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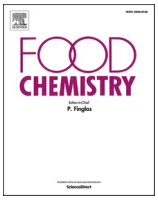

Effect of drying technology in *Saccharomyces cerevisiae* mannans: Structural, physicochemical, and functional properties

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ABSTRACT

Mannans are polysaccharides whose physicochemical and biological properties render them commercialization in several products. Since these properties are strongly dependent on production conditions, the present study aims to assess the impact of different drying technologies – freeze (FDM) and spray drying (SDM) – on the structural, physicochemical, and biological properties of mannans from *Saccharomyces cerevisiae*. Structural analysis was assessed by FT-IR, PXRD and SEM, whereas physicochemical properties were evaluated based on sugars, protein, ash and water contents, solubility, and molecular weight distribution. Thermal behaviour was analysed by DSC, and antioxidant activity by DPPH and ABTS assays. The parameters which revealed major differences, in terms of structural and physicochemical properties regarded morphology (SEM), physical appearance (colour), moisture (3.6 ± 0.1 % and 11.9 ± 0.6 % for FDM and SDM, respectively) and solubility (1 mg/mL for FDM and 25 mg/mL for SDM). Nevertheless, these differences were not translated into the antioxidant capacity.

1. Introduction

Polysaccharides are a very important class of biopolymers structurally characterized by the nature of monomer units, chain length, glycosidic linkages, and degree of branching.

Plant-derived polysaccharides have been extensively studied due to their numerous physiological benefits such as antioxidant, immunomodulatory, antitumor, anti-inflammatory, antidiabetic, and antiviral properties (Chen et al., 2018).

Mannans, long-chain carbohydrates mainly composed of mannose units, can be obtained from vegetables, microorganisms and seeds (Tester & Al-Ghazzewi, 2013). One important source is the cell wall of yeasts, particularly the baker's and brewer's yeast *Saccharomyces cerevisiae* (Orlean, 2012). Data from 2017 concerning the annual world production of beer pointed out to values near 193 billion litres; this production generated 46.26 billion tons of by-products, among which spent yeast corresponds to ca. 12.7 %: therefore, 1.5 – 3 kg of spent yeast are generated for each 100 L of beer produced (Brewers By-products Market Outlook, Share, Size, Forecast, Trends, Report, 2020). The recovery and reuse of yeast is an excellent example of circular economy, as this by-product may be used to extract functional compounds and develop new innovative products, whereas waste management and environmental impacts are improved (Rakowska et al., 2017).

Mannans present several physicochemical properties, which render them the property to be used as a hardening ingredient or emulsion stabilizer (Singh et al., 2018). They are also claimed to be used as antibiotic replacers in animal feed (Smith et al., 2020), limiting gastrointestinal infections. Additionally, the ability to scavenge radicals such as superoxide anions and hydroxyl radicals provides a potential antioxidant effect (Liu et al., 2018). All these properties reveal the potential applications of mannans in the areas of food, feed, and cosmetics.

Mannan's extraction from spent yeast usually includes several steps such as cell lysis, fractionation and purification; in addition to the extraction methods, drying steps are also included, as they increase the shelf life of mannans extracts. Drying technologies are a critical step as they can affect the structural, physicochemical and biological properties of the final product. Among the drying technologies, freeze drying (FD) and spray drying (SD) stand up as the most usually employed. SD was

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used at industrial scale since the 1920′s (Serra Costa et al., 2015). It presents the advantage of including only one processing step as opposite to FD (which requires a previous freezing of the extract), with yields ranging 50–70 % and a final extract with homogeneity in terms of size and morphology (Emami et al., 2018). The main drawback pointed out to this technology is that it can cause heat stress on extract's constituents, thus promoting undesirable physicochemical and/or functional changes. On the other hand, FD processing increases the stability of the extracts and their resistance to microorganism propagation (Shukla, 2011). However, this technology requires expensive equipment, being a time-consuming method and, as abovementioned, requiring additional processing (sample pre-freezing).

Although the processes pertaining to mannans extraction from yeast wall cells have been extensively reported in literature, the effect of drying step in the obtained extracts is usually poorly documented. Thus, the goal of the present work was to characterize the structural, physicochemical, and biological properties of mannans extracted from spent yeast *S. cerevisiae* and dried by FD or SD technologies. Additionally, those properties were compared to the ones exhibited by commercial pure mannans and duly discussed.

2. Materials and methods

2.1. Materials

Spent *S. cerevisiae* was obtained from Amyris Company (Emeryville, California, USA), and commercial *S. cerevisiae* mannan (M7504) was purchased from Sigma-Aldrich (USA). Trolox ((±)-6-hydroxy-2,5,7,8-tetramethylchromane-2-carboxylic acid), ABTS (2,2′-azino-bis (3-ethylbenzothiazoline-6-sulphonic acid) diammonium salt, ethanol, methanol and ascorbic acid (≥99 %) were also purchased from Sigma-Aldrich. DPPH (2,2-Diphenyl-1-picrylhydrazyl) was purchased from Thermo-Fisher Scientific (USA). All reagents were used as received without further purification.

2.2. Isolation and purification of mannans

2.2.1. Preparation of spent yeast

Spent yeast was centrifuged at 8000 rpm for 10 min (ThermoFisher Scientific, Massachusetts, USA), and the supernatant was removed. The pellet was washed twice with deionized water and centrifuged using the abovementioned conditions.

