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Freeze-thaw stability of konjac glucomannane-potato starch gels: stability from macroscopic to microscopic scale, using image processing

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ABSTRACT

Freeze-thaw (FT) stability is often used to assess the ability of a gel to support the damage induced by freezing; selected parameters such as drip loss, damage to structure etc can be used to assess the freeze tolerance of a gel. Konjac glucomannan (KGM) is a very specific hydrocolloid able to trap 100 times its weight in water; it has not been studied so far as an improver to enhance FT stability.

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 antioxidant trapping. FT cycles were used to accelerate the ageing of the product and to assess its stability. In addition to drip losses determination, the stability of carvacrol trapping was evaluated by the quantification of carvacrol in the syneresis liquid. Microscopic and macroscopic scales were considered with microscopy. 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 "cryoprotectant" 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 glucomannane potato starch gel.

Keywords: starch, carvacrol, konjac glucomannan, freeze-thaw stability

1. INTRODUCTION

The objective of this work 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 behavior of samples and determination of syneresis.

2. RESULTS AND DISCUSSION

2.1 Samples preparation

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 mmoles 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 sec 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.

2.2 Study of the stability of the trapped carvacrol by the mixed matrix of potato starch and Konjac glucomannane

The stability of carvacrol trapping was assessed by its assay in the syneresis fluid and no decomposition of carvacrol was observed on GCMS chromatograms. High carvacrol values assayed in the supernatant indicate poor carvacrol trapping stability.

Two parameters affect carvacrol entrapment stability: the presence of konjac glucomannan and the addition of carvacrol. The amount of carvacrol assayed in supernatants of PS-ELA and PS-LLA is higher than that assayed in SK-ELA and SK-LLA. The addition of a small amount of konjac glucomannan (0.2% (w/w)) enhances the stability of carvacrol trapping.

The moment of addition of carvacrol is not neutral. The amount of carvacrol entrapped remains stable for PS-LLA and SK-LLA regardless of the number of freeze / thaw cycles unlike that of PS-ELA and SK-ELA which is low and varies with the freeze / thaw cycle. However, after four cycles of freezing / thawing, SK-ELA has a better stability than PS-ELA. This study shows that SK-LLA has the best trapping stability of carvacrol.

Table 1: Amount of carvacrol in the syneresis liquid (% w/w) of potato starch suspensions (PS) and potato starch konjac glucomannan suspensions (SK) for each freeze-thaw cycle (FT). Ligands were added either before heating (ELA) or during the plateau at 60°C (LLA). Mean values in each column with different superscripts (a-c) are significantly different at $p < 0.05$ using Fisher t-test.Means values in each row with different superscripts (1-4) are significantly different at $p < 0.05$ using Fisher t-test.

sample	% carvacrol in syneresis(w)/initial content (w)			
	Cycle 1	Cycle 2	Cycle 3	Cycle 4
PS-ELA	$60.8^{d}_{2}\pm2.9$	$56.5^{a}_{2}\pm8.2$	$38.6^{b}_{1}\pm4.1$	$39.7^{b}_{1}\pm0.6$
PS-LLA	$53.4^{c}\pm4.3$	$51.2^{a}\pm4.5$	$46.6^{c}\pm3.8$	$52.1^{c}\pm3.8$
SK-ELA	$43.9^{b}_{3}\pm3.3$	$28.4^{b}_{1}\pm3.4$	$32.4^{a}_{12}\pm2.1$	$33.6^{a}_{2}\pm1.1$
SK-LLA	$24.8^{a}\pm4.1$	$29.1^{b}\pm5.0$	$31.2^{a}\pm1.9$	$33.0^{a}\pm1.7$

