Use of wheat bran arabinoxylans in chitosan-based films: Effect on physicochemical properties

Maria J. Costa^{a,*}, Miguel A. Cerqueira^{a,*}, Héctor A. Ruiz^b, Christian Fournies^c, Aurore Richel^d, António A. Vicente^a, José A. Teixeira^a, Mario Aguedo^d

^a Centre of Biological Engineering, University of Minho, Campus de Gualtar, 4710-057 Braga, Portugal

^b Biorefinery Group, Food Research Department, School of Chemistry, Autonomous University of Coahuila, Blvd. V. Carranza e Ing. José Cárdenas Valdés, 25280 Saltillo, Coah., Mexico

^c Cosucra Groupe Warcoing S.A., Rue de la Sucrierie 1, 7740 Warcoing, Belgium

^d Laboratory of Biological and Industrial Chemistry, University of Liège – Gembloux Agro-Bio Tech, Passage des Déportés 2, 5030 Gembloux, Belgium

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ABSTRACT

The aim of this work was to evaluate physicochemical properties of chitosan-based films with different fractions of arabinoxylans (AXs). Five fractions composed by AXs or mixtures of AXs and arabino-xylo-oligosaccharides (AXOS) obtained through three different processes were added to chitosan-based films. These films were obtained by solvent casting and characterized in terms of water vapor permeability (WVP), opacity, thickness, moisture content and mechanical properties (i.e., tensile strength – TS and elongation at break – EB), being the chemical interactions evaluated by FTIR.

Moisture content values ranged between 20 and 30% for all five studied films being the lowest value observed for chitosan films with the fraction F1 of AX/AXOS (21.1%). The films with incorporation of F3a, F3b and F4 AXs fraction were more opaque (>10%) than the other studied films. Regarding mechanical properties CH films presented EB and TS values of 31.26% and 8.25 MPa, respectively, both these values remain statistically equal with the incorporation of AX/AXOS except for films containing the fraction F1 and F2. For films with the F2 fraction were obtained higher values of EB (41.31%) while for CH films containing the F1 fraction the TS values (13.07 MPa) increased.

In conclusion, wheat bran AXs can be successfully incorporated into chitosan-based edible films providing, together with chitosan, an extra functional value to films, due their health benefits.

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1. Introduction

Is urgent the development and use of renewable and environmentally friendly bio-based materials aiming at the decrease of the environmental impact of petroleum-based materials used in food packaging. One of the solutions lies in the utilization of edible packaging replacing non-biodegradable synthetic packaging materials in some situations and/or decrease their use in others. They can act as a partial barrier to external influences such as: water vapor, oxygen and carbon dioxide, microbiological contamination, thus helping preserve food quality and safety (Cerqueira et al., 2014; Martins et al., 2013). The most used materials in bio-based films are proteins (used to provide mechanical stability),

polysaccharides (usually to control transmission of oxygen and other gases) (Cerqueira et al., 2014; Martins et al., 2012; Pereira et al., 2010), and lipids and resins (Cerqueira et al., 2012; Fadini et al., 2013) used to reduce water vapor permeability (Pavlath and Orts, 2009). Their chemical structures can differ widely, and therefore attributes of each component contribute to overall films properties (Martins et al., 2012; Soradech et al., 2012).

Edible packaging can also be used as a vehicle for functional compounds, which can include flavors, antimicrobials, antioxidants, nutrients, nutraceuticals or colorants that, once combined with film-forming biopolymers, may lead to structural modifications or new films functionalities (Cerqueira et al., 2010; Martins et al., 2012; Rojas-Graü et al., 2009).

The challenge for the successful use of edible packaging is to stabilize their physicochemical properties accordingly to the desired applications and to preserve them through processing and then when applied on food products. Being so, materials for edible

* Corresponding authors. Tel.: +351 253 601962; fax: +351 253 678986.

E-mail addresses: mariajcosta@ceb.uminho.pt (M.J. Costa), miguelcerqueira@deb.uminho.pt (M.A. Cerqueira).

packaging production must be chosen according to their mechanical, barrier and thermal properties, solubility and optical attributes.