2.2.2. Thermal hydrolysis

Subsequentially, pellet was resuspended in deionized water (10 % w/v) and the pH was adjusted to 7.0. This solution was then heated to 120 °C in autoclave (Prohs, Porto, Portugal) for 3 h. After cooling down, the solution was centrifuged (5000 rpm, 10 min) and the supernatant (rich in mannoproteins) was collected, precipitated with twice its volume with cold ethanol, and left overnight at 4 °C. The solid was collected by centrifugation and dried by freeze-drying for approximately 72 h (Alpha 2–4 LSCplus, Germany) or spray drying (Mini Spray Dryer B-290, Buchi, Switzerland) with the condition's inlet temperature 110 °C, aspirator 65 %, pump 13 %. The complete process is depicted in Fig. 1.

2.3. Structural characterization

2.3.1. Fourier-transform infrared spectroscopy – FT-IR

The Fourier-transform infrared spectroscopy (FT-IR) spectra were recorded using the Frontier™ MIR/FIR spectrometer from PerkinElmer in a scanning range of 550–4000 cm^{-1} for 16 scans at a spectral resolution of 4 cm^{-1}.

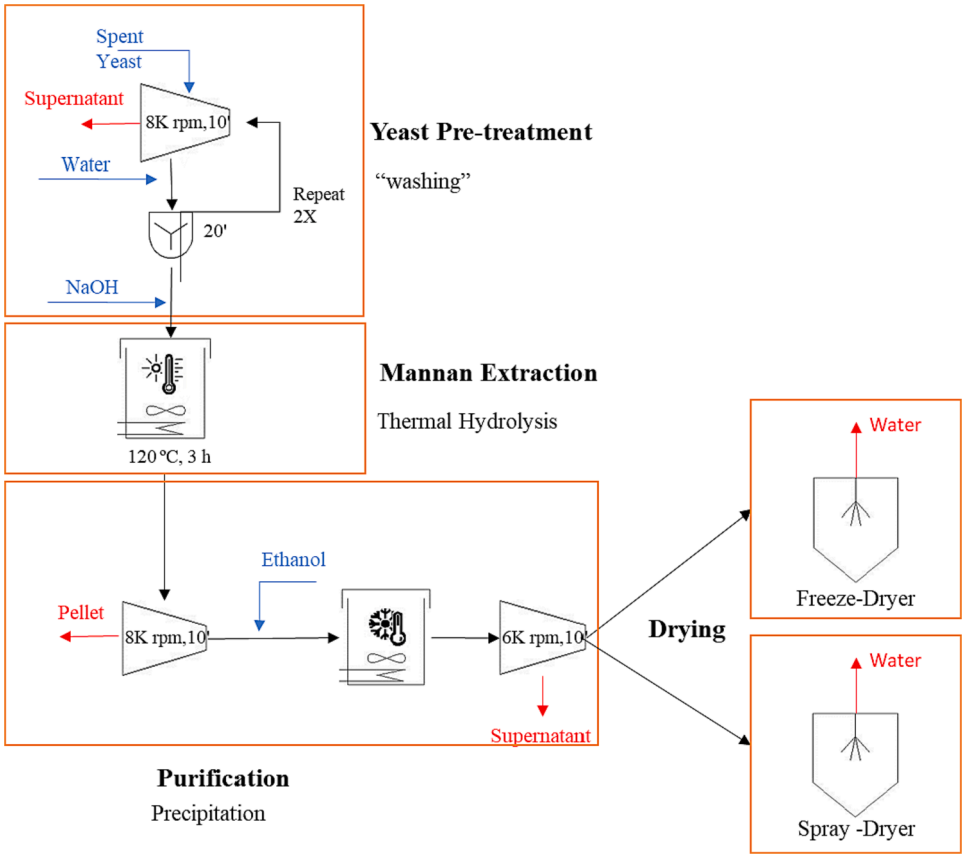


Fig. 1. Extraction process of mannan extracts from spent yeast *S. cerevisiae*.

2.3.2. Powder X-ray diffraction analysis – PXRD

Powder X-ray Diffraction Analysis (PXRD) was performed on Rigaku MiniFlex 600 diffractometer with Cu kα radiation, with a voltage of 40 kV and a current of 15 mA ($3° \leq 2\theta \geq 60°$; a step of 0.01 and speed rate of 3.0°/min). The evaluation of the crystallinity index (%CI) was performed using the program PDXL2 from Rigaku® (version 2.8.1.1) by the adjustment of the baseline and the profile of each diffractogram. The CI value is then obtained by considering the ratio of the total areas of crystalline peaks to the total area of all peaks. All measurements were done in duplicate.

2.3.3. Scanning Electron Microscopy – SEM

The morphology of all samples was evaluated by Scanning Electron Microscopy (SEM) on JSM-5600 LV Scanning Electron Microscope from JEOL, Japan. Prior to analysis, the mannans powdered samples were placed into observation stubs (covered with double-sided adhesive carbon tape (NEM tape, Nisshin, Japan) and coated with Aug/Pd using a Sputter Coater (Polaron, Bad Schwalbach, Germany). All observations were performed in the high-vacuum mode with an acceleration voltage of 20 kV, at a working distance of 9–10 mm and a spot-size of 27. The images presented are representative images of the morphology of each sample.

2.4. Physicochemical properties

2.4.1. Colour

The colour point was determined using a portable CR-410 Chroma meter (from Minolta Chroma, Osaka, Japan). The CIELAB (L*, a*, b*) colour system was employed to determine the colour point, where L* is the lightness coordinate (L* value of 100 for a white object and 0 to a black object); the a* defines the green–red coordinate, and b* the blue-yellow coordinate (Ordóñez-Santos et al., 2017). Colour was measured on the surface of the mannans samples in a petri dish placed on a white standard plate (L*= 93.22, a* = −0.08 and b*= 4.04). All measurements were done in triplicate.

