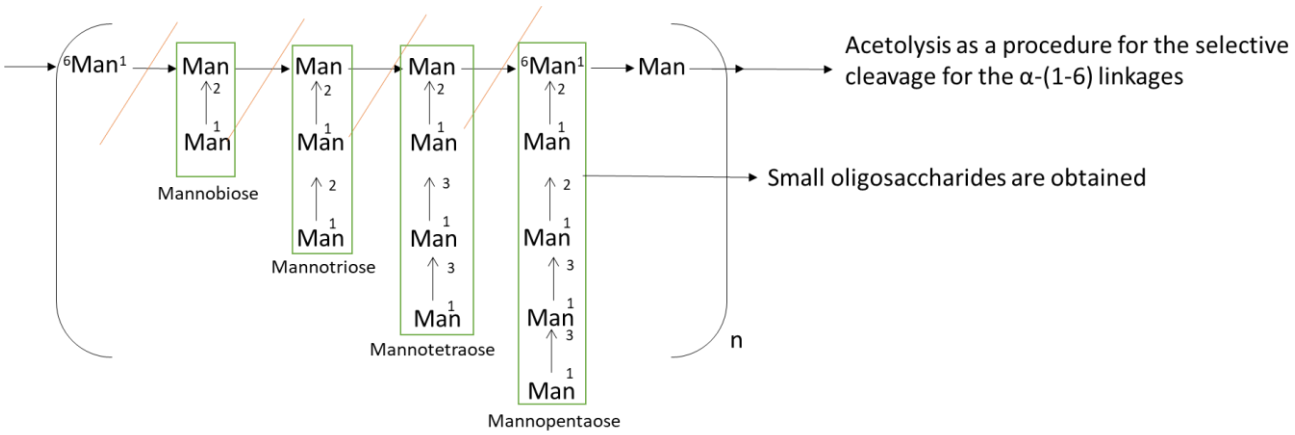

Figure 4: Acetolysis process of cleavage in the extracted mannan (red lines indicate the cleavage locations).

Acetolysis reaction was applied for the first time to yeast mannans by Gorin & Perlin (1956), and the mechanism has been studied in more detail ever since (Ballou, 1976; Kocourek & Ballou, 1969; Lee & Ballou, 1965; Marzaioli et al., 2014; Matsumoto et al., 1982; Stewart et al., 1968; Tanimoto et al., 2002; Vinogradov et al., 1998). The process of acetolysis consists of dissolving yeast mannans in a mixture of acetic anhydride, glacial acetic acid and concentrated sulfuric acid. The reaction has been reported to proceed in a wide range of conditions, from room temperature to 40 °C and with a duration that may extend from 2 h to 5 days. It is referred that the reaction is better controlled if mannans are firstly acetylated.

After acetolysis, the reaction must be stopped with a base, such as pyridine, and the solvents evaporated. The residue is dissolved by adding a chloroform:water solution. The organic solvent is evaporated and the residue is treated with sodium methoxide, usually for 20 min at room temperature. The precipitate formed from the previous reaction may be washed with methanol and dissolved in water. Neutralization and separation may then proceed by a number of methods (Ballou, 1976; Kocourek & Ballou, 1969; Lee & Ballou, 1965; Rosenfeld & Ballou, 1974, 1975; Stewart et al., 1968; Tanimoto et al., 2002).

3.1.1.2. Acid

According to Peat et al. (1961), acid hydrolysis may cleave mannans preferentially in the side chains, since the α-(1 -6) -glycosidic bonds has been reported to be much more stable to acid hydrolysis than the α-(1- 2) -glycosidic bonds.

In the study of Ogawa et al. (1994), mannans extracts were treated with 0.33 N sulfuric acid in a boiling water-bath for 12 h, followed by neutralization with barium carbonate and left overnight at 4 °C. The resultant products were mannans oligomers with 1-4 degree of polymerization (DP). Since these acid hydrolysates from mannans of *S. cerevisiae* and *Trichosporon aceleatum* were still branched, this suggests that acid hydrolysis does not cause preferential cleavage of side chains.

Young et al.(1998), proposed a methodology that encompasses an acetolysis and a partial acid hydrolysis to break down the backbone and side chains. For the partial hydrolysis mannans were incubated in 0.4 M sulphuric acid at 100 °C for 60 min, followed by neutralization with barium carbonate. The precipitate and the supernatant were filtered using a Microcon (10 kDa) membrane in order to eliminate the salt created by neutralization. The results showed a predominance of the disaccharide (mannobiose) with bonds Manα1-3Man and smaller amounts of the trisaccharides (mannotriose), Manα1-3Manα2/6Man, Manα1-2[Manα1- 6] Man with these bonds. In conclusion, the resultant products from the acidic method were mostly mannan oligomers with 1-4 degree of polymerization, *i.e.* monosaccharides, disaccharides, trisaccharides and tetrasaccharides.

3.1.1.3. Alkaline

Another chemical procedure used to obtain mannans and MOS is alkaline hydrolysis under specific conditions: the β-elimination effects occurs in the replaced serine and threonine residues, releasing mannose, mannobiose, mannotriose, and mannotetraose (Nakajima & Ballou, 1974). This alkaline extraction was described by the breakdown of the phosphodiester bonds or glycosyl-serine linkages (Ballou, 1974; Nakajima & Ballou, 1974). According to several studies (Nakajima & Ballou, 1974; Sentandreu & Northcote, 1968; Yen & Ballou, 1974; Young et al., 1998) the selective alkaline hydrolysis consists of mannans treatment with 0.05 - 0.1 M of sodium hydroxide or sodium hydroxide containing sodium borohydride (Yen & Ballou, 1974) at 25 °C-100 °C for 2-24 h. The reaction must be stopped by neutralization with acetic acid. By following this procedure, the authors (Nakajima & Ballou, 1974; Sentandreu & Northcote, 1968; Yen & Ballou, 1974; Young et al., 1998) claim to obtain di to tetra saccharides.

3.1.2. Enzymatic Methods

As mannans are structurally complex, a sequential action of various enzymes may be required to hydrolyze them into MOS (Singh et al., 2018). Table 4 summarizes different types of specific α-mannanases (to cleave the backbone of mannans into oligomers) and α-mannosidases (to hydrolyze mannans oligomers to mannose) (Dhawan & Kaur, 2007; Kommineni et al., 2019; Maruyama et al., 1994; Zordan et al., 2015; Zouchova et al., 1977).

According to Singh et al. (2018), enzymatic degradation is generally preferred in the food industry for partial degradation of commonly used food hydrocolloids (derived from plants and thus containing β-mannans). Since mannans derived from the yeast cell wall of *S. cerevisiae* are mainly composed of mannose structurally organized in α(1-6) linked backbone and α(1-2) and α(1-3) linked branches, enzymes available for plant mannans cleavage are not predicted to be applicable to yeast mannan. Indeed, the industrial use of α- mannanase enzymes, cleaving alpha bonds, is practically inexistent, which leads to its very high price, and turning their use almost exclusively for research.

As an example, an endo-1,4 β-mannanase (from *Cellvibrio japonicus*) containing 10,000 units costs 173€ (Megazyme) (*endo-1-4-beta*-mannanase *Cellvibrio japonicus Enzyme | Megazyme*, n.d.) whereas an α-D-Mannosidase (from *Bacteroides thetaiotaomicron*) containing 10 units costs 152€ (Megazyme) (*alpha-D-Mannosidase Bacteroides thetaiotaomicron Enzyme | Megazyme*, n.d.). Thus, considering the units present, the cost is roughly 1000 times higher. As such, the use of specific enzymes to catalyze the production of MOS is not an economically viable solution. Nevertheless, a potential solution could be the use of immobilized enzymes, which would allow their easier recovery and concomitant decrease in operational costs.

Table 4 – Different types of enzymes and their action mechanism in the mannan structure.

Enzyme	Action Mechanism	Optimal Conditions	Reference
Endo α-1,6 mannanase	Random hydrolysis of (1-6)-α-D-mannosidic linkages in unbranched (1-6)-mannans	Temperature 37 °C pH= 7.0	(Zordan et al., 2015)
α-1,2,3,6- mannosidase	Hydrolysis of the terminal (1-2)-linked α-D-mannose residues in the oligo-mannose oligosaccharide Man9 (GlcNAc)2	Temperature 37 °C pH= 4.5	(Kommineni et al., 2019)
α-1,2,3 - mannosidase	Exoglycosidase that catalyzes the hydrolysis of α-(1-2) and α-(1-3) linked mannose residues from oligosaccharides	Temperature 37 °C pH= 5.5	(Wong-Madden & Landry, 1995)
α- 1,6 mannosidase isolated from *Xanthomonas manihotis*	removes unbranched α-(1-6) linked D-mannopyranosyl residues from oligosaccharides	Temperature 37 °C pH= 5.5	(Wong-Madden & Landry, 1995)

Summarizing, acetolysis of yeast mannan preferentially cleaves α-(1-6) linkages, and has been reported to originate mannose, mannobiose, mannotriose and mannotretraose, containing α-(1-2) and α-(1-3) linkages (Stewart & Ballou, 1968). This procedure is applied to help determine the polysaccharide structures, although its application for MOS production is hampered by the chemical employed (e.g. pyridine and sodium methoxide). On the other hand, partial acid hydrolysis presents a lower specificity towards the linkages but remains an important technique to determine and elucidate the structure of the polysaccharides (Chiura et al., 1982). Despite not having a high selectivity as acetolysis, this procedure proves to be a simpler process. The alkaline hydrolysis was shown to cleave the linkages to serine and threonine residues in the oligomers (Nakajima & Ballou, 1974), resulting in the production of mannobiose, mannotriose and mannotretaose. This also proves to be a simple process without the use of highly toxic or environmentally harmful reagents. Finally, enzymatic methods for cleaving yeast mannan linkages, although very selective, depending on the chosen enzyme, and environmentally friendly, are at the present time particularly expensive.

