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Trapping of carvacrol by konjac glucomannan-potato starch gels: Stability from macroscopic to microscopic scale, using image processing

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ABSTRACT

The aim of the study was to show that the presence of a small quantity of konjac glucomannan (KGM) in potato starch suspension increased the stability of carvacrol trapping.

For that purpose, several freeze-thaw cycles were used to accelerate the ageing of the product and consequently drastically destabilize the gels, and induce syneresis. The stability of carvacrol trapping was evaluated by the quantification of carvacrol in the syneresis liquid. The stability of the starch gel structure was studied at microscopic and macroscopic scale.

The moment of the addition of carvacrol and the presence of KGM both had an effect on the stability of carvacrol trapping and of the structure of the gel.

KGM promoted amylose retrogradation but slowed down amylopectin retrogradation. The stability of potato starch gels can be improved by the addition of a small quantity of KGM which showed a 'cryo-protectant' behaviour.

New method to characterize the micro and macrostructure from SEM images processing has also been proposed. The processing of microscopy images was done using Generalized Fourier Descriptors and allowed the characterization of each sample. The carvacrol addition lowered the physical stability of the gel with larger pores and increased syneresis. On the contrary, the KGM addition increased the size of the pores but prevented the formation of very large pores and reduced syneresis. The most stable system was obtained by the addition of carvacrol at the end of heating, in a konjac glucomannan–potato starch gel.

1. Introduction

Carvacrol (5-isopropyl-2-methylphenol) is a phenolic monoterpene constituent of essential oils produced by aromatic plants as oregano and thyme. Carvacrol exhibits many effects of great interest for the food industry: antioxidant, antibacterial, antifungal (Ben Arfa, Combes, Prezosi-Belloy, Gontard, & Chalier, 2006; Burt, 2004). However, the addition of carvacrol in food products presents some difficulties: (i) it has an extremely low flavour threshold and can drastically change the sensory properties of the food, (ii) it is highly insoluble in water and (iii) it could be oxidized or evaporated when exposed to air, light, or heat.

Trapping of carvacrol in a starch gel could be a good way to overcome these problems. In fact, it is well known that starch may interact with small ligands such as aroma compounds or lipids to form molecular inclusion complexes (Conde-Petit, Escher, & Nuessli, 2006; Jouquand, Ducruet, & Le Bail, 2006). In fact, in presence of complexing molecules, amylose organizes in single helix, offering a large hydrophobic central cavity which can receive ligands. Another form of ligand trapping in starch matrix exists. It is based on a non-specific physical entrapment by the network gel established through the rearrangement of starch molecules.

Starch is an important ingredient for the food industry because

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of its wide availability, low cost and functional properties: thickener, gelling agent, stabilizer, and fat of substitution. However, many food applications of native starches are limited due to their tendency to retrograde during cooling and storage. This is usually associated with syneresis and changes in texture, decreasing the overall consumer acceptability of the product. A way to improve or maintain desirable textural properties and stability of most starchy products is the incorporation of hydrocolloids. Konjac glucomannan (KGM) may be used for this purpose. KGM is an essentially linear polysaccharide composed of β-1.4-linked D-glucosyl and D-mannosyl residues (in a molar ratio of 1.5:1) as main chain with branching β-1.4-glucosyl units. Degree of branching is about 8%. KGM contains acetyl group in the main chain, approximately 5–10% acetylation (Katsuraya et al., 2003; Khanna & Tester, 2006; Yoshimura, Takaya, & Nishirani, 1997). It was reported to interact with starches of various botanic origins: maize (Yoshimura et al., 1997), wheat (Funami et al., 2005; Zhou, Wang, Zhang, Du, & Zhou, 2008), potato (Khanna & Tester, 2006), tapioca (Muadklay & Charoenrein, 2008), and rice (S. Charoenrein, Tatirat, Rengsutti, & Thongngam, 2010; Huang, Kennedy, Li, Xu, & Xie, 2007). All these authors reported the high water holding capacity of KGM that prevented syneresis liquid occurring in starch gels and slowed down the retrogradation rate of starch during storage. Recently, Schwartz et al. (2014) demonstrated that the presence of KGM in a potato starch-water system could affect the gelatinization, the retrogradation and the complexation phenomena of potato starch. This phenomenon seemed to appear at low concentrations of KGM.

In this context, the objective was to study the stability of carvacrol trapping in a mixed gel of KGM and potato starch. Several freeze-thaw cycles (FT cycles) were used to accelerate the ageing of the product and consequently drastically destabilize the gels. In fact, the succession of FT cycles induced an increase of molecular associations between starch chains. More particularly, the retrogradation of amylose resulted in the expulsion of water from the gel structure. This phenomenon is named syneresis (Morris, 1990). The stability of carvacrol trapping was evaluated by the quantification of carvacrol in the syneresis using Head Space-Solid Phase Micro Extraction-Gas Chromatography Mass Spectrometry (HS-SPME-GCMS). The gel set-up and/or the physical stability of the starch gel were studied: (i) at microscopic scale using Scanning Electron Microscopy (SEM) followed with image processing analysis and (ii) at macroscopic scale with pasting behaviour of samples and determination of syneresis.