The total colour difference (ΔE*) was calculated according to Equation (1).

$$\Delta E^* = ((L_2^*\text{-}L_1^*)^2 + (a_2^*\text{-}a_1^*)^2 + (b_2^*\text{-}b_1^*)^2)^{1/2} \quad (1)$$

2.4.2. Bulk and tapped densities

The bulking properties and the tapped density of the powder mannan extracts was determined according to the European Pharmacopeia 7.0 (2010). Briefly, the bulk densities were determined using 10 mL graduated cylinders, where mannans' powders were gently introduced, without compacting, until the graduation of 10 mL. The bulk density of each sample was then obtained using the formula m/V0 (m – sample mass in grams and V0 – unsettled apparent volume in mL). After observing the initial powder volume (V0), the cylinders in the support were submitted to 20 continuous taps (no difference observed with taps increment) and the tapped density was obtained using the formula m/Vf (m – mass of each powder sample and Vf – final tapped volume in mL). All analyses were performed in triplicate. The Compressibility index and the Hausner Ratio are considered simple methods of predicting powder flow characteristics and can be calculated according to Equations 2 and 3, respectively.

$$\text{Compressibility Index} = 100 \times (\text{tapped density} - \text{bulk density}) / \text{tapped density} \quad (2)$$

$$\text{Hausner ratio} = \text{tapped density}/ \text{bulk density} \quad (3)$$

2.4.3. Total protein

Total protein content was assessed with the BCA Protein Assay Kit (Pierce, Bonn, Germany), used according to the manufacturer's instructions in microplates (25 μL sample/200 μL BCA working reagent; 37 °C/30 min; 562 nm). A BSA (Sigma, Munich, Germany) stock solution was prepared in bulk quantity prior to the experiments. BSA-dilution curves were made accordingly, and aliquots were stored to ensure an identical BSA-content for each experiment. For every microplate prepared, a BSA-dilution calibration curve was added, consisting of 8 points up to 1000 μg/ml BSA. Samples were measured in triplicate and averaged.

2.4.4. Neutral sugars

Poli- and oligosaccharides were converted into their monomer residues by a pre-hydrolysis with 72 % H_2SO_4 (w/w) for 3 h at room temperature, followed by 1 h hydrolysis in 1 M H_2SO_4 at 100 °C (Selvendran et al., 1979). Derivatization process was performed according to Blakeney et al., (1983). Neutral sugars were analysed by gas-chromatography-flame ionization detection (GC-FID). Samples were measured in triplicate and averaged.

2.4.5. Molecular weight distribution

Molecular weight distribution determination was carried out on Agilent 1260 Infinity II HPLC system equipped with Vialsampler, quaternary pump, thermostatic oven and Agilent 1290 Infinity II ELSD – Evaporative Light Scattering Detector. Data acquisition and analysis were performed with the OpenLaB CDS ChemStation from Agilent Technologies. The separation was performed on Agilent SE columns, PL Aquagel-OH Mixed – M (250 × 4.6 mm, 8 μm) and PL Aquagel-OH 20 (300 × 7.5 mm, 5 μm). Molecular weight was estimated against a calibration curve determined using pullulan standards. An aliquot of 10 μL of standards and test solutions was ejected and eluted with the solvent (Ammonium acetate 10 mM) at flow rate of 0.6 mL/min under isocratic conditions. ELSD Nebulizer temperature was set at 60 °C, evaporator temperature 80 °C and N_2 flow rate was kept at 1.25 SLM.

2.4.6. Dry weight and ashes

To evaluate the moisture content according to Association of Official Analytical chemists (AOAC, 2005), the mannans samples were placed at 105 °C in a convection oven for 24 h. To determine the ash content, samples were placed at 550 °C in a muffle for 36 h for incineration. The ash was weighed after equilibration at room temperature. Samples were measured in triplicate and averaged.

2.4.7. Solubility test

Tests were performed according to the European Pharmacopoeia (Pharmacopoeia, 2017): 1.0 g of finely ground compound was dissolved in increasing volumes of solvent (water), and its classification in terms of solubility was directly related with the volume of solvent necessary for complete solubilization. Substances that dissolve in 10–30 mL, 30–100 mL, 100–1000 mL or 1000–10000 mL of solvent are classified as soluble, sparingly soluble, slightly soluble or very slightly soluble, respectively. If the substance does not dissolve, it is classified as insoluble in this solvent.

2.4.8. Differential scanning calorimetry – DSC

Differential scanning calorimetry (DSC) measurements were performed under nitrogen atmosphere using a DSC 204 F1 Phoenix equipment from Netzsch, calibrated using an indium standard. The samples (3–6 mg) were placed into aluminium DSC pans, being an empty pan used as a reference, and heated from 20 to 500 °C at a heating rate of 10 °C/min.

2.5. Antioxidant capacity

2.5.1. ABTS scavenging assay

ABTS scavenging assay was performed in 96-well microplates, according to Gonçalves et al., (2009) with some modifications. This method can quantify antioxidants via direct production of the 2,2′-azino-bis (3-ethylbenzthiazoline-6-sulphonic acid) diammonium salt radical cation ($ABTS^{\bullet+}$) chromophore (blue/green) by reaction of 2,2′-azino-bis (3-ethylbenzthiazoline-6-sulphonic acid) diammonium salt (ABTS) and potassium persulphate ($K_2S_2O_8$) (Merck, Darmstadt, Germany), after incubation at room temperature in the dark for 16 h. The $ABTS^{\bullet+}$ working solution (freshly prepared) was filtered with a 0.45 μm syringe filter and diluted with solvent to an absorbance of 0.70 ± 0.02, at 734 nm. $ABTS^{\bullet+}$ (200 μL) was added to 15 μL of sample (at concentrations of 5 mg/mL for mannans extracts, 10 mg/mL for commercial mannan, and 0.05 mg/mL for ascorbic acid), Trolox (in the range 50–560 μM) or solvent (blank assay). The mixture was incubated for 5 min at 30 °C, and the absorbance at 734 nm was measured with a plate reader (Synergy H1, USA) controlled by Gen5 Biotek software (version 3.04). Scavenging activity was expressed as % reduction in absorbance as compared with the control. Regression equations between ABTS scavenging and Trolox concentration were calculated, and the results expressed as mmol TE (Trolox equivalent)/100 g of sample.

