The impact of sugar alcohols on the physical properties and short-term stability of fish gelatin

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ABSTRACT

Fish gelatin is a popular alternative to mammalian gelatins due to ecological and ethical concerns, religious and cultural preferences. Fish gelatin has lower gel strength and gelling/melting temperatures compared to mammalian gelatins. To improve the physical properties of fish gelatin gels, a combination of two different sugar alcohols, sorbitol and xylitol, was included in the gels. The gelling/melting kinetics as well as gel strength of these gels were analyzed using small amplitude oscillatory shear measurements. Short-term stability tests performed at 55 °C for 20 hours indicated that the heat stability of fish gelatin gels improved with sugar alcohols.

INTRODUCTION

Gelatin is a widely used ingredient in numerous industries such as food, pharmaceuticals, nutraceuticals, photography and cosmetics.[1] In food industry, gelatin offers a unique combination of functional properties being surface active, forming thermo-reversible gels, providing a melt-in-the-mouth texture, stabilization and chewiness.[2] Gelatin is produced by the partial hydrolysis of collagen found in the connective tissues of a wide variety of animals such as bovine, porcine, poultry or fish by acid or alkaline pre-treatment, yielding type A or type B gelatin, respectively.[3] Currently, porcine and bovine sources constitute the largest fraction of worldwide produced gelatin.[4] However, religious beliefs, cultural practices, ethical and ecological concerns increased the demand of gelatin obtained from other sources.[5] Fish gelatin plays an important role for this demand since its production highly relies on the by-products of fish industry.[6] On the one hand, the majority of the fisheries produce cold water fish but due to its much lower gelling power and gelling/meting temperature, compared to mammalian gelatins, cold water fish gelatin has a severely reduced application potential.[7] The suboptimal physical properties of cold water fish gelatin are due to the lower content of imino acids proline and hydroxyproline.[8,9] On the other hand, gelatin obtained from warm water fish species were reported to have more similar physical properties to mammalian gelatins.[10] Warm water fish gelatin have a higher amount of proline and hydroxyproline compared to cold water gelatin and therefore, offers an alternative to mammalian gelatins.[11] Despite having more similar properties to mammalian gelatins, warm water fish gelatin still has lower gel strength and gelling/melting temperature compared to mammalian gelatins.[12,13]

Sugar alcohols are commonly included in gelatin gels in the confectionery and nutraceutical industries to replace sugars and provide sweetness to the final product.[14] This inclusion is known to increase the gelling/melting temperature of gelatin gels.[15,16,17] Sugar alcohols also decrease the

water activity (A_w) of gelatin gels. Decreasing the A_w below 0.85 is recommended to inhibit pathogen microbial growth in food systems.[18] In addition, an optimal A_w improves chemical stability, textural stability and increases product shelf life.[19] Other benefits of using sugar alcohols are their low calorie content, contribution to dental health and convenience for diabetic people.[20] Although sugar alcohols are known to increase the gel strength of gelatin gels, a rheological study reported that sorbitol decreased the initial storage modulus of mammalian and fish gelatin gels.[21,22,15] Another important measure to maintain the stability of food products is to reduce the pH below 4.6.[18] However, lowering the pH will promote acid hydrolysis, causing cleavage of the peptide bonds, further impacting textural stability.[23,24]

The aim of this study was to improve the physical properties and stability of gels from warm water fish gelatin with the addition of sugar alcohols. The short-term (20 hours) stability of gelatin gels at pH close to 4.5 with and without sugar alcohols at 55 °C was analyzed by small amplitude oscillatory shear measurements to indicate the long-term effects of storage stability of fish gelatin gels.

MATERIALS AND METHODS

Materials

Fish gelatin 200 Bloom was provided by Lapi Gelatine, Italy. Xylitol was provided by Danisco, UK. Sorbitol was purchased from Food Innovation, Norway.

Composition and preparation of the gels

Gelatin gels were prepared using a warm water fish gelatin. Trisodium citrate dihydrate and malic acid were used as buffer salts to keep the pH stable and close to 4.5. The compositions of the gels with (FG-SA) and without (FG) sugar alcohols are given in Table 1. The gelatin/water ratio was kept constant for both of the gels at 0.5.

Table 1. Composition of the fish gelatin gels with (FG-SA) and without (FG) sugar alcohols. All ingredients are given as percentage (w/w).