Chitosan is the principal derivative of chitin, the material comprising the exoskeletons of crustaceans and molluscs, and is produced by alkaline deacetylation of chitin. These appealing features make chitosan widely applicable in food preservation, cosmetics, and wastewater treatment (Juang and Shao, 2002; Risbud et al., 2000). When compared with other polysaccharides, chitosan has several important properties such as emulsifying, antimicrobial, antioxidant and gelling properties, moreover it displays health claims regarding the improvement of the maintenance of normal levels of cholesterol in blood, while also acting as a functional fibre (Neyrinck et al., 2012). Due to chitosan's biodegradability, biocompatibility and bioactivity, chitosan has received much interest regarding applications in food industry (Zaritzky, 2011).

Hemicellulose polysaccharides are biopolymers usable in films (Luo et al., 2014), which can be obtained from the pre-treatment of low-cost agricultural residues (Liu et al., 2012; Ruiz et al., 2013a). Thus, arabinoxylans (AXs) appear in a wide range of cereal crops and wheat bran is a source of choice due its high availability (Aguado et al., 2014; Zhang and Whistler, 2004). AXs are constituted by a main chain of β -linked (1,4)-3-D-xylose substituted mainly by side chains of α -(O-2) and/or α -(O-3)-L-arabinose. Some AXs in wheat bran are also linked to lignin and cellulose through diferulate bridges and hydrogen bonds (Iiyama et al., 1994). The sugars from hemicellulose of destarched wheat bran amount to around 40% of the dry weight (Aguado et al., 2013). Arabino-xylo-oligosaccharides (AXOS) obtained through the breakdown of AXs have prebiotic properties providing health benefits (Neyrinck et al., 2012) showing the phenolic acids naturally associated with the AXs and AXOS interesting antioxidant benefits (Rivas et al., 2013). According to the extraction method used, the fractions of hemicellulose can present different compositions and consequently different properties (Aguado et al., 2014; Sárossy et al., 2013; Ruiz et al., 2013b).

The incorporation of AXs and AXOS with prebiotic properties into edible packaging can be an interesting way of extending chitosan-based films functional. Based on this view, the objectives of this work were to test the incorporation of five different fractions of AXs or AX/AXOS obtained from wheat bran into chitosan-based films and to evaluate their physicochemical properties.

2. Materials and methods

2.1. Materials

Chitosan was purchased from Golden-Shell Biochemical Co., Ltd. (Yuhuan, China) with high molecular weight and a degree of deacetylation of 95%. Glycerol 87% was obtained from Panreac (Barcelona, Spain). Tween 80 and L(β)-lactic acid 90% were obtained from Acros Organics (Geel, Belgium).

2.2. Production of AX/AXOS from wheat bran

The processes used to obtain the five different AX/AXOS samples from destarched wheat bran (DWB) were thoroughly described by Aguado et al. (2014). Briefly: (i) F1 was obtained by NaOH treatment of DWB followed by ultrafiltration on a 100 kDa membrane, (ii) F2 by NaOH treatment of DWB pre-treated with a xylanase, then 100 kDa ultrafiltration, (iii and iv) F3a and F3b by xylanolytic treatment of DWB, then ultrafiltration on 10 kDa and 100 kDa membranes, respectively, (v) F4 by hydrothermal treatment of DWB pre-treated with a xylanase, followed by ethanol precipitation. These five samples were also extensively characterized in Aguado et al. (2014). F1 and F2 were composed of high molecular mass AXs, whereas the three other fractions were mixtures of low molecular

mass oligosaccharides (AXOS) with various amounts of high-to-medium molecular mass AXs (Table 1) (Aguado et al., 2014).

2.3. Preparation of chitosan-based films with AX/AXOS incorporation

Film-forming solutions were prepared as described by Martins et al. (2012). Briefly, 1.5% (w/v) of chitosan was dissolved in 1% of lactic acid (v/v) under agitation during 1 h at 25 °C. After that of glycerol (0.5%) and of tween 80 (0.1%) were added and the solutions homogenized at 60 °C under agitation during 30 min. Then AX/AXOS (0.2%) were added and stirred until a homogeneous solution was obtained (approximately 72 h). Then, 28 mL of solution was cast into polystyrene Petri dishes of 90 mm, and dried at 30 °C during 60 h. Films were conditioned in a desiccator containing a saturated solution of $\text{Mg}(\text{NO}_3)_2 \cdot 6\text{H}_2\text{O}$ at 53% of relative humidity (RH) and 20 °C before analyses.