The $ABTS^{\bullet+}$scavenging effect percentage was determined according to Equation (4):

$$ABTS^{\bullet+}\text{scavenging effect } (\%) = [(A_{control} - A_{sample})/A_{control}] \times 100 \quad (4)$$

where $A_{control}$ and A_{sample} are the absorbances at 734 nm of control and sample, respectively. The assay was performed in triplicate.

2.5.2. DPPH scavenging assay

The 2,2-Diphenyl-1-picrylhydrazyl (DPPH)-free-radical scavenging activity was measured according to the method described by Schaich et al., (2015), with modifications for a 96-well microplate scale. The stock solution (600 μM) was prepared in methanol and stored in the dark at −20 °C. As for ABTS, a daily DPPH solution was prepared by dilution with methanol to 0.600 ± 0.100 at 515 nm. For the assay, 25 μL of sample (5 mg/mL for mannans extracts, 10 mg/mL for commercial mannan and 0.03 mg/mL for ascorbic acid), Trolox (7.5 – 240 μM) or solvent were added to 175 μL DPPH daily solution. The mixture was incubated for 30 min at 25 °C, and the absorbance at 515 nm was measured with a plate reader (Synergy H1, USA).

The scavenging activity was expressed as % reduction in absorbance related to the control. Regression equations between DPPH scavenging and Trolox concentration were calculated, and the results expressed as mmol TE (Trolox equivalent)/100 g of sample.

The DPPH scavenging effect percentage was determined according to Equation (5):

$$\text{DPPH scavenging effect } (\%) = [(A_{control} - A_{sample})/A_{control}] \times 100 \quad (5)$$

where $A_{control}$ and A_{sample} are the absorbances at 515 nm of control and sample, respectively. The assay was performed in triplicate.

2.6. Cytotoxicity evaluation

2.6.1. Cell line growth conditions

Human colon carcinoma (Caco-2) cells were obtained from the European Collection of Authenticated Cell Cultures and were grown using high glucose (4.5 g/L1) DMEM supplemented with 10 % (v/v) heat inactivated FBS, 1 % (v/v) penicillin–streptomycin–fungizone (Lonza, Verviers, Belgium), and 1 % (v/v) of non-essential amino acids 100× (Sigma, Germany). Cells were used between passages 53 and 54.

2.6.2. Cytotoxicity assay by presto blue

CaCo-2 viability assay with presto blue (PB) reagent was performed according to ISO 10993-5 (2009). The cells in suspension were seeded at 1×10^4 cells/well in a 96-well microtiter plate. After 24 h-treatment of the cells with samples, the PB reagent was added. Changes in cell viability were detected using fluorescence and absorbance spectroscopy. Absorbance was recorded at 570 nm after 2 h-incubation of endothelial cells with PB reagent, whereas fluorescence was read (excitation 570 nm; emission 610 nm) at recommended time of incubation (20 min- and 2 h-endpoint).

2.7. Statistical analysis

Data are expressed as the mean plus standard deviation of replicates. To analyse the differences between the different FDM, SDM and CM when a normal distribution was observed (Shapiro-Wilke normality tests), the one-way ANOVA test were carried out in association with Tukey's Multiple Comparison Test with a 95 % confidence interval. All tests were realized with a significance level of 5 % and using the Statistical Package for Social Sciences software (version 21, SPSS, Chicago, IL, USA).

3. Results and discussion

In order to extract functional compounds and develop innovative products in a circular economy concept, the recovery and reuse of spent yeast, namely *S. cerevisiae,* is an excellent opportunity. In this work we used a thermal extraction process with high temperature and the combination of pressure. The high temperatures used have the additional advantage of decontaminating a possible microbial presence.

In addition to the extraction methods, drying technologies are an important step to the final process since they can affect the structural, physicochemical, and biological properties of final products. The objective of the present work was to perform an integrated study on the structural, physiochemical, and biological (in terms of antioxidant capacity) characteristics of the freeze dry mannan extract (FDM) and spray dry mannan extract (SDM) obtained from spent *S. cerevisiae* of Amyris Company, as compared with commercial mannans.

3.1. Structural analysis

The structural analysis of the mannan extracts from *Saccharomyces cerevisiae* obtained by different drying methods – freeze drying (FDM) and spray drying (SDM) – was attained by the following set of solid-state techniques: Fourier-Transformed Infrared Spectroscopy (FT-IR), Powder X-ray Diffraction (PXRD) and Scanning Electron Microscopy (SEM). The functional groups, crystallinity indexes and the morphological analyses of both samples were compared with the mannan commercial sample, Mannan M7504 from Sigma (CM), as highlighted in Fig. 2.

The normalized FT-IR spectra of the mannan extracts from *Saccharomyces cerevisiae* (FDM and SDM) and the mannan benchmark (CM) are depicted in Fig. 2a. All spectra exhibit a strong and broad vibration band at 3000–3693 cm^{-1} correspondent to the stretching of the hydroxyl groups (Liu & Huang, 2018). The weak absorption band at ca. 2937 cm^{-1} is attributed to the C—H stretching vibration, and the band at 1404 cm^{-1} corresponds to the C—H bending vibration. The sharp band at 1645 cm^{-1} is assigned to the C—O asymmetric stretching vibration. The absorption peak at 1026 cm^{-1} corresponds to the O—H variable angle vibrations and the characteristic absorption of the mannan α-chain appeared at 810 cm^{-1} (Liu & Huang, 2018).