	FG	FG-SA
Fish gelatin	32.34	12.33
Xylitol	0	30
Sorbitol	0	30
de-ionized water	64.66	24.67
Trisodium citrate dihydrate	2	2
Malic acid	1	1

For the preparation of gels, gelatin, trisodium citrate dihydrate (2% (w/w)) and malic acid (1% (w/w)) were mixed with de-ionized water (18.2 MΩcm Stakpure OmniaPure, Germany) (with 0.02% (w/w) sodium azide (NaN_3, BDH, UK) for 30 minutes with magnetic stirring. The temperature throughout the preparation of the gels was kept constant at 70 °C. The concentration of trisodium citrate dihydrate and malic acid was optimized to obtain a pH close to 4.5. For the preparation of FG-SA, after mixing gelatin, water and buffer salts for 30 minutes, sorbitol (30% (w/w)) was added to the mixture and stirred for 20 minutes. Afterwards, xylitol (30% (w/w)) was added and stirred for 15 minutes. Witafrol (0.05 % (w/w)) was added to prevent foaming and the mixture was degassed twice with the Diaphragm Vacuum Pump machine (Vacuubrand, MZ 2C). The water loss was compensated after degassing. For the stability test, the gels were kept in a 55 °C water bath for 20 hours with constant magnetic stirring.

Rheology

The rheological analyses on the gels were performed with a rheometer (Malvern Kinexus ultra+, Westborough, United States). The lower plate was KNX0127, 50 mm diameter sandblasted lower plate with curved edges. The upper geometry was

CP4/40, 40 mm diameter 4° angle cone. The initial analysis after sample preparation is referred to as t_0. The rheometer was operated in 0.1% shear strain-controlled mode and the frequency was set to 1 Hz. The chosen strain was confirmed to be within the linear viscoelastic region for all samples. To avoid evaporation, the gel sample was covered with silicone oil (10 cS fluid, Dow Corning, UK) prior to and during measurement. The viscoelastic properties of the sample were obtained by using a temperature gradient of 2 °C/min, with a start and end temperature at 60 °C and a holding time of 15 min at 20 °C. 20 hours after gel preparation (t_{20}), approximately 2 grams of sample was removed from the gel and placed on the lower plate for analysis. The results were analyzed using the rSpace for Kinexus software.

The gelling temperature (T_g) and melting temperature (T_m) of the samples were estimated as the temperature at which the phase angle corresponds to 45°, i.e., $\delta = 45°$, in the cooling and heating process, respectively. The maximum storage modulus (G'_{max}) (Pa) was determined as the highest measurement point during curing at 20 °C.

Water activity

The A_w of the gels was measured with HygroPalm HC2-AW (Rotronic, Switzerland). 24 hours after curing at ambient temperature (approximately 22 °C), around 2 grams of gel was placed in the measurement chamber and the A_w was recorded after 45 minutes. Three replicates were analyzed for each gel.

RESULTS AND DISCUSSION

Water activity

The A_w of FG and FG-SA were compared. As expected, with the inclusion of sugar alcohols to the gelatin gel system, the water activity (A_w) decreased (Table 1).

Table 1. A_w of FG and FG-SA. The results are given as the average of three replicates ± standard deviation.

	FG	FG-SA
A_w	0.97 ± 0.01	0.70 ± 0.00

Rheology

The gelling/melting kinetics of FG and FG-SA were investigated at t_0. FG exhibited higher G'_{max} compared to FG-SA (Table 2).