In conclusion, although acetolysis and enzymatic hydrolysis both seem to be the most selective processes to obtain MOS from mannans hydrolysis, neither of them appears to be applicable at industrial scale, due to the abovementioned reasons. Therefore, acid or alkaline hydrolysis processes remain the possible, despite less effective choices. This may explain why in many commercial products claimed to be MOS, their compositional analysis shows a mixture of mannans and MOS (unpublished data from authors).

4. Bioactivities and potential applications of mannans, MOS and mannose residues

Mannans, MOS and mannose residues from yeast present specific bioactivities which tailor their potential use in pharmaceutical and animal feed industries (Singh et al., 2018). This section will cover some of the bioactivities present in these three compounds.

4.1. Mannans

In the literature, yeast mannans have been mainly shown to present prebiotic activity, when incorporated into animal diet, limiting gastrointestinal infections (Browne et al., 2019). Indeed, it has been reported (Oba et al., 2020) that mannans extracted from baker's yeast selectively increased the abundance of *Bacteroides thetaiotaomicron* and *B. ovatus* in the fermentation of rat feces *in vitro*, thus increasing the production of acetate, propionate and other beneficial short chain fatty acids, and improving intestinal environment. In the same context, mannans have been shown to limit gastrointestinal infection in susceptible animals, including pigs, broilers and cows, by blocking the mechanism by which pathogenic gram-negative bacteria adhere and invade intestine, an effect measured by a reduction in animal pro-inflammatory responses (Browne et al., 2019).

Concerning pathogenic control, *S. cerevisiae* mannans were also reported to enhance collateral sensitivity of *Escherichia coli* to antibiotics, both in susceptible and antibiotic resistant strains, through modulation of bacterial metabolism (Smith et al., 2020).

According to Korcová et al. (2015) mannans could present immunomodulatory activity through the stimulation of macrophages (*via* interaction with their mannose receptors), which eliminate circulating atherogenic lipoproteins such as low density lipoproteins (LDLs). Additionally, mannans, as well as β-glucans, have shown antitumor and anti-metastatic effects (Joseph et al., 2013; Kogan et al., 2008).

Another activity reported was the ability to scavenge radicals, where mannans exhibited a good scavenging ability to superoxide anions and hydroxyl radicals, suggesting a potential antioxidant effect (Liu et al., 2018); these properties enhance mannans possible applications in food, feed and cosmetic areas.

Despite its beneficial effects, there are also some reports on the undesirable responses recorded after mannans ingestion. Khmaladze et al. (2014) found that intraperitoneal *S. cerevisiae* mannans injection resulted in the induction and exacerbation of psoriasis and psoriasis arthritis (production of IL-17, a pathway regulated by ROS in macrophages).

Another study from Cuskin et al. (2015), in a group of patients with Crohn's disease, described a disturbed humoral and cellular immune response to yeast mannans. In older studies (Young et al., 1998) it has been shown that a fraction (Sc500) of mannans stimulates the production of T and B cells in patients with Crohn's disease.

4.2. MOS

MOS are reported to serve as prebiotics for beneficial bacteria in the gut, also showing certain biological effects such as enhancement of protection in intestinal mucosal layer,

and boost of the immunity, antimutagenic and antioxidant defenses (Teng & Kim, 2018). According to Spring et al. (2015) and Spring et al. (2000), MOS are known for their ability to bind and limit the colonization of enteric pathogens such as *Salmonella*, *Escherichia coli*, *Campylobacter*, and others (Koc et al., 2010).The α-(1-3) and α-(1-6) branched of MOS present in the cell wall of *S. cerevisiae* are particularly effective at binding pathogens Firon et al. (1987). According to Spring et al. (2015), when the attachment and colonization of the intestine by gram-negative pathogenic bacteria is blocked with MOS, the autogenous population can flourish, making the gut more efficient, and liberating more nutrients for lean tissue growth and improve immunity in the host animal. However, as reported on the studies of Baurhoo et al. (2007), Sims et al. (2004), and Spring et al. (2000) MOS effects on promoting beneficial bacteria, such as *Lactobacilli* and *Bifidobacteria* are more variable. The overall known different action modes of MOS are exemplified in Figure 5.

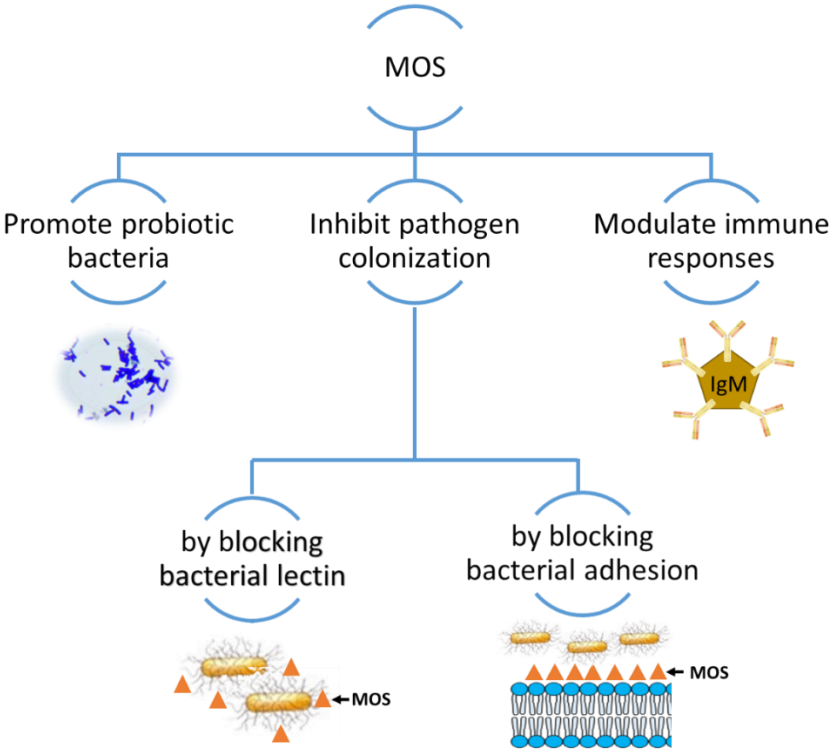

Figure 5: Potential mechanisms of action of MOS in pathogen colonization inhibition.

Apart from effects on caecal microbiota, MOS also improved microbial community in other intestine sections of broilers – jejunum, ileum, jejunal mucosa, ileal mucosa and ileocecal junction (Chee et al., 2010; Geier et al., 2009; Kim et al., 2011; Wang et al., 2016; Yang et al., 2008). Studies by Corrigan et al. (2015) and Lee et al. (2016) report that MOS increased caecal Bacteroidetes population in broilers, known for their strong metabolic activity in ferment indigestible saccharides (as MOS) to short-chain fatty acids and, consequently, improve nutrient absorption and protect the host from pathogen infection (Wexler, 2007).

Although not so clear in many studies, as previously mentioned, the effect of MOS on the promotion of increased prevalence of beneficial bacteria has also been reported by several authors. Indeed, ileal *Lactobacillus acetotolerans*, *L. delbrueckii* subsp. *lactis*, *L. sakei* subsp. *Sakei*, and caecal *L. ingluviei*, *L. mucosae*, *L. salivarius*, and *L. crispatus* populations in broilers were reported to increase with the inclusion of MOS in the diet (Corrigan et al., 2011, 2015). Among these species, *L. crispatus* was reported in Chen et al. (2007) and Zhang et al. (2007) to have anti-*E. coli* and anti-*Salmonella* activities. In the same way, *L. salivarius* was mentioned by Chen et al. (2007) and Zhang et al. (2007) as limiting *Salmonella* colonization. The anti-pathogenic characteristics of *Lactobacillus* may be the reason why MOS indirectly reduced the numbers of *E. coli* or *Salmonella* in the intestine, ameliorating bacterial infection in pathogen-challenged broilers (Baurhoo et al., 2007, 2009; Spring et al., 2000). It has also been reported that MOS are considered prebiotics that increase the efficiency of microbial elimination by phagocytosis through the agglutination process in various strains of *E. coli*, *Salmonella typhimarium* and *S. enteritidis in vitro* (Spring et al., 2000).

MOS can also be used as substitutes for antibiotic growth promoters (AGP) (antibiotics in sub-therapeutical doses), widely used in animal feed due to their growth-promoting effects, but banned in European Union since 2006, due to concerns regarding the link between usage of antibiotics in animal feed and an increase of bacterial antibiotic resistance (Al-Khalaifah, 2018). Furthermore, consumers prefer to reduce the use of antibiotics and other therapeutic chemicals in animal feed. In this perspective, MOS can be a greener approach to simultaneously prevent disease outbreaks and enhance animal health. When supplemented in animal feed, MOS contribute to mortality decrease and to the improvement of the levels of bactericidal (destroys bacteria) and lysozyme (destroys the protective layer of bacteria) activities (Chacher et al., 2017; Torrecillas et al., 2007; Torrecillas et al., 2014). In summary, there are countless advantages in using MOS in animal feed: they have the ability to modify microbiota composition, prevent pathogenic bacteria adhesion (as described above), can be associated with the proliferation of beneficial microorganisms such as lactic bacteria, and show positive effects in decreasing diarrhea incidence (Agazzi et al., 2020; Al-Khalaifa et al., 2019; Leblebicier & Aydoğan, 2018; Valpotić et al., 2016; Zhao et al., 2012).

Nevertheless, despite the several benefits of MOS consumption and utilization reported, a more systematic research on the mechanisms behind these beneficial effects on the human body, as well as the safety of its use, still needs to be carried out in depth. However, there is still a large ambiguity on the definition of MOS, mannans or yeast cell wall extracts, especially within market products, usually presented as MOS, although being composed of cell wall extracts or mannans, most of the times. The clear characterization and labelling of these components is essential to better understand the relation between composition and activity.

4.3. Mannose

D-mannose is found in nature as a component of mannans from vegetable and microbial origin, hemicellulose and cellulose in dietary fiber (Hu et al., 2016). Its structure is very similar to that of other sugars such as D-glucose and D-fructose; in fact, D-mannose is an epimer of D-glucose in the C-2 position and is an aldose isomer of D-fructose (Hu et al., 2016; Wu et al., 2019).