In order to study the crystallinity differences resulting from the drying processes, both yeast mannan extracts (FDM and SDM) were analysed by Powder X-ray Diffraction (PXRD). The crystallinity of the commercial mannan sample (CM) was also evaluated, since the crystallinity may affect physicochemical/biological properties, namely the solubility, being expected the decrease of solubility with an increase of crystallinity (Guo et al., 2017). All PXRD patterns highlighted in Fig. 2b exhibit a single broad reflection centered at $2\theta = 18.8°$. Besides the

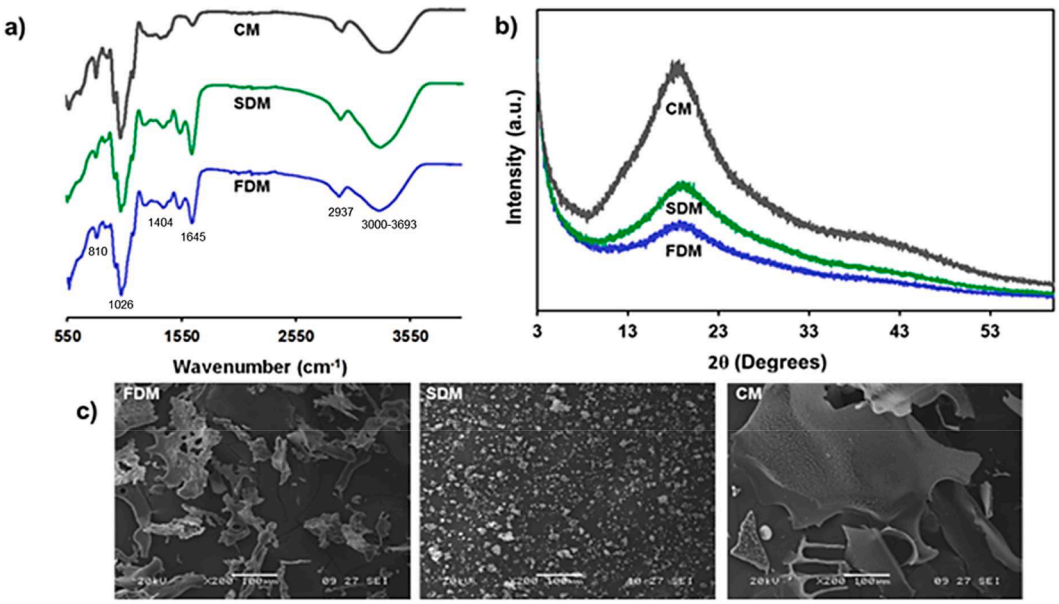


Fig. 2. Structural characterization of the freeze (FDM) and spray dried (SDM) mannan extracts, and the commercial mannan (CM) by a) Fourier- Transform Infrared Spectroscopy; b) Powder X-ray Diffraction analysis and c) Scanning electron microscopy.

predominant amorphous pattern of all samples, the crystallinity index (CI in %) was calculated using the PDXL2 da Rigaku® (version 2.8.1.1) software. Briefly, the baseline and the diffractogram profile were properly adjusted for each analysis, followed by the modelling of the amorphous phase. The CI (%) is then obtained by the ratio of the crystalline area to the total area. The mannan benchmark exhibited the highest crystallinity (33.8 % (±1.0)), followed by the spray dried mannan extract SDM (26.4 % (±0.9)), and the freeze-dried product FDM (23.7 % (±0.8)).

The different drying methods clearly affected the morphology and size of the products, as represented in Fig. 2c. The morphology of the mannan benchmark was also studied, since morphology/size may affect physicochemical properties, such as wettability and solubility (Padma Ishwarya & Anandharamakrishnan, 2015). The spray dried product SDM exhibit a round shape with a smaller and more uniform size than the freeze-dried product FDM, or even the commercial mannan (CM). The FDM is irregular in shape and exhibits larger particles than the SDM sample, while the CM sample is characterized by large plates along with smaller grains. These observations are in agreement with the morphology described in the literature for the spray (Emami et al., 2018) and freeze drying (Liapis & Bruttini, 2020) methods.

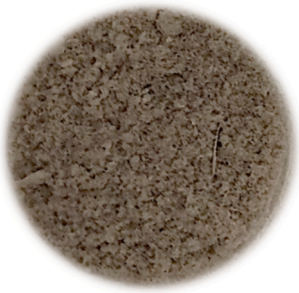
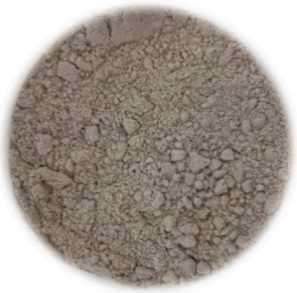
3.2. Physicochemical characterization

The integrated and comparative analysis of the physical appearance and powder flow properties of all mannan products (FDM, SDM and CM), along with their physicochemical properties and thermal analysis, is presented in this section.

3.2.1. Physical appearance

The powders' physical appearance is clearly different, as depicted in Table 1. While the SDM appeared as a homogeneous fine powder, the FDM product presents granules that difficult the maceration process after drying. The mannan benchmark CM appeared as little white sheets,

Table 1
Results of physical appearance, colour characteristics, and powder flow properties of the freeze (FDM) and spray dried (SDM) mannan extracts and the mannan benchmark (CM).