2.4. Fourier transform infrared (FTIR) spectroscopy

FTIR spectra of the films were recorded with a Jasco Infrared Spectrometer (FT/IR-4100) FTIR spectrometer in the wavelength range 4000–400 cm^{-1} at a resolution of 4 cm^{-1} , using attenuated total reflection mode (ATR). The absorbance of each FTIR spectrum was normalized between 0 and 1. The measurements were performed in triplicate.

2.5. Moisture content

To determine the moisture content (MC) of films about 50 mg of film were dried at 105 °C during 24 h (until the equilibrium weight). The weight loss of the sample was determined, from which the moisture content was calculated using Eq. (1).

$$\text{Moisture content} = \left(\frac{M_i - M_f}{M_i} \right) \times 100 \quad (1)$$

where M_i and M_f are the masses of initial and dried samples, respectively.

2.6. Water vapour permeability (WVP)

Water vapour permeability (WVP) was measured based on the methodology described by Casariego et al. (2009). Films were sealed on cups containing distilled water and placed inside a desiccator containing silica gel (0% RH; 20 °C). To maintain uniform conditions for all samples, a fan was used inside the desiccator. Periodical cup weightings (2 h) were performed to monitor the weight loss over time until steady state was reached. Finally, water vapour transmission rate (WVTR) was calculated by dividing the slope of a linear regression of weight loss versus time by film area, and WVP ($\text{g m}^{-1} \text{s}^{-1} \text{Pa}^{-1}$) as follows:

$$\text{WVP} = \frac{(\text{WVTR} \times L)}{\Delta P} \quad (3)$$

where L is the film thickness (m) and ΔP is the water vapour partial pressure difference (Pa) across the two sides of the film. For each measurement, at least three replicates were made for each film sample.

2.7. Film thickness measurements

The thicknesses of the films were measured at ten different points for each sample using a digital micrometer (No. 293-5, Mitutoyo, Japan) with ± 0.001 mm accuracy.

Table 1

Description of the AXs and AX/AXOS fractions. Molecular masses at the peak (Mp), percent of the main populations and degrees of polymerization (DP), calculated by dividing Mp values by 132 which is the average mass of a polymerized pentose.

Fractions	Mp (kDa) ^a	% HPSEC surface ^a	DP
F1	484.4 ± 23.2	100	3670 ± 176
F2	462.4 ± 23.3	100	3503 ± 177
F3a	4.9 ± 0.1	81.0 ± 1.7	37 ± 1
	143.3 ± 8.6	19.0 ± 1.7	1085 ± 65
F3b	10.6 ± 0.4	78.6 ± 0.9	80 ± 3
	137.4 ± 11.6	21.4 ± 0.9	1041 ± 88
F4	0.4 ± 0.0	2.1 ± 0.1	3 ± 0
	8.9 ± 0.3	84.5 ± 1.2	67 ± 2
	163.4 ± 13.1	13.4 ± 1.3	1238 ± 99

^a Determined from three HPSEC analyses as described in Aguedo et al. (2014).

2.8. Mechanical properties

Mechanical properties of the films were measured with an Instron Universal Testing Machine (Model 4500, Instron Corporation, Canton, USA) following the methodology described by ASTM D882-10. The samples were conditioned at the same temperature (20 °C) and relative humidity (53%). Samples were clamped between grips with an initial distance of 100 mm. The force and deformation were recorded during extension at 50 mm min⁻¹. Tensile strength (TS) and elongation-at-break (EB) were expressed in MPa and percentage, respectively. For each measurement, at least three replicates were made for each film sample.

2.9. Opacity and color

Opacity was determined according to the Hunter Lab method, as the relationship between the opacity of each sample on a black standard (Y_b) and the opacity of each sample on a white standard (Y_w) (Eq. (4)). The measurements were repeated five times for each film sample.

$$\text{Opacity} = \left(\frac{Y_b}{Y_w} \right) \times 100 \quad (4)$$

The color of the films was determined with a Minolta colorimeter (Cr 400; Minolta, Japan). A white standard color plate ($Y=93.9$, $x=0.3158$, $y=0.3321$) was used for calibration and as a background for color measurements of the films, and the L^* , a^* , b^* values of each film were evaluated by reflectance measurements. In this system L^* indicates the lightness (ranging from black to white), and the horizontal axes, indicated by a^* and b^* , are the chromatic

coordinates (ranging from $-a^*$: greenness, $-b^*$: blueness to $+a^*$: redness, $+b^*$: yellowness). The values of a^* and b^* approach zero for neutral colors and increase as the color becomes more chromatic and more saturated.