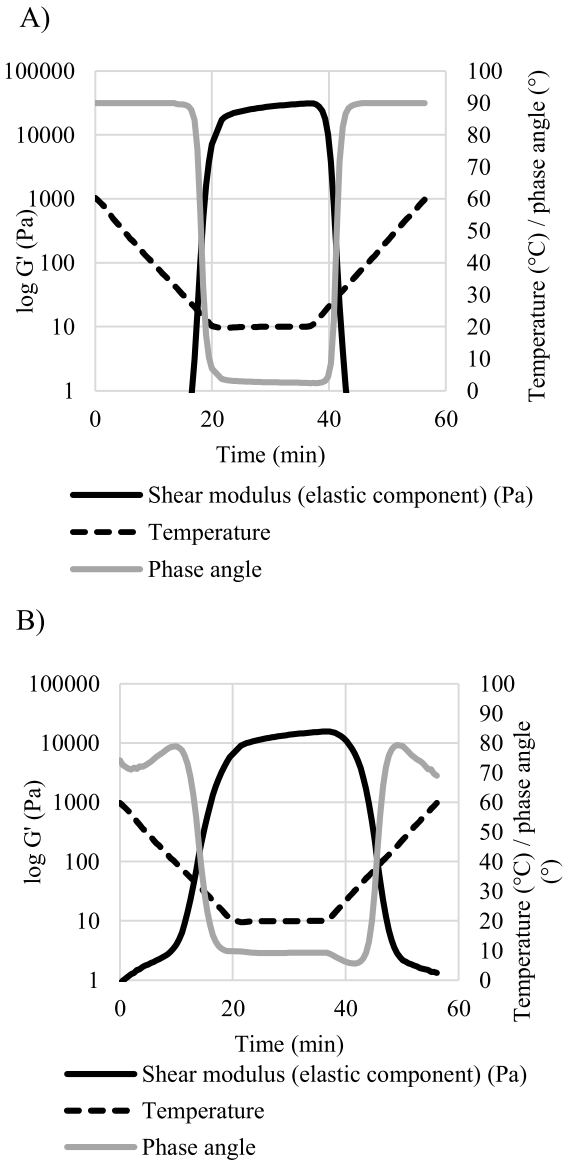

Figure 1. Small amplitude oscillatory shear measurements of A) FG and B) FG-SA at t_0.

This is in accordance with the literature[15] and possibly due to the lower gelatin per total volume in FG-SA. The gelling/melting curves were steeper for FG (Fig. 1A and Fig. 1B). This indicates slower gelling/melting kinetics upon sugar alcohol addition into the system, which can be due to reduced kinetics in both the formation as well as the dissolution of ordered triple helical junction zones.

As expected, FG-SA had much higher T_m and T_g values compared to FG (Table 2).

Short-term stability test

The effect of sugar alcohols on the short-term thermal stability of fish gelatin solutions was investigated by keeping both samples at 55 °C for 20 hours and comparing rheological parameters at t_{20} to their values at t_0.

Table 2: The comparison of the G'_{max}, T_m and T_g of FG and FG-SA at t_0 and after being kept at 55 °C for 20 hours (t_{20}).

	Time	FG	FG-SA
G'_{max} (kPa)	t_0	31.4	15.7
	t_{20}	22.8	13.5
T_m (°C)	t_0	29.1	38.5
	t_{20}	28.4	37.2
T_g (°C)	t_0	23.7	31.5
	t_{20}	22.8	30.0

As expected, both gels had a lower G'_{max} after the stability test compared to their t_0 value since 55 °C is well above the sol-gel transition temperature of both gels. At temperatures exceeding the sol-gel transition temperature of the gelatin gel, the majority of the gelatin molecules will be in the disordered state, i.e., the sol fraction will dominate.[25]

The decrease in G'_{max} ($\Delta G'_{max}$) was larger for FG compared to FG-SA, with 27% and 14% reduction, respectively (Fig. 2). This indicates that the gel with sugar alcohols was able to preserve its structure to a larger extent. Both gels had lower T_m and T_g values after the test. Although, the decrease was slightly larger for FG-SA it still had considerably higher T_m and T_g values compared to FG (Table 2).

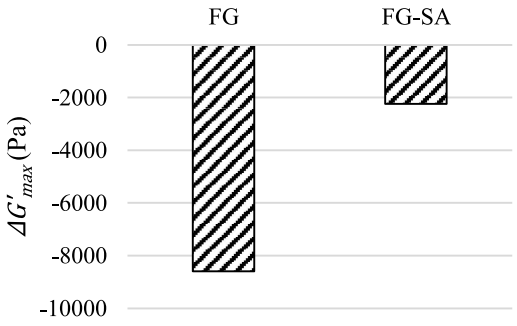

Figure 2. $\Delta G'_{max}$ of FG and FG-SA after being kept at 55 °C for 20 hours.

There are different theories on the stabilization mechanisms of gelatin gels by sugar alcohols. For example the reduced A_w was hypothesized to increase the intra- and intermolecular hydrogen bonding and hydrophobic interactions among protein molecules.[26] Another theory states that the exclusion of sugar alcohols from the surface of the gelatin molecules promotes gelation by driving protein-protein interactions.[27] In addition, it was stated that sugar alcohols stabilize gelatin due to a large decrease in the entropy of gel melting[22] and by increasing the thermodynamic stability of the collagen fold.[21] All these theories indicate that sugar alcohols increase the degree of ordering within gelatin gels which would lead to a higher degree of stability to resist depolymerization.

CONCLUSIONS

The inclusion of sugar alcohols in fish gelatin gels resulted in slower gelling/melting kinetics. The short-term stability tests showed that the fish gelatin gel with sugar alcohols preserved its structure at temperatures above its sol-gel transition temperature better compared to the gel without sugar alcohols. These findings

indicate that sugar alcohols may be combined with warm water fish gelatin in nutraceutical/pharmaceutical formulations or confectionery products to achieve a more ordered and stable gel which will exhibit higher gelling/melting temperatures and lower A_w.

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