	Freeze Dry Mannan Extract (FDM)			Spray Dry Mannan Extract (SDM)			Mannan M7054 from Sigma (CM)		
Physical appearance									
Colour Characteristics	L*	a*	b*	L*	a*	b*	L*	a*	b*
	75.15 ± 0.21	1.49 ± 0.05	9.54 ± 0.09	83.67 ± 0.01	1.54 ± 0.00	7.12 ± 0.04	90.08 ± 0.03	−0.23 ± 0.01	7.73 ± 0.01
Bulk Density (g/ml)	0.09 ± 0.00 [a]			0.09 ± 0.01 [a]			0.03 ± 0.00[b]		
Tapped Density (g/ml)	0.16 ± 0.01 [a]			0.21 ± 0.01 [a]			0.08 ± 0.01[b]		
Hausner Ratio	1.89 ± 0.04[b]			2.29 ± 0.03[a]			2.78 ± 0.08[c]		
Compressibility (%)	47.00 ± 1.00[b]			56.33 ± 0.58 [a]			64.00 ± 1.00[c]		

[a,b,c] means within the same line, marked with the same letter, do not differ from each other ($p > 0.05$).

while both yeast mannan extracts exhibit a brown colour. The colour of products constitutes a key criterion for their commercialization, since the colours accepted by the consumers are very scarce. The colour point of all mannan products was measured using the system CIELAB (L*, a*, b*) (Ordóñez-Santos et al., 2017). The benchmark product CM exhibits the highest L* value (90.08 ± 0.03), followed by the yeast mannan products SDM (83.67 ± 0.01) and FDM (75.15 ± 0.21), respectively. The total colour difference (ΔE*) was determined to allow the quantification of the colour change between the yeast mannan products and the mannan commercial product, and even between both *Saccharomyces cerevisiae* mannan extracts. The ΔE* (1.50 < ΔE* > 3.0) between the SDM and FDM samples corresponds to 8.86, while the ΔE* between the FDM or SDM samples and the benchmark CM was 15.13 and 6.68, respectively. Since the determined total colour difference were higher than 3.0, this corroborates the distinct visual perception among all mannan samples (Bellary et al., 2016).

The flow properties of all powder samples were studied using the compressibility index and the closely related Hausner ratio, calculated from the bulk and tapped densities using Equations 2 and 3, respectively. The different sizes and shapes of the mannan extracts FDM and SDM do not contribute to significant differences in the observed bulk (0.09 ± 0.01 g/mL) or tapped densities (0.21 ± 0.01 g/mL). The mannan benchmark CM revealed the lowest tapped and bulk densities of 0.03 ± 0.00 g/mL and 0.08 ± 0.01 g/mL, respectively. According to the European Pharmacopeia 7.0 (2010), all powder mannan samples exhibit a flow character of very, very poor, according to the generally accepted scale of flowability (Compressibility index > 38 and Hausner ratio > 1.60). The FDM extract has a larger particle size (Fig. 2) and consequently presents an increase in interparticle void spaces with smaller contact surface areas per unit volume, that may have led to it having an apparently lower density (Caparino et al., 2012) than the SDM sample.

3.2.2. Physicochemical properties

The physicochemical analyses performed for the mannan extracts from *S. cerevisiae* (FDM and SDM) and for the mannan benchmark CM are summarized in Table 2. All mannan products were evaluated regarding to their composition – protein and total sugars (mannose and glucose); molecular weight by HPLC-*SEC*; moisture; ash content and solubility.

Both mannan extracts revealed to have a similar composition. The different drying processes revealed not to have a significant impact on the mannan content, since both extracts exhibit a mannan content of 42.7 ± 6.6 % and 42.0 ± 0.0 % for the FDM and SDM samples, respectively. The analyses of the FDM extract present higher standard deviations, which could be indicative of the higher heterogeneity of the powder. The CM sample revealed the highest purity, since has the lowest protein content (0.5 ± 0.0 %), no-glucose residue detected and a mannose content of 98.0 ± 0.0 %.

The molecular weight (MW) of samples usually has a direct relationship with their bioactivity (Liu et al., 2021). According to Hashim et al., (2018), mannan extracts with a MW of 12.9 kDa and 50 % (w/w) of sugar demonstrated to stimulate immunity in broiler chicken. Galinari et al., (2018) extracted mannans from *Kluyveromyces marxianus* and obtained 5 different fractions with MW ranging from 10 to 203 kDa, all exhibiting antioxidant activity. Table 2 shows the MW fractions present in both mannan extracts and CM. As observed, there are no significant differences between FDM and SDM (Supplementary Material: Fig. S1); on the other side, CM only exhibits one single population of 34 – 62.5 kDa, as expected due to its high purity.

According to the results obtained in Table 2, a significant difference found between FDM and SDM concerned the moisture content, although for the ash content this difference was not significant. It was also noted that SDM had a higher moisture content when compared with FDM and CM. For the inorganic matter, CM demonstrated a smaller quantity of matter in comparison to FDM and SDM, which may indicate that CM had a higher purity. A solution to achieve a more homogenous content between samples could be increase the purification process (e.g. through protein removal).

The solubility of polysaccharides plays a key role in their applications, since most of their functions are achieved in an aqueous medium, namely, emulsion capacity, drug delivery, among others. The FDM exhibited the lowest solubility limit (1 mg/mL), followed by the mannan benchmark CM (10 mg/mL) and the SDM sample (25 mg/mL). The morphological analyses showed in Fig. 2 corroborated the easiest solubility of the SDM product, since the lowest size may increase the surface area, and consequently the rate of solubility (Anandharamakrishnan et al., 2010). Besides the morphology, it was possible to verify that the crystallinity indexes of the different samples do not have a significant impact on the solubility. In fact, the FDM sample, which exhibits the highest amorphous nature (CI of 23.7 %), revealed to have the hardest sample to dissolve and not the easiest soluble character – compounds with higher amorphous content must exhibit higher solubility. Regarding to the sample composition, summarized in Table 2, it was possible to infer that the CM benchmark, exhibiting the lowest molecular weight of 69 kDa, was not the easiest product to solubilize, contrasting the tendency of polysaccharides solubility based on the promotion of solubility for low molecular weight molecules (Guo et al., 2017).