D-mannose is widely used in food, medicine, cosmetics, and food additives industries. Among its physiological health benefits, those more referred concern the immune system, diabetes mellitus, intestinal diseases and urinary tract infections (Wei et al., 2020; Wu et al., 2019).

Mannose residues act in lock-and-key interactions with carbohydrate-binding proteins found on the surface of certain bacteria (Zopf & Roth, 1996). For example, D-mannose inhibits the adhesion of enteric pathogenic bacteria related to urinary tract infections (UTI) to uroepithelial cells, thus reducing bacteriuria levels (Kranjčec et al., 2014). More specifically, the inhibition mechanism refers to the binding of free mannose in the bladder to the type 1 fimbriae (FimH) of pathogens such as uropathogenic *Escherichia coli* (UPEC) (Flores-Mireles et al., 2015; Pigrau & Escolà-Vergé, 2020; Ruggieri et al., 1985; Schilling et al., 2001; Scribano et al., 2020). Fimbriae structure presents lectins, glycoproteins which allow the bacteria to bind to the cellular receptors present on the surface of uroepithelial cells (Flores-Mireles et al., 2015; Pigrau and Escolà-Vergé, 2020; Scribano et al., 2020). By binding to free mannose molecules instead of the uroepithelial cells, pathogens are eliminated from the urinary tract by the flow of urine, resulting in a reduction in the number of UTI, without the need to resort to antibiotics; this approach may even be applied to prevent UTI thus paving the way for commercialization as a health supplement. Indeed, it has been described that after ingesting therapeutic amounts of D-mannose, only a fraction was metabolized by the body (Kyriakides et al., 2020; Lenger et al., 2020), and the remaining was excreted through the kidneys and bladder ureters, to eventually be eliminated from the system in urine. Such amounts of mannose in the bladder may promote the desired effect of preventing UTI. In a study by Kranjcec et al. (2014), D-mannose powder was shown effective in preventing UTI during 6-month prophylaxis. The rate of recurrence of infection did not differ between patients taking standard medication (Nitrofurantion) and those taking D-mannose powder.

D-mannose is also reported to inhibit the binding of *S. typhimurium* to intestinal cells in chickens (Oyofo, DeLoach, et al., 1989; Oyofo, Droleskey, et al., 1989). This sugar, for now, is the most effective in blocking colonization to inhibit the growth of intestinal pathogens (Berge & Wierup, 2012; Galiş et al., 2013).

5. Commercial Products

A wide range of yeast mannans and MOS have been commercially available since the 1990s and some of them, such as the Bio-MOS (Alltech), with a substantial repertoire of

621 scientific articles and practical examples of its effectiveness (Baurhoo et al., 2007; Spring
622 et al., 2015). Nowadays, the use of MOS (or yeast cell wall extract or mannans, depending
623 on the effective composition of the commercial product) in animal feed has become more
624 prominent, mainly due to the European ban on antibiotics as prophylactic growth
625 promoters in animal feed.
626 There are still very few commercial products of MOS and mannans obtained by *S.*
627 *cerevisiae* targeted for humans. In some of the products already commercialized,
628 mannans are extracted from plants (Konjac), such as GLUCOMANNAN from brands such
629 as PROZIS, NOW and Nutricost. Still in the line of supplements with mannans, prebiotics
630 and probiotics have been recently incorporated into whey proteins. The Gold Nutrition
631 brand incorporated fenomannans (which are galactomannans from fenugreek seeds) in
632 one of its whey proteins available on the market. Another product for human health is a
633 probiotic supplement from the brand Jarrow Formulas that contains S*. boulardii* and
634 MOS. Table 5 lists some of these products currently on the market with MOS and
635 mannans obtain from *S. cerevisiae.* The majority of these products are used for animal
636 feed and supplements.
637

638 Table 5 – Mannans and MOS from yeast *S. cerevisiae* commercially available.

Company	Product	General Composition	Claims	Extraction Process	Website
Ohly - GO	MOS	Yeast cell walls derived from autolyzed cultivated baker's yeast (*S. cerevisiae*)	• Benefits of application of yeast cell walls in animal and used by the animal feed industry as antibiotic replacers.	Autolysis	https://www.ohly.com/en/feed-health/health-promoting-polysaccharides/ohly-go-mos/
	SoluMannan	Purified soluble mannoproteins product containing ca. 65% mannans	• Higher solubility • Effective at a dosage that is some 10 times lower (50–250 g/tonne feed) than that of conventional yeast cell wall/MOS products		https://www.ohly.com/en/feed-health/health-promoting-polysaccharides/
	Wall	Mixture of beta-glucans and MOS from the outside of the yeast cell walls	• Immune modulators of both the innate and adaptive immune systems. • MOS are able to bind and limit the colonization, of gut pathogens like *Salmonella*, *E. coli* and *Clostridia* spp.	Hydrolysis	https://www.ohly.com/en/feed-health/health-promoting-polysaccharides/ohly-go-wall/
YES	BioWall	Contains a high concentration of	• Glucans: Stimulate the immune system, making the		https://www.yes.ind.br/en/biosolutions/

		β-glucans and mannans	animal more resistant to infections. • Mannans modulate the intestinal microbiota, because in addition to being used as a substrate by beneficial bacteria, they agglutinate pathogenic bacteria such as *Salmonella* and *E.coli*, ensuring the animal a better health status		
	GUTBIO MOS		• Promotes intestinal microbiota balance; 95% *Salmonella* and 100% *E. coli* (*in vitro*) agglutination. Better intestinal integrity (villi/crypts)		https://www.yes.ind.br/en/gutbio/
Alltech	BIO-MOS	A unique product derived from a selected strain of *Saccharomyces cerevisiae* yeast	• Enhances feed efficiency • Contributes to immune system development • Participates in normalizing gut microflora		https://www.alltech.com/bio-mos

			• Stimulates the natural defenses of the organism • Reinforces the function of the digestive system • Contributes to meat, egg and milk marketability		
	Actigen	Second-generation, unique bioactive product derived from *Saccharomyces cerevisiae* (Mannan Rich Fraction)	• Participates in normalizing gut microflora and promoting microbiome diversity • Maintains gastrointestinal integrity and stability • Aids nutrient utilization		https://www.alltech.com/actigen
Leiber	BIOLEX MB40	Characterized by its high levels of 1.3 -1.6 beta-D-glucan and MOS	• Active support and relief of the immune system • High bonding power and inactivation of pathogens/toxins in the intestinal lumen • Prebiotic effects on the intestinal and ruminal microflora	Obtained during the production of soluble brewers´ yeast extracts	https://www.leibergmbh.de/int/feed/products/biolexmb40/
Super Mannan	Supermannan	MOS from *S. cerevisae* and	• Take SuperMannan at the first		https://www.amazon.com/Belvedere-Environmentals-SuperMannan/dp/B00FAWEUTA

		Candida guillermondii.	indication of a UTI attack		
Nutritech	EquiMOS	Nutritional supplement by horses with modified MOS	• Reduction of pathogenic bacteria, viruses and mycotoxins. • Gastrointestinal health and performance by stimulating the growth of beneficial intestinal bacteria • Supporting the immune system		https://www.nutritech.co.nz/product/equimos/
Lallemand Animal Nutrition	AGRIMOS	Feed ingredient produced from a high-quality *Saccharomyces cerevisiae* yeast cell wall. Combination of MOS and β-(1,3 and 1,6)-poly-D-glucose	• Maintain favorable intestinal microflora balance in farm animals		https://lallemandanimalnutrition.com/en/europe/our-products/product-details/agrimos/
	ALKOSEL MOS	Source of organic selenium with higher bioavailability than inorganic forms of selenium. Also part of its composition is MOS extracted	• Prebiotic that favors the beneficial natural microbiota of the rumen and intestine • Increases the Selenium content		https://lallemandanimalnutrition.com/pt-br/brazil/our-products/product-details/alkosel-mos/

		from the yeast cell wall			
Angel Yeast	Fubon MOS	A functional oligosaccharide rich in MOS	• Prebiotic additive, derived from a selected yeast cell wall • Promote beneficial bacteria to grow and reproduce • Adjust microflora balance in intestine and stomach, absorb pathogens and control diseases		https://en.angelyeast.com/products/animal-nutrition/fubon-mos.html
ORFFA	ActiveMOS	Prebiotic rich in MOS extracted from specially selected strain of the yeast *Saccharomyces cerevisiae*	• Highly efficient in the agglutination (binding) of pathogens, thereby reducing the risk of pathogen colonization to the gut wall		https://orffa.com/products/activemos/
Vet Spirit	SymbioIMMUNE	Contains inulin, 1,3-1,6 beta-D-glucan and MOS. These two are obtain from brewer's yeast	• Support the immune and gastrointestinal functions • Complementary prebiotic and probiotic feed for dogs and cats of all ages		https://vetspirit.com/product/symbioimmune-supplement-80-tablets/

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6. Conclusions

Yeast mannans are cell wall structural carbohydrates mainly composed of large numbers of mannose residues, assembled in a fairly complex structure.

Mannans extraction can be performed through several different processes (physical, chemical, mechanical or enzymatic), each one presenting advantages and disadvantages. They reveal a high potential for use in several applications due to their bioactive properties, such as immunomodulation and prebiotic activity, as well as antioxidant potential. This latter property can be improved by functionalization processes, which can also provide new interesting bioactivities.

MOS can be fractionated from mannans using different chemical (acetolysis, acidic or alkaline), or enzymatic processes. Since acetolysis and enzymatic processes, despite their selectivity, are not adequate for industrial scale production because of the toxic chemicals employed and high cost, respectively, the best options with likely be to use acid or alkaline hydrolysis; even if they do not present the same level of selectivity, they are more straightforward and less toxic processes.