2.10. Scanning electron microscopy

The surface morphology of the film was examined using scanning electron microscopy (SEM) (Nova NanoSEM 200, Eindhoven, Netherlands) with an accelerating voltage of 10–15 kV. Before analysis, all samples were mounted on aluminium stubs using carbon adhesive tape and sputter-coated with gold and then where shredded.

2.11. Statistical analysis

Statistical analysis was carried out using single-factor analysis of variance (ANOVA), while multiple comparison tests were used to determine the statistical significance with a 95% confidence level. For the data analyses, MATLAB software was used.

3. Results and discussion

3.1. Incorporation of AX and AX/AXOS in chitosan-based films

Very high molecular mass AXs of more than 450 kDa (fractions F1 and F2), or mixtures of AXOS and high molecular masses AXs (around 150 kDa) (fractions F3 and F4) were incorporated at 0.2% in the chitosan-based films (Table 1). This content in AX fractions was chosen based on preliminary studies which allowed the

Fig. 1. FTIR spectra of chitosan-based films without (CH) or with the incorporation of AXs or AX/AXOS (CH + F), CH, CH + F1, CH + F2, CH + F3a, CH + F3b, CH + F4.

Table 2

Values of moisture content and water vapour permeability (WVP) of the films without (CH) or with the incorporation of AXs or AX/AXOS (CH + F).

Film samples	Moisture content (%)	WVP $\times 10^{-10}$ (g (m s Pa) $^{-1}$)
CH	25.88 $\pm$ 0.75 ^a	3.32 $\pm$ 0.68 ^a
CH + F1	21.09 $\pm$ 0.99 ^b	4.63 $\pm$ 0.54 ^a
CH + F2	29.20 $\pm$ 1.43 ^c	5.17 $\pm$ 0.96 ^a
CH + F3a	26.38 $\pm$ 1.62 ^{a,d}	5.21 $\pm$ 0.94 ^a
CH + F3b	28.08 $\pm$ 1.53 ^{c,d}	4.55 $\pm$ 0.93 ^a
CH + F4	25.52 $\pm$ 2.33 ^a	3.86 $\pm$ 0.50 ^a

Values reported are the means $\pm$ sd.

Different letters (a–d) in the same column indicate a statistically significant difference ($p < 0.05$).

determination of AX/AXOS contents that, when added to film-forming solution did not affect their processability and led to the formation of films with good homogeneity. Higher contents led to immiscibility and non-homogeneous films (results not shown).

3.2. FTIR spectroscopy

The effect of the addition of AX/AXOS in chitosan films was evaluated by FTIR spectroscopy analysis. When different compounds are mixed, physical bonds and chemical interactions are reflected by changes in characteristic spectra peaks; these changes can reflect polymer miscibility (e.g., shifting of absorption bands). Fig. 1 shows FTIR spectra of film samples before and after addition of AX/AXOS in chitosan films. Chitosan spectra are in good agreement with the results presented by Bourbon et al., 2011; Martins et al., 2012 and Rubilar et al., 2013 concerning peaks and characteristic bands of chitosan. The broad peak between 3700 and 3000 cm^{-1} is related with the stretching vibrations of the hydroxyl groups. Absorption peaks at 2880 cm^{-1} are related to $-\text{CH}$ stretching and residual lactic acid and the peak at 1740 cm^{-1} is related to carbonyl vibration of the carboxylic acid. The spectra of the films containing AXs or AX/AXOS were similar to the reference CH films (Fig. 1) reflecting no significant changes in the structure with the addition of AXs or AX/AXOS. However, the appearance of a new peak at the region of 2380 cm^{-1} is related to the presence of the AX/AXOS being representative of the phosphorus present in AX/AXOS mixtures (Lin-Vien et al., 1991 Żyła et al., 1999).