3.2.3. Thermal analysis

The thermal properties of the mannan extract from *Saccharomyces cerevisiae* dried by freeze (FDM) or spray drying (SDM), and the mannan benchmark (CM) were studied by differential scanning calorimetry (DSC), as depicted in Fig. 3.

The DSC thermograms of the FDM and the SDM products exhibit a broad endothermic peak centered at 87.43 and 84.94 °C, respectively, which can be attributed to the evaporation of the residual water (Harish Prashanth, 2002). This endothermic event was also observed for the

Table 2
Results of physicochemical characterization of same extract of mannan dried by different methods freeze drying (FDM) and spray drying (SDM) and compare with commercial mannan (CM).

	Freeze Dry Mannan Extract (FDM)				Spray Dry Mannan Extract (SDM)				Mannan M7054 from Sigma (CM)
Protein Content (% w/w)	23.4 ± 1.5 [a]				21.1 ± 1.4 [a]				0.52 ± 0.04 [b]
Total Sugars (% w/w) *	46.9 ± 6.1 [a]				44.9 ± 1.2 [a]				98.0 ± 0.0 [b]
Mannose (% w/w)	42.7 ± 6.6 [a]				42.0 ± 0.0 [a]				97.0 ± 0.0 [b]
Glucose (% w/w)	3.0 ± 0.8 [a]				2.0 ± 0.1 [a]				Not detected
Molecular Weight (kDa)	244 ± 3 [a]	119 ± 3 [b]	23 ± 0 [a]	10 ± 0 [a]	241 ± 6 [a]	105 ± 4 [a]	24 ± 2 [a]	10 ± 0 [a]	34–62.5
Area %	79	17	2	3	81	16	1	2	100
Moisture (% w/w)	3.6 ± 0.1 [b]				11.9 ± 0.6 [a]				8.1 ± 0.4 [c]
Ash (% w/w)	8.0 ± 0.5 [a]				8.0 ± 0.5 [a]				0.4 ± 0.1 [b]
Solubility	Very slightly soluble (approx. 1 mg/mL)				Sparingly soluble (approx. 25 mg/ml)				Sparingly soluble (approx. 10 mg/ml)

*Total sugars are the sum of the glucose content (% w/w) plus the mannose content (% w/w).
** information provided by Sigma Aldrich.
[a,b,c] means within the same line, marked with the same letter, do not differ from each other ($p > 0.05$).

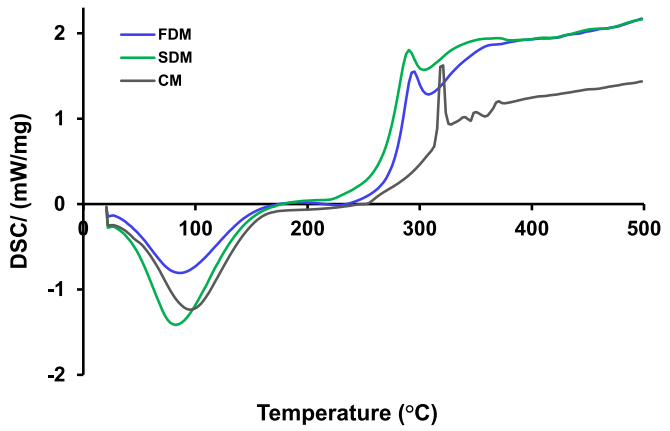


Fig. 3. DSC thermograms of the freeze (FDM) and spray drying (SDM) mannan extracts, and the commercial mannan (CM) obtained at a heating rate of 10 °C/min under nitrogen atmosphere.

benchmark sample (CM) – peak centered at 99.89 °C. An exothermic event is observed at around 290 °C in the extracts FDM and SDM, which is also detected for CM, but in the latter, the event takes place at approximately 320 °C; this event also can be related with polysaccharide thermal decomposition. The FDM and SDM products are a mixture of different compounds, so it is likely to observe the contribution of the events of each single component. One possibility to explain the position of the exothermic peak at around 300 °C was typical for chitosan, a deacetylated derivative of chitin (Roca et al., 2012), spent yeast in the cell wall content 1–2 % of chitin and chitin bonded to β-(1–3)-D-glucan, (Orlean, 2012), consequently, some residues of chitin bonded β-glucan may be present in the mannan extract after the extraction of the spent yeast. The presence of the β-glucan on the mannan extract is also possible, since as shown above in Table 2, there is a percentage of glucose in the extract which can be associated with the presence of β-glucan residues (Novák et al., 2012).

According to Ospina Álvarez et al. (2014) events close to or above 300 °C are typically observed during degradation of the saccharide structure and may be involved in the dehydration of saccharide rings. Even with the events not explained at molecular level, the DSC analysis allowed to infer about the close thermal stability of the mannans samples (FDM and SDM), and the mannan commercial sample (CM).