Most of the commercial products with mannans and/or MOS claim beneficial effects in modulating the intestinal microbiota and are targeted for animal feed, with a concomitant relatively low price, which strongly limits their production costs. To increase the added value of the products and expand their market area to alternative targets, such as those of human functional foods, cosmeceuticals or even pharmaceuticals, the commercialized extracts must present clear and scientifically validated claims for additional beneficial effects. This endeavor has been complicated in several studies by the heterogenicity of the extracts, both in terms of composition (apart from mannose they also present other sugars, proteins, and lipids) and size dispersion of the molecules (mannans or MOS). This translates into distinct bioactive effects, thus misleading the analysis of effective relationships between composition and mode of action. In fact, systematic research on the mechanisms of these beneficial effects on the human body remains to be performed. Thus, to deepen those studies, it is extremely important to use selective extraction/fractionation processes, so that purer extracts can be used in studies, and strong evidence can be build relating the ingestion of extracts and corresponding beneficial effects.

The technologies currently available to extract mannans from yeast cell wall and to obtain MOS by mannans cleavage are not very selective, except for the ones using enzymes, which present a prohibitive cost. This gap may however be surpassed by using immobilized enzymes, which would allow their easier recovery and concomitant decrease in operational costs. Nevertheless, additional studies are necessary, eventually exploiting green extraction processes based on waves (e.g. ultrasounds and pulsed electric fields) or pressure (e.g. supercritical and subcritical extraction technologies).

Apart from the beneficial properties of mannans already stated, other positive impacts of these molecules on health are on the horizon, helped by the (still incipient) knowledge

about their functionalization, a growing area of research. Indeed, the field of chemical modification in mannans has not yet been deeply studied, but it reveals to be a very promising process to improve their inherent bioactive properties or even provide new ones.

By enlarging the range of potential applications of yeast mannans and MOS, not only their generation during fermentative processes no longer presents an environmental issue (in a circular economy concept), but also those different applications may promote the emergence of new market players, specialized in their exploitation.

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7. References

Abbott, D. W., Martens, E. C., Gilbert, H. J., Cuskin, F., & Lowe, E. C. (2015). Coevolution of yeast mannan digestion: Convergence of the civilized human diet, distal gut microbiome, and host immunity. *Gut Microbes*, *6*(5), 334–339. https://doi.org/10.1080/19490976.2015.1091913

Agazzi, A., Perricone, V., Omodei Zorini, F., Sandrini, S., Mariani, E., Jiang, X.-R., Ferrari, A., Crestani, M., Nguyen, T. X., Bontempo, V., Domeneghini, C., & Savoini, G. (2020). Dietary Mannan Oligosaccharides Modulate Gut Inflammatory Response and Improve Duodenal Villi Height in Post-Weaning Piglets Improving Feed Efficiency. *Animals*, *10*(8), 1283. https://doi.org/10.3390/ani10081283

Al-Khalaifa, H., Al-Nasser, A., Al-Surayee, T., Al-Kandari, S., Al-Enzi, N., Al-Sharrah, T., Ragheb, G., Al-Qalaf, S., & Mohammed, A. (2019). Effect of dietary probiotics and prebiotics on the performance of broiler chickens. *Poultry Science*, *98*(10), 4465–4479. https://doi.org/10.3382/ps/pez282

Al-Khalaifah, H. S. (2018). Benefits of probiotics and/or prebiotics for antibiotic-reduced poultry. *Poultry Science*, *97*(11), 3807–3815. https://doi.org/10.3382/ps/pey160

alpha-D-Mannosidase Bacteroides thetaiotaomicron Enzyme | Megazyme. (n.d.). Retrieved June 21, 2021, from https://www.megazyme.com/alpha-d-mannosidase-bacteroides-thetaiotaomicron

Bacon, J. S. D., Farmer, V. C., Jones, D., & Taylor, I. F. (1969). The glucan components of the cell wall of baker's yeast (Saccharomyces cerevisiae) considered in relation to its ultrastructure. *Biochemical Journal*, *114*(3), 557–567. https://doi.org/10.1042/bj1140557

Ballou, C. (1974). Some aspects of the structure, biosynthesis and genetic control of yeasts mannans. *Advances in Enzymology*, *40*, 239–270.

Ballou, C. (1976). Structure and Biosynthesis of the Mannan Component of the Yeast Cell Envelope. *Advances in Microbial Physiology*, *14*(C), 93–158. https://doi.org/10.1016/S0065-2911(08)60227-1

Baurhoo, B., Ferket, P. R., & Zhao, X. (2009). Effects of diets containing different concentrations of mannanoligosaccharide or antibiotics on growth performance, intestinal development, cecal and litter microbial populations, and carcass parameters of broilers. *Poultry Science*, *88*(11), 2262–2272. https://doi.org/10.3382/ps.2008-00562

Baurhoo, B., Phillip, L., & Ruiz-Feria, C. A. (2007). Effects of Purified Lignin and Mannan Oligosaccharides on Intestinal Integrity and Microbial Populations in the Ceca and Litter of Broiler Chickens. *Poultry Science*, *86*(6), 1070–1078. https://doi.org/10.1093/ps/86.6.1070

Berge, A. C., & Wierup, M. (2012). Nutritional strategies to combat Salmonella in mono-gastric food animal production. *Animal*, *6*(4), 557–564. https://doi.org/10.1017/S1751731111002217

Bonaly, R., Moulki, H., Benjelloun, A. T., & Pierfitte, M. (1971). Étude des parois de levures du genre rhodotorula II. influence des conditions de culture sur la composition chimique des parois. *Biochimica et Biophysica Acta (BBA) - General Subjects*, *244*(2), 484–494. https://doi.org/10.1016/0304-4165(71)90254-6

Brewers By-products Market Outlook, Share, Size, Forecast, Trends, Report. (2020). https://www.beroeinc.com/category-intelligence/brewers-by-products-market/

748 Browne, N., Traynor, A., & Horgan, K. A. (2019). Mannan rich fraction from yeast
749 modulates inflammatory responses in intestinal cells (HT-29) exposed to
750 Escherichia coli. *Journal of Applied Animal Nutrition*, *7*, e5.
751 https://doi.org/10.1017/jan.2019.5
752 Butylina, S., Shataeva, L., & Nystrom, M. (2007). Separation of nucleoprotein complexes
753 with antioxidant activity from yeast Saccharomyces cerevisiae. *Separation and*
754 *Purification Technology*, *53*(1), 64–70.
755 https://doi.org/10.1016/j.seppur.2006.06.014
756 Cameron, D. R., Cooper, D. G., & Neufeld, R. J. (1988). The mannoprotein of
757 Saccharomyces cerevisiae is an effective bioemulsifier. *Applied and Environmental*
758 *Microbiology*, *54*(6), 1420–1425. https://doi.org/10.1128/aem.54.6.1420-
759 1425.1988
760 Capek, P., Kubačková, M., Alföldi, J., Bilisics, L., Lišková, D., & Kákoniová, D. (2000).
761 Galactoglucomannan from the secondary cell wall of Picea abies L. Karst.
762 *Carbohydrate Research*, *329*(3), 635–645. https://doi.org/10.1016/S0008-
763 6215(00)00210-X
764 Cawley, T. N., Harrington, M. G., & Letters, R. (1972). A study of the phosphate linkages
765 in phosphomannan in cell walls of Saccharomyces cerevisiae. *Biochemical Journal*,
766 *129*(3), 711–720. https://doi.org/10.1042/bj1290711
767 Chacher, M. F. A., Kamran, Z., Ahsan, U., Ahmad, S., Koutoulis, K. C., Qutab Ud Din, H. G.,
768 & Cengiz, Ö. (2017). Use of mannan oligosaccharide in broiler diets: an overview of
769 underlying mechanisms. *World's Poultry Science Journal*, *73*(4), 831–844.
770 https://doi.org/10.1017/S0043933917000757
771 Charalampopoulos, D., & Rastall, R. A. (2012). Prebiotics in foods. *Current Opinion in*
772 *Biotechnology*, *23*(2), 187–191. https://doi.org/10.1016/j.copbio.2011.12.028
773 Chee, S. H., Iji, P. A., Choct, M., Mikkelsen, L. L., & Kocher, A. (2010). Characterisation
774 and response of intestinal microflora and mucins to manno-oligosaccharide and
775 antibiotic supplementation in broiler chickens. *British Poultry Science*, *51*(3), 368–
776 380. https://doi.org/10.1080/00071668.2010.503477
777 Chen, X., Xu, J., Shuai, J., Chen, J., Zhang, Z., & Fang, W. (2007). The S-layer proteins of
778 Lactobacillus crispatus strain ZJ001 is responsible for competitive exclusion against
779 Escherichia coli O157:H7 and Salmonella typhimurium. *International Journal of*
780 *Food Microbiology*, *115*(3), 307–312.
781 https://doi.org/10.1016/j.ijfoodmicro.2006.11.007
782 Chiura, H., Iizula, M., & Yamamoto, T. (1982). A glucomannan as an extracellular
783 product of Candida utilis. II Structure of a glucomannan. Characterization of
784 oligosaccharides obtained by partial hydrolysis. *Agricultural and Biological*
785 *Chemistry*, *46*(7), 1733–1742. https://doi.org/10.1271/bbb1961.46.1733
786 Čížová, A., Csomorová, K., Rychlý, J., & Bystrický, S. (2019). Stability of cationic and
787 amphoteric derivatives of mannan from the yeast Candida albicans. *Carbohydrate*
788 *Polymers*, *207*(November 2018), 440–446.
789 https://doi.org/10.1016/j.carbpol.2018.11.101
790 Čížová, A., Neščáková, Z., Malovíková, A., & Bystrický, S. (2016). Preparation and
791 characterization of cationic and amphoteric mannans from Candida albicans.
792 *Carbohydrate Polymers*, *149*, 1–7. https://doi.org/10.1016/j.carbpol.2016.04.083
793 Corrigan, A., de Leeuw, M., Penaud-Frézet, S., Dimova, D., & Murphy, R. A. (2015).
794 Phylogenetic and Functional Alterations in Bacterial Community Compositions in

Broiler Ceca as a Result of Mannan Oligosaccharide Supplementation. *Applied and Environmental Microbiology*, *81*(10), 3460–3470. https://doi.org/10.1128/AEM.04194-14

Corrigan, A., Horgan, K., Clipson, N., & Murphy, R. A. (2011). Effect of Dietary Supplementation with a Saccharomyces cerevisiae Mannan Oligosaccharide on the Bacterial Community Structure of Broiler Cecal Contents. *Applied and Environmental Microbiology*, *77*(18), 6653–6662. https://doi.org/10.1128/AEM.05028-11