2.3 Study of the physical stability of the mixed matrix of potato starch - Konjac glucomannane

On the macroscopic scale, the physical stability of the gels was studied by the syneresis volume measurements collected. All samples, regardless of the number of freeze / thaw cycles, exhibited syneresis. High syneresis values reflect a gel that is not stable over time. Indeed, the succession of freeze / thaw cycles accelerates the retrogradation of the starch. Associations between the chains of starch molecules increase, expelling water, syneresis, outside the structure. Two parameters influence the stability of the matrices: the presence of konjac glucomannan and the presence of carvacrol. The addition of konjac glucomannan reduces the syneresis of PS subjected to accelerated aging. However, this effect is only visible from the third cycle of freezing / thawing. For the first two cycles, a "syneresis accelerator" effect is observed. Lafarge and al (Lafarge, Bou-Marouna, Pontoire, Le Bail, & Cayot, 2017) showed that 0.2% (w/w) of konjac glucomannan accelerates the retrogradation of amylose, which takes place in the early hours of the starch retrogradation and slows the retrogradation of amylopectin, which takes place over several days or even weeks. This effect is due to the physicochemical changes of the continuous phase (viscosity, volume fraction) in the presence of gluconannan konjac. In fact, according to our previous results, in the presence of konjac glucomannan, the amylose is concentrated in the continuous phase, favoring the amylose-amylose interactions and therefore the retrogradation of the amylose. On the other hand, the presence of konjac glucomannan in the continuous phase inhibits the association of the ghosts of the starch grains and thus slows down the retrogradation of the amylopectin. These results seem promising for the application of Konjac glucomannan to preserve the long-term quality of frozen starch-based foods.

PS-ELA and PS-LLA have significantly higher volumes of syneresis than those of PS. Carvacrol therefore decreased the physical stability of the starch gels and increased the retrogradation of the starch. Our results are in agreement with the literature. Nevertheless, the effect of the aroma compounds on the retrogradation of the starch varies. Indeed, Cayot, Lafarge et al (Cayot, Lafarge, Arvisenet, & Taisant, 2000) showed during accelerated aging (24 hours at -26 ° C, then 24 hours at room temperature) by penetrometry tests that the retrogradation of standard corn starch was slowed down in the presence of isoamyl acetate (non-complexing aroma compound). Following natural aging for 28 days at 6 ° C, study of the same matrices of standard corn starch flavored with isoamyl acetate, octanol (complex with V6II amyloidosis) or linalool (complex with V6III-type amyloidosis), by dynamic rheological analysis, also showed that the presence of isoamyl acetate or octanol slowed the retrogradation of standard corn starch (Reparet, Moine, Arvisenet, Le Bail, & Cayot, 2006). On the other hand, no impact of linalool on the retrogradation of the standard corn starch gel has been demonstrated. The syneresis volumes collected for SK-ELA and SK-LLA vary with the freeze / thaw cycles and are greater than SK from the third freeze / thaw cycle. As a conclusion of the study of the physical stability of the matrices, on the four matrices tested, SK-LLA is the matrix presenting the best physical stability after four cycles of freeze / thaw.