3.3. Cytotoxicity

Cytotoxicity of samples was assayed against CaCo-2 cells, by evaluating their impact upon cell metabolism using a viable dye. According to ISO 10993–5 (2009), a sample is cytotoxic when a metabolic inhibition percentage above 30 % is observed. Both FDM (-31.8 ± 3.9 %) and SDM (-67.3 ± 12.4 %) exhibited no cytotoxicity up to 10 mg/mL (the highest concentration tested) with no metabolic inhibitions being observed. In fact, the negative values illustrate an increase in cell metabolism observed when in the presence of SDM and FDM. This indicates that the differences in drying process, while resulting in alterations in microstructure, do not negatively impact their interactions with CaCo-2 cells. Moreover, the highest metabolic stimuli of SDM could be a result of its better solubility. This possibility is substantiated by the fact that its metabolic inhibition percentages fall within the range of those observed for the benchmark CM (-79.4 ± 14.8 %), which has similar solubility.

3.4. Antioxidant capacity

Polysaccharides from various sources have been reported to present strong antioxidant capacities (Machová & Bystrický, 2013), turning their incorporation in food products an interesting way to prevent oxidative damage in human body. However, processing conditions during their extraction, purification and drying may lead to structural/physicochemical modifications, thus interfering with their bioactivities. Therefore, the impact of the drying process on mannan extracts antioxidant capacity was assessed. Antioxidants play their beneficial role by deactivating reactive species through the transference of a hydrogen atom or an electron to those species. Therefore, the two mechanisms used to assess antioxidant capacity of compounds are Hydrogen Atom Transfer (HAT) and Electron Transfer (ET). ABTS (also known as TEAC assay) and DPPH are mixed-mode assays, as both HAT and ET mechanisms occur (with H atom donors and reducing agents, respectively) depending on the corresponding reaction conditions (such as pH and solvent) (Munteanu & Apetrei, 2021). Antioxidant ability was measured in the test compounds by ABTS and DPPH assays and compared with Ascorbic acid (vitamin C), as standard.

From Table 3 and concerning ABTS, it can be observed that the use of distinct drying processes didn't promote significant variations in the antioxidant capacity of the extracts. Ascorbic acid presented a significantly higher radical scavenging capacity than SDM and FDM extracts, and the CM exhibited the lowest values. Regarding DPPH assay, the same trend is observed, although the difference in the radical scavenging activity was statistically significant within the two drying processes (13 % for SDM and 19 % for FDM, at a concentration of 5 mg/mL). Machová & Bystrický, (2013) also reported lower DPPH assay results for *S. cerevisiae* mannans than for ascorbic acid (2.9–6.5 % for *S. cerevisiae* mannans, and 62–100 % for ascorbic acid, for concentrations of 0.125–1 mg/mL, respectively). Although the relative scavenging inhibition percentage of our mannan extracts (i.e. normalized for an equivalent concentration of extract) was lower in our study, it has to be kept in mind that the results from Machová & Bystrický, (2013) were assessed in mannans prepared as mannan-cooper complexes, which may interfere with the determination.

The large difference found between the commercial mannan and the extracts in both antioxidant assays may be tentatively explained by their MW profile, as bioactivity is usually inversely related with molecular size: whereas CM presents only one fraction of 34 – 62.5 kDa, SDM and FDM extracts present several fractions with MW ranging from 244 to 10 kDa. Nevertheless, this assumption must be validated with further studies. Regarding the difference found between SDM and FDM in DPPH assay, it may be tentatively explained by the differences found within the structural and/or physicochemical properties studied (namely differences in morphology, moisture or solubility). Alternatively, we wonder if this difference could be due to the intrinsic characteristics of the assays, such as the reactant solvents; although we did not measure extracts solubility in methanol (solvent mixture used in DPPH assay), maybe a poor solubility of FDM could explain its lower performance in this assay.

4. Conclusion

The present study reports the effect of different drying technologies

Table 3
ABTS radical and DPPH radical scavenging activity expressed as % scavenging activity and concentration mmol of Trolox/100 g in freeze drying (FDM) and spray drying (SDM) mannan extracts, commercial mannan (CM) and Ascorbic acid (Positive control).

	% Scavenging activity		Concentration mmol of Trolox/100 g	
	ABTS	DPPH	ABTS	DPPH
FDM	67 ± 0.77 [a]	13 ± 2.0 [a]	9.9 ± 0.11[a]	0.70 ± 0.15[b]
SDM	66 ± 1.1[a]	19 ± 0.59 [a]	9.3 ± 0.15 [a]	2.2 ± 0.008[a]
CM	2.0 ± 1.0[c]	8 ± 0.050[c]	0.08 ± 0.17[c]	0.30 ± 0.050 [d]
Ascorbic Acid	94 ± 0.16[b]	92 ± 0.070[b]	463 ± 25[b]	424 ± 17[c]

[a,b and c] means within the same column, labelled different subscripts, differ significantly ($p < 0.05$).

in the structural, physicochemical and biological characteristics of mannans obtained from spent yeast *S. cerevisiae*. From the parameters evaluated it can be concluded that the structural analysis in FT-IR and PXRD showed no significant variations between the freeze drying and spray drying methods. However, these drying technologies clearly affected the morphology and size, apart from physical appearance in terms of colour (as measured by colour difference). Furthermore, differences in moisture content and solubility were also observed. These differences may dictate the choice of the drying process for mannans extracts, depending on their intended final application.

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CRediT authorship contribution statement

Margarida Faustino: Investigation, Methodology, Writing – original draft. **Carla F. Pereira:** Supervision, Validation, Writing – review & editing. **Joana Durão:** Conceptualization, Supervision, Writing – review & editing. **Ana Sofia Oliveira:** Investigation. **Joana Odila Pereira:** Investigation. **Carlos Ferreira:** Investigation. **Manuela E. Pintado:** Resources, Project administration, Funding acquisition. **Ana P. Carvalho:** Supervision, Validation, Writing – review & editing.

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Data availability

Data will be made available on request.

Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.foodchem.2023.135545.

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