Cuskin, F., Lowe, E. C., Temple, M. J., Zhu, Y., Cameron, E. A., Pudlo, N. A., Porter, N. T., Urs, K., Thompson, A. J., Cartmell, A., Rogowski, A., Hamilton, B. S., Chen, R., Tolbert, T. J., Piens, K., Bracke, D., Vervecken, W., Hakki, Z., Speciale, G., … Gilbert, H. J. (2015). Human gut Bacteroidetes can utilize yeast mannan through a selfish mechanism. *Nature*, *517*(7533), 165–169. https://doi.org/10.1038/nature13995

Dhawan, S., & Kaur, J. (2007). Microbial Mannanases: An Overview of Production and Applications. *Critical Reviews in Biotechnology*, *27*(4), 197–216. https://doi.org/10.1080/07388550701775919

Ďurana, R., Lacík, I., Paulovičová, E., & Bystrický, S. (2006). Functionalization of mannans from pathogenic yeasts by different means of oxidations - Preparation of precursors for conjugation reactions with respect to preservation of immunological properties. *Carbohydrate Polymers*, *63*(1), 72–81. https://doi.org/10.1016/j.carbpol.2005.08.003

Eggensperger; Wilker. (1997). *Multiaktiv wirksame Polysaccharide. Teil I: Pilzextrakte*. https://pascal-francis.inist.fr/vibad/index.php?action=getRecordDetail&idt=2748376

endo-1-4-beta-Mannanase Cellvibrio japonicus Enzyme | Megazyme. (n.d.). Retrieved June 21, 2021, from https://www.megazyme.com/endo-1-4-beta-mannanase-cellvibrio-japonicus

Feuillat, M. (2003). Yeast Macromolecules: Origin, Composition, and Enological Interest. *American Journal of Enology and Viticulture*, *54*(3).

Firon, N., Ashkenazi, S., Mirelman, D., Ofek, I., & Sharon, N. (1987). Aromatic alpha-glycosides of mannose are powerful inhibitors of the adherence of type 1 fimbriated Escherichia coli to yeast and intestinal epithelial cells. *Infection and Immunity*, *55*(2), 472–476. http://www.ncbi.nlm.nih.gov/pubmed/3542836

Fleet, G. H. (1985). Composition and structure of yeast cell walls. *Current Topics in Medical Mycology*, *1*(132), 24–56. https://doi.org/10.1007/978-1-4613-9547-8_2

Fleet, G. H., & Manners, D. J. (1977). The Enzymic Degradation of an Alkali-soluble Glucan from the Cell Walls of Saccharomyces cerevisiae. *Journal of General Microbiology*, *98*(2), 315–327. https://doi.org/10.1099/00221287-98-2-315

Flores-Mireles, A. L., Walker, J. N., Caparon, M., & Hultgren, S. J. (2015). Urinary tract infections: Epidemiology, mechanisms of infection and treatment options. In *Nature Reviews Microbiology* (Vol. 13, Issue 5, pp. 269–284). https://doi.org/10.1038/nrmicro3432

Fritsche, W. (1971). Structure and biosynthesis of the yeast cell envelope. In A. H. Rose & J. S. Harrison (Eds.), *The Yeasts-Physiology and Biochemistry* (Vol. 2, pp. 135–210). Wiley-Blackwell. https://doi.org/10.1002/jobm.19720120411

Galiş, A. M., Marcq, C., Marlier, D., Portetelle, D., Van, I., Beckers, Y., & Théwis, A. (2013). Control of Salmonella Contamination of Shell Eggs-Preharvest and

Postharvest Methods: A Review. *Comprehensive Reviews in Food Science and Food Safety*, *12*(2), 155–182. https://doi.org/10.1111/1541-4337.12007

Gaspar, L. R., Camargo, F. B., Gianeti, M. D., & Maia Campos, P. M. B. G. (2008). Evaluation of dermatological effects of cosmetic formulations containing Saccharomyces cerevisiae extract and vitamins. *Food and Chemical Toxicology*, *46*(11), 3493–3500. https://doi.org/10.1016/j.fct.2008.08.028

Geier, M. S., Torok, V. A., Allison, G. E., Ophel-Keller, K., & Hughes, R. J. (2009). Indigestible carbohydrates alter the intestinal microbiota but do not influence the performance of broiler chickens. *Journal of Applied Microbiology*, *106*(5), 1540–1548. https://doi.org/10.1111/j.1365-2672.2008.04116.x

Ghasemian, M., & Jahanian, R. (2016). Dietary mannan-oligosaccharides supplementation could affect performance, immunocompetence, serum lipid metabolites, intestinal bacterial populations, and ileal nutrient digestibility in aged laying hens. *Animal Feed Science and Technology*, *213*, 81–89. https://doi.org/10.1016/j.anifeedsci.2015.12.012

Gibson, G. R., Hutkins, R., Sanders, M. E., Prescott, S. L., Reimer, R. A., Salminen, S. J., Scott, K., Stanton, C., Swanson, K. S., Cani, P. D., Verbeke, K., & Reid, G. (2017). Expert consensus document: The International Scientific Association for Probiotics and Prebiotics (ISAPP) consensus statement on the definition and scope of prebiotics. *Nature Reviews Gastroenterology and Hepatology*, *14*(8), 491–502. https://doi.org/10.1038/nrgastro.2017.75

Gibson, G. R., Probert, H. M., Loo, J. Van, Rastall, R. A., & Roberfroid, M. B. (2004). Dietary modulation of the human colonic microbiota: updating the concept of prebiotics. *Nutrition Research Reviews*, *17*(2), 259–275. https://doi.org/10.1079/nrr200479

Gorin, P. A. J., & Perlin, A. S. (1956). A mannan produced by Saccharomyces rouxii. *Canadian Journal of Chemistry*, *34*(12), 1796–1803. https://doi.org/10.1139/v56-232

Gorin, P. A. J., Spencer, J. F. T., & Magus, R. J. (1969). Comparison of proton magnetic resonance spectra of cell-wall mannans and galactomannans of selected yeasts with their chemical structures. *Canadian Journal of Chemistry*, *47*(19), 3569–3576. https://doi.org/10.1139/v69-590

Haworth, W. N., Hirst, E. L., & Isherwood, F. A. (1937). 160. Polysaccharides. Part XXIV. Yeast mannan. *Journal of the Chemical Society (Resumed)*, *Vii*, 784. https://doi.org/10.1039/jr9370000784

Hu, X., Shi, Y., Zhang, P., Miao, M., Zhang, T., & Jiang, B. (2016). D -Mannose: Properties, Production, and Applications: An Overview. *Comprehensive Reviews in Food Science and Food Safety*, *15*(4), 773–785. https://doi.org/10.1111/1541-4337.12211

Ikuta, K., Shibata, N., Kanehiko, H., Kobayashi, H., Suzuki, S., & Okawa, Y. (1997). NMR assignment of the galactomannan of Candida lipolytica. *FEBS Letters*, *414*(2), 338–342. https://doi.org/10.1016/S0014-5793(97)01028-4

Jana, U. K., Suryawanshi, R. K., Prajapati, B. P., & Kango, N. (2020). Prebiotic mannooligosaccharides: Synthesis, characterization and bioactive properties. *Food Chemistry*, 128328. https://doi.org/10.1016/j.foodchem.2020.128328

Joseph, M. M., Aravind, S. R., George, S. K., Varghese, S., & Sreelekha, T. T. (2013). A galactomannan polysaccharide from Punica granatum imparts in vitro and in vivo

anticancer activity. *Carbohydrate Polymers*, *98*(2), 1466–1475. https://doi.org/10.1016/j.carbpol.2013.07.023

Juhani Saarinen, H., Ritva Niemela, H., Jari Helin, R., Jari Natunen, V., & Jukka Hiltunen, H. (2016). *Yeast cell material Optional homogenization, heat, pneumatic, hydrostatic* (Patent No. US 9,320,291 B2). https://patentimages.storage.googleapis.com/58/a1/d3/c466adc66f1a76/US9320291.pdf

Kagimura, F. Y., da Cunha, M. A. A., Barbosa, A. M., Dekker, R. F. H., & Malfatti, C. R. M. (2014). Biological activities of derivatized D-glucans: A review. In *International Journal of Biological Macromolecules* (Vol. 72, pp. 588–598). Elsevier B.V. https://doi.org/10.1016/j.ijbiomac.2014.09.008

Kath, F., & Kulicke, W. (1999). Mild enzymatic isolation of mannan and glucan from yeastSaccharomyces cerevisiae. *Die Angewandte Makromolekulare Chemie*, *268*(1), 59–68. https://doi.org/10.1002/(SICI)1522-9505(19990701)268:1<59::AID-APMC59>3.0.CO;2-F

Khmaladze, I., Kelkka, T., Guerard, S., Wing, K., Pizzolla, A., Saxena, A., Lundqvist, K., Holmdahl, M., Nandakumar, K. S., & Holmdahl, R. (2014). Mannan induces ROS-regulated, IL-17A-dependent psoriasis arthritis-like disease in mice. *Proceedings of the National Academy of Sciences*, *111*(35), E3669–E3678. https://doi.org/10.1073/pnas.1405798111

Kim, G.-B., Seo, Y. M., Kim, C. H., & Paik, I. K. (2011). Effect of dietary prebiotic supplementation on the performance, intestinal microflora, and immune response of broilers. *Poultry Science*, *90*(1), 75–82. https://doi.org/10.3382/ps.2010-00732

Klis, F. M., Boorsma, A., & De Groot, P. W. J. (2006). Cell wall construction in <i> Saccharomyces cerevisiae <i>. *Yeast*, *23*(3), 185–202. https://doi.org/10.1002/yea.1349

Koc, F., Samli, H., Okur, A., Ozduven, M., Akyurek, H., & Senkoylu, N. (2010). Effects of Saccharomyces cerevisiae and/or Mannanoligosaccharide on performance, blood parameters and intestinal microbiota of broiler chicks. *Bulgarian Journal of Agricultural Science*, *16*(5), 643–650.