3.3. Moisture content

Moisture contents (MC) were determined in order to evaluate in what extent the incorporation of AX/AXOS in chitosan films influences the water affinity to the CH film matrix.

Table 2 presents the MC values of films, showing that the incorporation of the AX fraction F1 in chitosan-based films led to a decrease ($p < 0.05$) of the MC (21.1%) when compared with CH films. For the films with the incorporation of F3b and F2 the MC increased ($p < 0.05$). Such differences are hardly explainable, since F1 and F2 for instance have very close composition and structure (Table 1), except a higher acetylation and phytate content in F1 (Aguedo et al., 2014). With the incorporation of the fractions F3a and F4 in the films, the moisture content was not significantly changed ($p > 0.05$) showing that the incorporation of these two AX/AXOS fractions, did not influence water affinity properties observed for the films. The values obtained are in agreement with those presented by other authors for chitosan-based films (Casariego et al., 2009; Cerqueira et al., 2012).

Table 3

Values of elongation-at-break and tensile strength of chitosan-based films without (CH) or with the incorporation of AXs or AX/AXOS (CH + F).

Film samples	Elongation-at-break (%)	Tensile strength (MPa)
CH	31.26 $\pm$ 2.99 ^a	8.25 $\pm$ 1.43 ^{ac}
CH + F1	28.85 $\pm$ 2.76 ^a	13.07 $\pm$ 2.15 ^b
CH + F2	41.31 $\pm$ 3.79 ^b	10.90 $\pm$ 3.74 ^{ab}
CH + F3a	26.36 $\pm$ 4.16 ^a	5.94 $\pm$ 1.33 ^c
CH + F3b	30.61 $\pm$ 3.33 ^a	9.17 $\pm$ 1.55 ^{abc}
CH + F4	27.98 $\pm$ 1.85 ^a	7.61 $\pm$ 0.52 ^{ac}

Values reported are the means $\pm$ sd. Different letters (a–c) in the same column indicates a statistically significant difference ($p < 0.05$).

3.4. Water vapor permeability

The evaluation of water vapor permeability (WVP) of the films allows to understand how changes in film composition (i.e., incorporation of AXs or AX/AXOS) can influence parameters such as sorption, solubility and diffusion of water molecules in the film matrix (Cerqueira et al., 2014). Table 2 shows the WVP values of the studied films. The permeability of the films was not significantly changed ($p > 0.05$) by the incorporation of AXs or AX/AXOS fractions. The value achieved for chitosan-based film (3.32×10^{-10} g (m s Pa) $^{-1}$) is in agreement with the literature (Bourbon et al., 2011). Despite some differences observed in MC values (films with F1, F2 and F3b), the permeability values did not present any statistical difference. The present results showed that it is possible to incorporate AXs or AX/AXOS (within the concentration range used) in chitosan-based films without changing their WVP.

3.5. Mechanical properties

Table 3 shows the values of elongation-at-break (EB) and tensile-strength (TS) for the studied films. The values for the reference chitosan films were very similar to the values reported by Ojagh et al. (2010). The incorporation of AXs or AX/AXOS compounds in chitosan film matrices led to a general maintenance of EB values, except for CH + F2 film that presents higher values ($p < 0.05$) (Table 3). This suggests that the AXs with higher molecular mass and the pre-treatment with xylanase increased the intermolecular forces within the chitosan-based films resulting in a stronger and more flexible material (Höije et al., 2008) (Table 1). Regarding TS values, they remained statistically not different when compared with CH films except for the film with F1 for which a significant increase in TS values happened, which can be explained by the higher molecular mass of the CH + F1 combined with lower MC, that lead to higher values of TS. It is clear the influence of AX and AXOS molecular mass in films structures, where the lower molecular mass AX/AXOS fractions showed to interrupt bonds between chitosan molecules more pronouncedly than the AX/AXOS with higher molecular mass.

3.6. Opacity and color

The color and opacity of films are extremely important in food packaging. Good optical properties of films are achievable through the control of the incidence of light in the food product or/and by ensuring that the consumer can clearly see the food product.