2. Materials and methods

2.1. Materials

Potato starch was obtained from Sigma Aldrich and purified konjac glucomannan was kindly provided by Georges Srzednick (University of New South Wales of Sydney, Australia). All suspensions were made using deionized MilliQ water.

Carvacrol (SAFC, Saint-Louis, United States of America, purity 98%) and propylene glycol (Aldrich, Saint-Louis, United States of America, purity 99.5%, food grade) have the following physico-chemical characteristics at 25 °C (estimation program EPI suite ™):

- Carvacrol ($C_{10}H_{14}O$, CAS n°499-75-2), Log $P_{oct\text{-}w} = 3.49$, vapour pressure = 3.09 Pa, solubility in water = 1.25 g L^{-1}.
- Propylene glycol ($C_3H_8O_2$, CAS n°57-55-6, Log $P_{oct\text{-}w} = -0.92$, vapour pressure = 38.80 Pa, solubility in water = 811 g L^{-1}.

The extraction standard for quantification of carvacrol was 4-sec-butylphenol (Aldrich, Saint-Louis, United States of America, CAS n°99-71-8, purity 97% prepared at 50 mg L^{-1} in dimethyl sulfoxide (DMSO, Aldrich, Saint-Louis, United States of America, (CAS n°67-68-5).

Glutaraldehyde (Aldrich, Saint-Louis, United States of America, CAS n°111-30-8, grade 1 prepared at 2.5% in buffer 7) and absolute ethanol (Aldrich, Saint-Louis, United States of America, CAS n°64-17-5) were used to prepare the samples for scanning electron microscopy.

2.2. Samples preparation and pasting profile

Aqueous suspensions containing potato starch (PS samples, 25 g of water plus 1.25 g of potato starch by batch), or both starch and konjac glucomannan (SK samples, 25 g of water plus 1.25 g of starch and 0.05 g of konjac glucomannan per batch) were prepared using a Rapid Visco Analyzer™ (model RVA-super 4, Newport Scientific, Australia) equipped with the Thermocline™ software. The mixture was put in the aluminium flask and manually stirred to avoid sedimentation. Then, the mixture was held at 50 °C for 1 min, heated to 95 °C at a constant rate of 12 °C• min^{-1}, held at 95 °C for 3.5 min and finally cooled to 60 °C at the same rate and held at this temperature for 3 min. A constant stirring of 160 rpm was applied, except at the beginning of the pasting profile when the mixture was stirred at 960 rpm for the first 10 s at 50 °C and during cooling step for 10 s at 60 °C. Viscosity profiles were recorded to check reproducibility of the preparation. Four parameters were taken from the RVA curves: pasting temperature (°C) (corresponding to the beginning of the increase in viscosity), peak viscosity (mPa.s), setback (final viscosity minus trough viscosity, mPa.s) and final viscosity at 60 °C (mPa.s).

A stock solution of carvacrol was prepared by dissolving 1.807 g of carvacrol in 100 mL of propylene glycol at 25 °C under stirring. The concentration of carvacrol was chosen in order to obtain a final concentration of 2 mmol of carvacrol per glucose equivalent of starch considered as sufficient to induce complex formation (Arvisenet, Le Bail, Voilley, & Cayot, 2002). The stock solutions were stored at 4 °C before use. One hundred μL of stock solution was added to the suspensions, either before heating (Early Ligand Addition: ELA), or after heating (30 s after the start of the plateau at 60 °C, Late Ligand Addition: LLA). Six samples were made in triplicate: PS, SK, PS-ELA, PS-LLA, SK-ELA, SK-LLA.

2.3. Freeze-thaw cycles

Immediately after preparation, suspensions were sampled as such into centrifuge tubes or jars for further analyses. Then, they were frozen 30 min after the end of pasting profile at −18 °C for 24 h then thawed at 30 °C in water bath for two hours. This FT cycle was repeated up to four times.

2.4. Syneresis measurement

The samples were centrifuged at 8000*g* at 25 °C for 15 min. The liquid exuded from the suspension was cautiously collected and weighed. The percentage of syneresis was calculated as the ratio of the weight of liquid exuded from the suspension to the total weight of the suspension before centrifugation. The result was multiplied by 100. The data were reported as the average of three measurements.

2.5. Quantification of carvacrol in syneresis

Carvacrol was quantified in syneresis by Head Space-Solid Phase Micro Extraction-Gas Chromatography Mass Spectrometry HS-SPME-GCMS, using a calibration curve (linear regression of six points).

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