Kocourek, J., & Ballou, C. E. (1969). Method for fingerprinting yeast cell wall mannans. *Journal of Bacteriology*, *100*(3), 1175–1181. http://www.ncbi.nlm.nih.gov/pubmed/5361210

Kogan, G., Pajtinka, M., Babincova, M., Miadokova, E., Rauko, P., Slamenova, D., & Korolenko, T. A. (2008). Yeast cell wall polysaccharides as antioxidants and antimutagens: Can they fight cancer? # Minireview. In *NEOPLASMA* (Vol. 55).

Kollár, R., Reinhold, B. B., Petráková, E., Yeh, H. J. C., Ashwell, G., Drgonová, J., Kapteyn, J. C., Klis, F. M., & Cabib, E. (1997). Architecture of the Yeast Cell Wall. *Journal of Biological Chemistry*, *272*(28), 17762–17775. https://doi.org/10.1074/jbc.272.28.17762

Kommineni, V., Markert, M., Ren, Z., Palle, S., Carrillo, B., Deng, J., Tejeda, A., Nandi, S., McDonald, K. A., Marcel, S., & Holtz, B. (2019). In Vivo Glycan Engineering via the Mannosidase I Inhibitor (Kifunensine) Improves Efficacy of Rituximab Manufactured in Nicotiana benthamiana Plants. *International Journal of Molecular Sciences*, *20*(1), 194. https://doi.org/10.3390/ijms20010194

Korcová, J., Machová, E., Filip, J., & Bystrick, S. (2015). Biophysical properties of carboxymethyl derivatives of mannan and dextran. *Carbohydrate Polymers*, *134*, 6–

936 11. https://doi.org/10.1016/j.carbpol.2015.07.008
937 Kranjčec, B., Papeš, D., & Altarac, S. (2014). D-mannose powder for prophylaxis of
938 recurrent urinary tract infections in women: A randomized clinical trial. *World*
939 *Journal of Urology*, *32*(1), 79–84. https://doi.org/10.1007/s00345-013-1091-6
940 Kurakake, M., Sumida, T., Masuda, D., Oonishi, S., & Komaki, T. (2006). Production of
941 Galacto-manno-oligosaccharides from Guar Gum by β-Mannanase from Penicillium
942 oxalicum SO. *Journal of Agricultural and Food Chemistry*, *54*(20), 7885–7889.
943 https://doi.org/10.1021/jf061502k
944 Kyriakides, R., Jones, P., & Somani, B. K. (2020). Role of D-Mannose in the Prevention of
945 Recurrent Urinary Tract Infections: Evidence from a Systematic Review of the
946 Literature. In *European Urology Focus*. Elsevier B.V.
947 https://doi.org/10.1016/j.euf.2020.09.004
948 Latgé, J.-P. (2007). The cell wall: a carbohydrate armour for the fungal cell. *Molecular*
949 *Microbiology*, *66*(2), 279–290. https://doi.org/10.1111/j.1365-2958.2007.05872.x
950 Lazzari, F. (1999). *A product based on polysaccharides from bakers' yeast and its use as*
951 *a technological coadjuvant for bakery products* (Patent No. EP 0 950 716 B1).
952 https://patentimages.storage.googleapis.com/55/36/4b/de55f1e5d6d144/EP0950
953 716B1.pdf
954 Leblebicier, Ö. D. Y., & Aydoğan, İ. (2018). The Effects of Mannan Oligosaccharide and
955 Chitosan Oligosaccharide on Performance and Blood Parameters of Broilers.
956 *Journal of Poultry Research*, *15*(1), 18–22.
957 Lee, S. I., Park, S. H., & Ricke, S. C. (2016). Assessment of cecal microbiota, integron
958 occurrence, fermentation responses, and Salmonella frequency in conventionally
959 raised broilers fed a commercial yeast-based prebiotic compound. *Poultry Science*,
960 *95*(1), 144–153. https://doi.org/10.3382/ps/pev322
961 Lee, T., & Dugoua, J.-J. (2011). Nutritional Supplements and Their Effect on Glucose
962 Control. *Current Diabetes Reports*, *11*(2), 142–148.
963 https://doi.org/10.1007/s11892-010-0175-6
964 Lee, Y.-C., & Ballou, C. E. (1965). Preparation of Mannobiose, Mannotriose, and a New
965 Mannotetraose from Saccharomyces cerevisiae Mannan. *Biochemistry*, *4*(2), 257–
966 264. https://doi.org/10.1021/bi00878a013
967 Lenger, S. M., Bradley, M. S., Thomas, D. A., Bertolet, M. H., Lowder, J. L., & Sutcliffe, S.
968 (2020). D-mannose vs other agents for recurrent urinary tract infection prevention
969 in adult women: a systematic review and meta-analysis. *American Journal of*
970 *Obstetrics and Gynecology*, *223*(2), 265.e1-265.e13.
971 https://doi.org/10.1016/j.ajog.2020.05.048
972 Li, J., & Karboune, S. (2018). A comparative study for the isolation and characterization
973 of mannoproteins from Saccharomyces cerevisiae yeast cell wall. *International*
974 *Journal of Biological Macromolecules*, *119*, 654–661.
975 https://doi.org/10.1016/j.ijbiomac.2018.07.102
976 Li, J., Karboune, S., & Asehraou, A. (2020). Mannoproteins from inactivated whole cells
977 of baker's and brewer's yeasts as functional food ingredients: Isolation and
978 optimization. *Journal of Food Science*, *85*(5), 1438–1449.
979 https://doi.org/10.1111/1750-3841.15054
980 Li, S., Xiong, Q., Lai, X., Li, X., Wan, M., Zhang, J., Yan, Y., Cao, M., Lu, L., Guan, J., Zhang,
981 D., & Lin, Y. (2016). Molecular Modification of Polysaccharides and Resulting
982 Bioactivities. *Comprehensive Reviews in Food Science and Food Safety*, *15*(2), 237–

250. https://doi.org/10.1111/1541-4337.12161
Libjaková, L., Bystrický, S., Ližičárová, I., Paulovičová, E., & Machová, E. (2007). Evaluation of different mannan polysaccharide usage in enzyme-linked immunosorbent assay for specific antibodies determination. *Journal of Pharmaceutical and Biomedical Analysis*, *45*(3), 521–525. https://doi.org/10.1016/j.jpba.2007.06.007
Liu, Y., & Huang, G. (2018). The derivatization and antioxidant activities of yeast mannan. *International Journal of Biological Macromolecules*, *107*(PartA), 755–761. https://doi.org/10.1016/j.ijbiomac.2017.09.055
Liu, Y., Huang, G., & Lv, M. (2018). Extraction, characterization and antioxidant activities of mannan from yeast cell wall. *International Journal of Biological Macromolecules*, *118*, 952–956. https://doi.org/10.1016/j.ijbiomac.2018.06.145
Machová, E., Bystrický, P., Malovíková, A., & Bystrický, S. (2014). Preparation and characterization of carboxymethyl derivatives of yeast mannans in aqueous solutions. *Carbohydrate Polymers*, *110*, 219–223. https://doi.org/10.1016/j.carbpol.2014.03.079
Machová, E., Čížová, A., & Bystrický, P. (2014). Effect of carboxymethylation on antioxidant properties and radical degradation of mannans and glucans. *Carbohydrate Polymers*, *112*, 603–607. https://doi.org/10.1016/j.carbpol.2014.06.050
Maekaji, K. (1974). The Mechanism of Gelation of Konjac Mannan. *Agricultural and Biological Chemistry*, *38*(2), 315–321. https://doi.org/10.1080/00021369.1974.10861161
Martínez, J. M., Cebrián, G., álvarez, I., & Raso, J. (2016). Release of mannoproteins during saccharomyces cerevisiae autolysis induced by pulsed electric field. *Frontiers in Microbiology*. https://doi.org/10.3389/fmicb.2016.01435
Maruyama, Y., Nakajima, T., & Ichishima, E. (1994). A 1,2-α-d-mannosidase from a bacillus sp.: purification, characterization, and mode of action. *Carbohydrate Research*, *251*(C), 89–98. https://doi.org/10.1016/0008-6215(94)84278-7
Marzaioli, A. M., Bedini, E., Lanzetta, R., Parrilli, M., & De Castro, C. (2014). Conversion of yeast mannan polysaccharide in mannose oligosaccharides with a thiopropargyl linker at the pseudo-reducing end. *Carbohydrate Research*, *383*, 43–49. https://doi.org/10.1016/j.carres.2013.10.016
Matsumoto, T., Mikami, T., Takanohashi, M., Okubo, Y., Shibata, N., Suzuki, S., & Suzuki, M. (1982). Action of alkaline sodium borohydride on fractions of d-mannans from bakers' yeast (Saccharomyces cerevisiae). *Carbohydrate Research*, *99*(1), 93–98. https://doi.org/10.1016/S0008-6215(00)80981-7
Matsumoto, T., Takanohashi, M., Okubo, Y., Suzuki, M., & Suzuki, S. (1980). Growth-inhibitory activity of the d-mannan of saccharomyces cerevisiae X2180-1A-5 mutant strain against mouse-implanted sarcoma 180 and ehrlich-carcinoma solid tumor. *Carbohydrate Research*, *83*(2), 363–370. https://doi.org/10.1016/S0008-6215(00)84548-6
Miadoková, E., Svidová, S., Vlčková, V., Dúhová, V., Nad'ová, S., Rauko, P., & Kogan, G. (2006). Diverse biomodulatory effects of glucomannan from Candida utilis. *Toxicology in Vitro*, *20*(5), 649–657. https://doi.org/10.1016/j.tiv.2005.12.001
Mikkonen, K. S., Tenkanen, M., Cooke, P., Xu, C., Rita, H., Willför, S., Holmbom, B., Hicks, K. B., & Yadav, M. P. (2009). Mannans as stabilizers of oil-in-water beverage

emulsions. *LWT - Food Science and Technology*, *42*(4), 849–855. https://doi.org/10.1016/j.lwt.2008.11.010

Millane, R. P., & Hendrixson, T. L. (1994). Crystal structures of mannan and glucomannans. *Carbohydrate Polymers*, *25*(4), 245–251. https://doi.org/10.1016/0144-8617(94)90050-7

Nakajima, T., & Ballou, C. E. (1974). Characterization of the carbohydrate fragments obtained from Saccharomyces cerevisiae mannan by alkaline degradation. *Journal of Biological Chemistry*.