Table 4 shows the values for opacity and color parameters L^* , a^* and b^* with the incorporation of AXs or AX/AXOS fractions. The presence of 0.2% of AX/AXOS (fractions F3a, F3b and F4) in the chitosan films led to an increase ($p < 0.05$) of opacity values when compared to the control films but it remained the same for the films with F1 and F2. This can be explained by the conditions used during the process used to produce the different AX/AXOS: the process using NaOH (F1 and F2) led to fractions with less phenolic

Fig. 2. SEM images of chitosan-based films with addition of AXs or AX/AXOS (CH + F): CH, CH + F1, CH + F2, CH + F3a, CH + F3b, CH + F4.

Fig. 3. SEM cross-sectional images of chitosan-based films with addition of AXs or AX/AXOS (CH + F): CH, CH + F1, CH + F2, CH + F3a, CH + F3b, CH + F4.

Table 4

Values of opacity, L^* , a^* and b^* of chitosan-based films without (CH) or with the incorporation of AXs or AX/AXOS (CH + F).

Film samples	Opacity	L^*	a^*	b^*
CH	6.10 ± 0.81 ^a	93.22 ± 1.29 ^a	−2.23 ± 0.22 ^a	19.83 ± 3.63 ^a
CH + F1	9.59 ± 2.68 ^a	88.48 ± 2.20 ^a	1.14 ± 0.87 ^a	21.81 ± 4.65 ^a
CH + F2	7.53 ± 2.00 ^a	87.52 ± 3.32 ^a	1.66 ± 1.53 ^a	29.51 ± 5.00 ^b
CH + F3a	16.49 ± 7.99 ^b	65.86 ± 6.41 ^b	19.48 ± 5.39 ^b	52.77 ± 2.42 ^c
CH + F3b	14.94 ± 5.75 ^b	67.49 ± 6.54 ^b	16.92 ± 3.73 ^b	52.51 ± 2.66 ^c
CH + F4	15.80 ± 3.08 ^b	61.84 ± 3.79 ^b	17.50 ± 2.44 ^b	54.47 ± 1.41 ^c

Values reported are the means ± sd. Different letters (a–c) in the same column indicates a statistically significant difference ($p < 0.05$).

compounds (Aguedo et al., 2014) which can influence the color and opacity of the films. There was a clear relation between the phenolic content of the AX/AXOS samples and the opacity of the films (Aguedo et al., 2014). Regarding the color parameters L^* , a^* and b^* , the incorporation of AXs (F2) or of AX/AXOS (F3a, F3b and F4) had a statistically significant influence ($p < 0.05$) on L^* , a^* and b^* . The presence of AXs or AX/AXOS led to the increase of a^* values, meaning an increase of red and a decrease of green color. Moreover, the presence of AXs or AX/AXOS led to an increase of b^* (indicating that the films became more yellowish). The observed results are again related to the phenolic content of the AX/AXOS (Aguedo et al., 2014) that contributes to a darkening of the products (Table 4).

3.7. Scanning electron microscopy

SEM was used to characterize the morphology of the films before and after incorporation of AXs or AX/AXOS in chitosan-based films. Figs. 2 and 3 shows SEM images of the films without and with the addition of AXs or AX/AXOS of the surface and of the cross-sectional image, respectively. It is clear that the roughness of the surface of the films increases when AXs or AX/AXOS are added to the chitosan-based films being more clear for fractions F1 and F2, explained by the high molecular mass and degree of polymerization of the AX/AXOS used in these films (Izydorczyk and Dexter, 2008) (Table 1). Regarding type F3a and F3b it is very clear that the roughness of F3b is higher due to treatment used, since between these two types (F3a and F3b) the only difference is the ultrafiltration membrane used, where for F3a is used a membrane of 10 kDa and for F3b is used a membrane of 100 kDa. This leads to a fraction F3b with a greater proportion of high Mp AXs (Aguedo et al., 2014) in CH films and the increase of the film's roughness.

4. Conclusion

This work shows that high molecular mass AXs or mixtures of AXOS and AXs can be successfully added to chitosan-based films. Despite their influence on the color and the mechanical properties of the films, the WVP and morphologies were not changed. The incorporation of 0.2% of AXs or AX/AXOS into the chitosan matrix enabled to obtain films with added potential original functionalities in addition to those of chitosan (potential maintenance of blood cholesterol levels), namely prebiotic and/or dietary fibre properties of AXOS and/or AXs, respectively. Such films with potential added health benefits can be considered a promising avenue worth exploring.

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