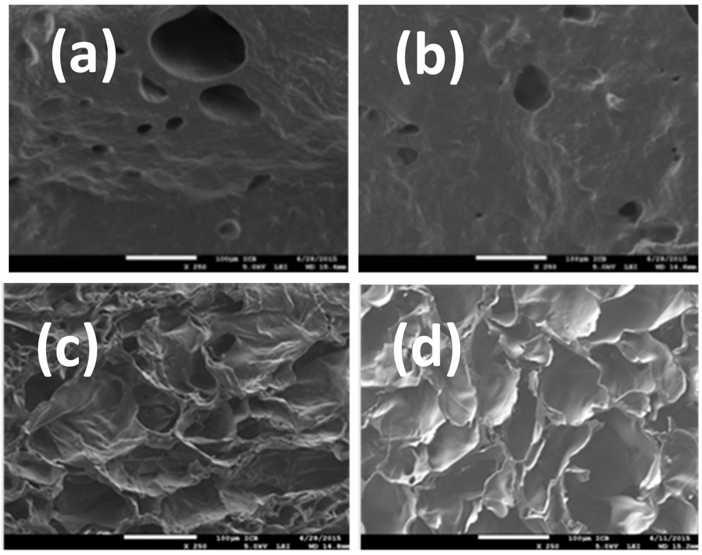

Figure 1: Typical SEM images of potato starch gels (5% (w/w)) with or without konjac glucomannan (0.2% (w/w)) and with or without carvacrol (x 250, bar = 100µm). (a) Unfrozen potato starch gel, (b) Unfrozen potato starch-konjac glucomannan gel, (c) Potato starch gel with carvacrol added at the end of heating, after four freeze-thaw cycles, (d) Potato starch - konjac glucomannan gel with carvacrol added at the end of heating, after four freeze-thaw cycles.

The analysis of the SEM images by the Fourier transform clearly demonstrated the effect of freeze / thaw cycles, carvacrol and gluconannan of Konjac on the textural surface characteristics of the samples.
After 4 cycles of freezing / thawing, PS exhibits textural properties of heterogeneous surface but with homogeneous and small pore surfaces. The pore surface reflects the size and shape of ice crystals formed during freeze / thaw cycles. The heterogeneity of the surface of the samples reflects the effect of successive freezing, which is in agreement with the increase of the volume of syneresis observed. By contrast, SK has a more homogeneous texture than PS which is in agreement with the results of the difference in physical stability between PS and SK. The distribution of the pore surface is more heterogeneous for SK.

The heterogeneity of the surface of the samples reflects the effect of successive freezing, which is in agreement with the increase of the volume of syneresis observed. By contrast, SK has a more homogeneous texture than PS which is in agreement with the results of the difference in physical stability between PS and SK. The distribution of the pore surface is more heterogeneous for SK. This distribution reflects the adverse effect of konjac glucomannan on the organization of amylose and amylopectin chains during the retrogradation.

However, the presence of large pore surface is not correlated with an increase in syneresis. The high water retention capacity of konjac glucomannan ensured the physical stability of the gels during accelerated aging by limiting the appearance of syneresis without preventing the formation of large ice crystals. This result is in agreement with the study of Ni, Ke et al, (Ni, Ke, Xiao, Wu, Kuang, Corke, et al., 2016). The authors showed that in a matrix consisting of a mixture of potato starch (5% w / v), hydroxypropyl methylcellulose (1.4% w / v), carrageenan (0.3% w / w), / v), pectin (0.4% w / v), agar (0.5% w / v), the presence of 0.3% (w / v) or 0.5% (w / v)) of Konjac glucomannan limited the formation of large ice crystals. On the other hand, this effect is not visible with a 0.1% (m / v) addition of konjac glucomannan.

PS-LLA has a much more heterogeneous surface than PS, with large pore surfaces. These results reveal that ice formation is more marked in the presence of carvacrol. This reinforces our previous observations of the detrimental effect of carvacrol on the physical stability of PS-ELA and PS-LLA. In our experimental conditions, carvacrol has a destabilizing effect resulting in significant volumes of syneresis.

Comparison of surface textural characteristics of PS-LLA with that of SK-LLA showed that these two matrices have large pore surfaces but that the presence of konjac glucomannan limits the size of these pores. This explains why the SK-LLA matrix has higher physical stability and carvacrol trapping stability compared to the other three matrices in the study (PS-ELA, PS-LLA, SK-ELA).
This study of the stability of carvacrol trapping over time was carried out by choosing drastic conditions: the freezing / thawing cycles. The latter accelerated the retrogradation of the starch, which was all the more amplified that the rate of freezing applied was low. The results obtained determine the stability limits of the samples.

3. CONCLUSION

The konjac glucomannan, added in a very low quantity (0.2%) into a potato starch gel, has shown its efficiency as agent to ensure the physical stability of the gel subjected to repeated freeze-thaw cycles. This phenomenon could be explained by the result of the konjac glucomannan water-holding capacity and by the competition for water between KGM and starch. This minor addition of KGM (0.2%) was also effective to ensure the stability of carvacrol trapping.

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