Nanyang Techological University. (2017). *Scientists use brewery waste to grow yeast needed for beer making -- ScienceDaily*. ScienceDaily. https://www.sciencedaily.com/releases/2017/08/170829091256.htm

Northcote, D. H., & Horne, R. W. (1977). *The Chemical Composition and Structure of the Yeast Cell Wall*.

Oba, S., Sunagawa, T., Tanihiro, R., Awashima, K., Sugiyama, H., Odani, T., Nakamura, Y., Kondo, A., Sasaki, D., & Sasaki, K. (2020). Prebiotic effects of yeast mannan, which selectively promotes Bacteroides thetaiotaomicron and Bacteroides ovatus in a human colonic microbiota model. *Scientific Reports*, *10*(1), 1–11. https://doi.org/10.1038/s41598-020-74379-0

Ogawa, K., Nishikori, J., Ino, T., & Matsuda, K. (1994). Chemical Structures of Oligosaccharides Obtained from Partial Acid Hydrolysates of Saccharomyces cerevisiae Mannan. *Bioscience, Biotechnology, and Biochemistry*, *58*(3), 560–562. https://doi.org/10.1271/bbb.58.560

Oka, S., Kumano, N., & Kurita, K. (1972). Antitumor activity of the yeast mannan preparation in relation to effect of chemical modification. *The Research Institute for Tuberculosis, Leprosy and Cancer*, *980*(3), 365–369.

Olaniyi, O. O., Igbe, F. O., & Ekundayo, T. C. (2013). Optimization studies on mannanase production by Trichosporonoides oedocephalis in submerged state fermentation. *E3 Journal of Biotechnology and Pharmaceutical Research*, *4*(7), 110–116.

Onitake, T., Ueno, Y., Tanaka, S., Sagami, S., Hayashi, R., Nagai, K., Hide, M., & Chayama, K. (2015). Pulverized konjac glucomannan ameliorates oxazolone-induced colitis in mice. *European Journal of Nutrition*, *54*(6), 959–969. https://doi.org/10.1007/s00394-014-0772-2

Orlean, P. (2012). Architecture and Biosynthesis of the Saccharomyces cerevisiae Cell Wall. *Genetics*, *192*(3), 775–818. https://doi.org/10.1534/genetics.112.144485

Oyofo, B. A., DeLoach, J. R., Corrier, D. E., Norman, J. O., Ziprin, R. L., & Mollenhauer, H. H. (1989). Prevention of Salmonella typhimurium Colonization of Broilers with D-Mannose. *Poultry Science*, *68*(10), 1357–1360. https://doi.org/10.3382/ps.0681357

Oyofo, B. A., Droleskey, R. E., Norman, J. O., Mollenhauer, H. H., Ziprin, R. L., Corrier, D. E., & Deloach, J. R. (1989). Inhibition by Mannose of In Vitro Colonization of Chicken Small Intestine by Salmonella typhimurium. *Poultry Science*, *68*(10), 1351–1356. https://doi.org/10.3382/ps.0681351

Peat, S., Whelan, W. J., & Edwards, T. E. (1961). 6. Polysaccharides of baker's yeast. Part IV. Mannan. *Journal of the Chemical Society (Resumed)*, *0*, 29. https://doi.org/10.1039/jr9610000029

Phaff, H. J. (1963). Cell Wall of Yeasts. *Annual Review of Microbiology*, *17*(1), 15–30. https://doi.org/10.1146/annurev.mi.17.100163.000311

Pigrau, C., & Escolà-Vergé, L. (2020). Recurrent urinary tract infections: From pathogenesis to prevention. *Medicina Clínica (English Edition)*, *155*(4), 171–177. https://doi.org/10.1016/j.medcle.2020.04.015

Pinto, M., Coelho, E., Nunes, A., Brandão, T., & Coimbra, M. A. (2015). Valuation of brewers spent yeast polysaccharides: A structural characterization approach. *Carbohydrate Polymers*, *116*, 215–222. https://doi.org/10.1016/j.carbpol.2014.03.010

Rakowska, R., Sadowska, A., Dybkowska, E., & Świderski, F. (2017). Spent yeast as natural source of functional food additives. *Roczniki Panstwowego Zakladu Higieny*, *68*(2), 115–121.

Rosenfeld, L., & Ballou, C. E. (1974). ELsevier Scientific Publishing Company, Amsterdam - Printed in Beigium. *Carbohydrate Research*, *32*, 287–298.

Rosenfeld, L., & Ballou, C. E. (1975). An approach to the sequencing of yeast mannan. *Biochemical and Biophysical Research Communications*, *63*(3), 571–579. https://doi.org/10.1016/S0006-291X(75)80422-0

Ruggieri, M. R., Hanno, P. M., & Levin, R. M. (1985). Mannose inhibition of Escherichia coli adherence to urinary bladder epithelium: Comparison with yeast agglutination. *Urological Research*, *13*(2), 79–84. https://doi.org/10.1007/BF00261571

Russell, I., Garrison, I. F., & Stewart, G. G. (1973). Studies on the formation of spherolasps from stationary phase cells of Saccharomyces cerevisiae. *Journal of the Institute of Brewing*, *79*(1), 48–54. https://doi.org/10.1002/j.2050-0416.1973.tb03499.x

Scheller, H. V., & Ulvskov, P. (2010). Hemicelluloses. *Annual Review of Plant Biology*, *61*(1), 263–289. https://doi.org/10.1146/annurev-arplant-042809-112315

Schilling, J. D., Mulvey, M. A., & Hultgren, S. J. (2001). Structure and Function of Escherichia coli Type 1 Pili: New Insightinto the Pathogenesis of Urinary Tract Infections . *The Journal of Infectious Diseases*, *183*(s1), S36–S40. https://doi.org/10.1086/318855

Schröder, R., Atkinson, R. G., & Redgwell, R. J. (2009). Re-interpreting the role of endo-β-mannanases as mannan endotransglycosylase/hydrolases in the plant cell wall. *Annals of Botany*, *104*(2), 197–204. https://doi.org/10.1093/aob/mcp120

Scribano, D., Sarshar, M., Prezioso, C., Lucarelli, M., Angeloni, A., Zagaglia, C., Palamara, A. T., & Ambrosi, C. (2020). D-Mannose treatment neither affects uropathogenic Escherichia coli properties nor induces stable fimh modifications. *Molecules*, *25*(2). https://doi.org/10.3390/molecules25020316

Sedmark, J. J. (2014). *Production of beta-glucans and mannans* (Patent No. US 8,753,668 B2). https://patentimages.storage.googleapis.com/6d/96/fd/1fb6ea16072986/US8753668.pdf

Sentandreu, R., & Northcote, D. H. (1968). The structure of a glycopeptide isolated from the yeast cell wall. *The Biochemical Journal*, *109*(3), 419–432. https://doi.org/10.1042/bj1090419

Shibata, N., Mizugami, K., Takano, K., & Suzuki, S. (1983). Isolation of mannan-protein complexes from viable cells of Saccharomyces cerevisiae X2180-1A wild type and Saccharomyces cerevisiae X2180-1 A-5 mutant strains by the action of Zymolyase-60,000. *Journal of Bacteriology*, *156*(2), 552–558. https://doi.org/10.1128/JB.156.2.552-558.1983

Sims, M. D., Dawson, K. A., Newman, K. E., Spring, P., & Hoogell, D. M. (2004). Effects of Dietary Mannan Oligosaccharide, Bacitracin Methylene Disalicylate, or Both on the Live Performance and Intestinal Microbiology of Turkeys. *Poultry Science*, *83*(7), 1148–1154. https://doi.org/10.1093/ps/83.7.1148

Singh, S., Singh, G., & Arya, S. K. (2018). Mannans: An overview of properties and application in food products. *International Journal of Biological Macromolecules*, *119*, 79–95. https://doi.org/10.1016/j.ijbiomac.2018.07.130

Smith, H., Grant, S., Parker, J., & Murphy, R. (2020). Yeast cell wall mannan rich fraction modulates bacterial cellular respiration potentiating antibiotic efficacy. *Scientific Reports*, *10*(1), 21880. https://doi.org/10.1038/s41598-020-78855-5

Snyman, C., Mekoue Nguela, J., Sieczkowski, N., Marangon, M., & Divol, B. (2021). Optimised Extraction and Preliminary Characterisation of Mannoproteins from Non-Saccharomyces Wine Yeasts. *Foods*. https://doi.org/10.3390/foods10050924

Spring, P., Wenk, C., Connolly, A., & Kiers, A. (2015). A review of 733 published trials on Bio-Mos®, a mannan oligosaccharide, and Actigen®, a second generation mannose rich fraction, on farm and companion animals. *Journal of Applied Animal Nutrition*, *3*, 1–11. https://doi.org/10.1017/jan.2015.6

Spring, P., Wenk, C., Dawson, K. A., & Newman, K. E. (2000). The Effects of Dietary Mannanoligosaccharides on Cecal Parameters and the Concentrations of Enteric Bacteria in the Ceca of Salmonella-Challenged Broiler Chicks. *Poultry Science*, *79*(2), 205–211. https://doi.org/10.1093/ps/79.2.205

Stewart, T. S., & Ballou, C. E. (1968). A comparison of yeast mannans and phosphomannans by acetolysis. *Biochemistry*, *7*(5), 1855–1863. https://doi.org/10.1021/bi00845a033

Stewart, T. S., Mendershausen, P. B., & Ballou, C. E. (1968). Preparation of a Mannopentaose, Mannohexaose, and Mannoheptaose from Saccharomyces cerevisiae Mannan. *Biochemistry*, *7*(5), 1843–1854. https://doi.org/10.1021/bi00845a032

Stynen, D., Sarfati, J., Goris, A., Prevost, M.-C., Lesourd, M., Kamphuis, H., Darras, V., & Latge2, J.-P. (1992). Rat Monoclonal Antibodies against Aspergillus Galactomannan. In *Infection and Immunity*. http://iai.asm.org/

Tanimoto, T., Ikuta, A., Sugiyama, M., & Koizumi, K. (2002). HPLC analysis of manno-oligosaccharides derived from Saccharomyces cerevisiae mannan using an amino column or a graphitized carbon column. *Chemical and Pharmaceutical Bulletin*, *50*(2), 280–283. https://doi.org/10.1248/cpb.50.280

Teng, P.-Y., & Kim, W. K. (2018). Review: Roles of Prebiotics in Intestinal Ecosystem of Broilers. *Frontiers in Veterinary Science*, *5*, 245. https://doi.org/10.3389/fvets.2018.00245

Tester, R. F., & Al-Ghazzewi, F. H. (2013). Mannans and health, with a special focus on glucomannans. *Food Research International*, *50*(1), 384–391. https://doi.org/10.1016/j.foodres.2012.10.037

Theis, T. V., Queiroz Santos, V. A., Appelt, P., M. Barbosa-Dekker, A., Vetvicka, V., F. H. Dekker, R., & A. Cunha, M. A. (2019). Fungal Exocellular (1-6)-β-d-glucan: Carboxymethylation, Characterization, and Antioxidant Activity. *International Journal of Molecular Sciences*, *20*(9), 2337. https://doi.org/10.3390/ijms20092337

Torrecillas, S., Makol, A., Caballero, M. J., Montero, D., Robaina, L., Real, F., Sweetman, J., Tort, L., & Izquierdo, M. S. (2007). Immune stimulation and improved infection

resistance in European sea bass (Dicentrarchus labrax) fed mannan oligosaccharides. *Fish and Shellfish Immunology*, *23*(5), 969–981. https://doi.org/10.1016/j.fsi.2007.03.007

Torrecillas, Silvia, Montero, D., & Izquierdo, M. (2014). Improved health and growth of fish fed mannan oligosaccharides: Potential mode of action. *Fish & Shellfish Immunology*, *36*(2), 525–544. https://doi.org/10.1016/j.fsi.2013.12.029

Valentin, E., Herrero, E., Javier Pastor, F. I., & Sentandreu, R. (1984). Solubilization and Analysis of Mannoprotein Molecules from The Cell Wall of Saccharomyces cerevisiae. In *Journal of General Microbiology* (Vol. 130).

Valpotić, H., Samardžija, M., Terzić, S., Vince, S., Šperanda, M., Lacković, G., Habrun, B., Mas, N., Đuričić, D., Kočila, P., Marković, F., & Valpotić, I. (2016). Effect of mannan oligosaccharide supplementation on blood and intestinal immune cells, bacteria numbers and performance in weaned pigs. *Acta Veterinaria Brno*, *85*(3), 267–276. https://doi.org/10.2754/avb201685030267

Van Loo, J. (2004). The specificity of the interaction with intestinal bacterial fermentation by prebiotics determines their physiological efficacy. *Nutrition Research Reviews*, *17*(1), 89–98. https://doi.org/10.1079/NRR200377

Vazquez-Reyna, A. B., Ponce-Noyola, P., Calvo-Mendez, C., Lopez-Romero, E., & Flores-Carreon, A. (1999). Purification and biochemical characterization of two soluble -mannosidases from Candida albicans. *Glycobiology*, *9*(6), 425–432. https://doi.org/10.1093/oxfordjournals.glycob.a018867

Vieira, E. F., Carvalho, J., Pinto, E., Cunha, S., Almeida, A. A., & Ferreira, I. M. P. L. V. O. (2016). Nutritive value, antioxidant activity and phenolic compounds profile of brewer's spent yeast extract. *Journal of Food Composition and Analysis*, *52*, 44–51. https://doi.org/10.1016/j.jfca.2016.07.006

Vinogradov, E., Petersen, B., & Bock, K. (1998). Structural analysis of the intact polysaccharide mannan from Saccharomyces cerevisiae yeast using H and C NMR spectroscopy at 750MHz. *Carbohydrate Research*, *307*(1–2), 177–183. https://doi.org/10.1016/S0008-6215(98)00042-1

Wang, X., Farnell, Y. Z., Peebles, E. D., Kiess, A. S., Wamsley, K. G. S., & Zhai, W. (2016). Effects of prebiotics, probiotics, and their combination on growth performance, small intestine morphology, and resident Lactobacillus of male broilers. *Poultry Science*, *95*(6), 1332–1340. https://doi.org/10.3382/ps/pew030

Wei, Z., Huang, L., Cui, L., & Zhu, X. (2020). Mannose: Good player and assister in pharmacotherapy. In *Biomedicine and Pharmacotherapy* (Vol. 129). Elsevier Masson SAS. https://doi.org/10.1016/j.biopha.2020.110420

Wexler, H. M. (2007). Bacteroides: the Good, the Bad, and the Nitty-Gritty. *Clinical Microbiology Reviews*, *20*(4), 593–621. https://doi.org/10.1128/CMR.00008-07

Wong-Madden, S. T., & Landry, D. (1995). *Purification and characterization of novel glycosidases from the bacterial genus Xanthomonas* (Vol. 5, Issue 1). https://academic.oup.com/glycob/article-abstract/5/1/19/559079

World beer production | Statista. (2020). https://www.statista.com/statistics/270275/worldwide-beer-production/

Wu, H., Zhang, W., & Mu, W. (2019). Recent studies on the biological production of D-mannose. In *Applied Microbiology and Biotechnology* (Vol. 103, Issues 21–22, pp. 8753–8761). Springer Verlag. https://doi.org/10.1007/s00253-019-10151-3

Xie, L., Shen, M., Hong, Y., Ye, H., Huang, L., & Xie, J. (2020). Chemical modifications of

polysaccharides and their anti-tumor activities. *Carbohydrate Polymers*, *229*(October 2019), 115436. https://doi.org/10.1016/j.carbpol.2019.115436

Xu, Y., Wu, Y., Sun, P., Zhang, F., Linhardt, R. J., & Zhang, A. (2019). Chemically modified polysaccharides: Synthesis, characterization, structure activity relationships of action. *International Journal of Biological Macromolecules*, *132*, 970–977. https://doi.org/10.1016/j.ijbiomac.2019.03.213

Yamabhai, M., Sak-Ubol, S., Srila, W., & Haltrich, D. (2016). Mannan biotechnology: From biofuels to health. *Critical Reviews in Biotechnology*, *36*(1), 32–42. https://doi.org/10.3109/07388551.2014.923372

Yang, Y., Iji, P. A., Kocher, A., Thomson, E., Mikkelsen, L. L., & Choct, M. (2008). Effects of mannanoligosaccharide in broiler chicken diets on growth performance, energy utilisation, nutrient digestibility and intestinal microflora. *British Poultry Science*, *49*(2), 186–194. https://doi.org/10.1080/00071660801998613

Yen, P. H., & Ballou, C. E. (1974). Structure and immunochemistry of Hansenula wingei Y-2340 mannan. *Biochemistry*, *13*(11), 2420–2427. https://doi.org/10.1021/bi00708a029

Yin, M., Zhang, Y., & Li, H. (2019). Advances in Research on Immunoregulation of Macrophages by Plant Polysaccharides. *Frontiers in Immunology*, *10*(FEB), 145. https://doi.org/10.3389/fimmu.2019.00145

Young, M., Davies, M. J., Bailey, D., Gradwell, M. J., Smestad-Paulsen, B., Wold, J. K., Barnes, R. M. R., & Hounsell, E. F. (1998). Characterization of oligosaccharides from an antigenic mannan of Saccharomyces cerevisiae. *Glycoconjugate Journal*, *15*(8), 815–822. https://doi.org/10.1023/A:1006968117252

Yu, X., Li, Z., Yu, M., Yao, J., & Zhang, Y. (2008). Method and Preparing Glucan and Mannan, Glucan Preparation and Mannan Preparation Produced Thereby and Use Thereof. In *US Patent App. 12/990,206*. http://www.google.com/patents?hl=en&lr=&vid=USPATAPP12990206&id=49InAQAAEBAJ&oi=fnd&dq=Method+and+preparing+glucan+and+mannan,+glucan+preparation+and+mannan+preparation+produced+thereby+and+use+thereof&printsec=abstract

Yu, X., Li, Z., Yu, M., Yao, J., & Zhang, Y. (2011). *Method and preparing glucan and mannan, glucan preparation and mannan preparation produced thereby and use thereof.* (Patent No. US 8,679,797 B2). https://patentimages.storage.googleapis.com/90/4c/e8/58668bea5178f0/US20110045545A1.pdf

Zhang, G., Ma, L., & Doyle, M. P. (2007). Salmonellae Reduction in Poultry by Competitive Exclusion Bacteria Lactobacillus salivarius and Streptococcus cristatus. *Journal of Food Protection*, *70*(4), 874–878. https://doi.org/10.4315/0362-028X-70.4.874

Zhao, P. Y., Jung, J. H., & Kim, I. H. (2012). Effect of mannan oligosaccharides and fructan on growth performance, nutrient digestibility, blood profile, and diarrhea score in weanling pigs 1. *J. Anim. Sci*, *90*, 833–839. https://doi.org/10.2527/jas.2011-3921

Zopf, D., & Roth, S. (1996). Oligosaccharide anti-infective agents. *The Lancet*, *347*(9007), 1017–1021. https://doi.org/10.1016/S0140-6736(96)90150-6

Zordan, R. E., Beliveau, B. J., Trow, J. A., Craig, N. L., & Cormack, B. P. (2015). Avoiding the Ends: Internal Epitope Tagging of Proteins Using Transposon Tn7. *Genetics*, *200*(1), 47–58. https://doi.org/10.1534/genetics.114.169482

Zouchova, Z., Kocourek, J., & Musilek, V. (1977). Separation and Properties of alpha-Mannosidase and Mannanase from the Basidiomycete Phellinus abietis. In *Folia Miorobiol* (Vol. 22).