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Evaluation of tilapia skin gelatin as a mammalian gelatin replacer in acid milk gels and low-fat stirred yogurt

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ABSTRACT

Tilapia skin gelatin (TSG) was studied in a 3-stage process (cooling, annealing, and heating) for pure gelatin gels and in a 4-stage process (acidification, cooling, annealing, and heating) for acid milk gels and cultured yogurt. The aim was to evaluate the use of TSG as a replacement for mammalian gelatin in yogurt. In pure TSG gels, stronger gels with higher melting temperatures were formed with increasing TSG concentrations. Compared with bovine gelatin (BG), which gelled at a concentration of 2.5%, TSG gels had lower gelling (14.1°C) and melting (24°C) temperatures but comparable storage moduli during annealing. In acid milk gels, addition of TSG increased the firmness of the gels with increasing concentration. Gelling and melting points of TSG in milk gels were observed at sufficient concentrations during cooling and heating. Strands and sheets were observed in the electron micrographs of milk gels with 1% TSG and a very dense structure was observed with 2.5% TSG. Yogurt with 0.4% TSG had similar viscosity, consistency, pseudoplasticity, and thixotropy as yogurt containing 0.4% BG; no difference was perceived by sensory panelists according to a triangle test. Addition of 0.4% TSG completely prevented whey separation from the acid milk gel and yogurt. The results suggest that TSG could be a suitable replacement for mammalian gelatin in low-fat stirred yogurt.
Key words: tilapia skin gelatin, yogurt, rheology, microstructure, sensory

INTRODUCTION

Gelatin is a multi-functional and most favored stabilizer in yogurt. It increases the gel strength, viscosity and water binding capacity of the yogurt, modifies the texture of the yogurt, stabilizes the yogurt system and, most uniquely, its melt-in-mouth property provides fat-like sensory perception to low-fat yogurt (Kalab et al., 1975; Fiszman and Salvador, 1999; Karim and Bhat, 2009). However, gelatin is mainly produced from pigskin, cattle bones, and cattle hide. Consumer groups with certain religious beliefs, such as Jews and Muslims, do not accept products made with such mammalian gelatin (Karim and Bhat, 2009).

There are also concerns about bovine spongiform encephalopathy (BSE), foot-and-mouth disease (FMD), and avian influenza with gelatin derived from mammals. Therefore, fish gelatin has been considered as a possible alternative to mammalian gelatin, especially since the outbreak of BSE in the 1980s. It meets the demands of the majority of consumers and complements the increasing global demand for gelatin (Karim and Bhat, 2009). The use of fish gelatin as an alternative to mammalian gelatin could reduce the volume of waste materials in the fish industry. Research has been carried out on methods of production of fish gelatin and its properties (Haug et al., 2004). In general, fish gelatin has some suboptimal physical properties compared with mammalian gelatin, particularly its low gelling and melting temperatures. The differences between fish and mammalian gelatins are due to the lower content of the imino acids proline (Pro) and hydroxyproline (Hyp) in fish gelatin. However, it was also found that the gel strength, gelling and melting temperature, and rheological properties are greatly influenced by the source of the fish gelatin (Zhou et al., 2006). In general, due to their higher imino acid content, the properties of gelatin from warm-water fish (e.g., tilapia, catfish, shark, and Nile perch), are closer to those of mammalian gelatin than those from cold-water fish (e.g., cod, salmon, and Alaska pollock; Zhou et al., 2006; Mahmoodani et al., 2014).

Tilapia is a warm-water fish species that is an important fishery resource. It is commonly farm raised, supplying large quantity of fish skins as by-products, which have become the raw materials for gelatin pro-

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duction. Gelatin from tilapia contains around 79 Hyp and 119 Pro residues per 1,000 total residues (Sarabia et al., 2000), compared with 91 Hyp and 132 Pro in pork gelatin (Eastoe and Leach, 1977), and 96 Hyp and 123 Pro in bovine gelatin (Jellouli et al., 2011); it has physical properties similar to those of mammalian gelatin (Sarabia et al., 2000).

Fish gelatin has been suggested for use in yogurt (Karim and Bhat, 2009) but to date little has been reported on the effect of fish gelatin on yogurt properties. Therefore, in this work, we studied a fish gelatin from tilapia skin (**TSG**) with relatively high bloom and evaluated its potentiality for application in yogurt. The aim of this study was to evaluate TSG in yogurt as a possible replacement for mammalian gelatin. The study included an investigation of the behavior of pure TSG and the effects of TSG on acid milk gels.

MATERIALS AND METHODS

Evaluation of Pure TSG Gel

Commercial tilapia fish gelatin (200 Bloom) was purchased from Jiangxi Cosen Biology Co. Ltd. (Yingtan, China). The gelatin is a type A gelatin produced from tilapia fish skin. The mammalian gelatin, which was used in previous research and used for comparison in this study, was supplied by Gelita (Beaudesert, Australia); it was a light-colored edible bovine skin (type B) gelatin powder (200 Bloom). Solutions with 3 concentrations (0.4, 1, and 2.5%, wt/wt) of TSG were prepared by allowing the gelatin to swell in distilled water overnight (about 15 h) followed by heating at 45°C for 30 min to dissolve it.

Dynamic oscillatory measurements were performed on a stress-controlled rheometer (model AR-G2, TA Instruments, Elstree, UK). Test samples were poured at 45°C onto the bottom plate of the rheometer, and a cone (4 cm, diameter; 2° angle) and plate geometry was used. A strain sweep revealed that 0.5% strain at a frequency of 1 Hz was within the linear viscoelastic region for the samples. The measurements were carried out in a 3-stage process—cooling, annealing, and heating—as described by Pang et al. (2015) with some modification: cooling = equilibration at 30°C and a temperature sweep to 10°C at a cooling rate of 1°C/min to promote gelatin gel formation; annealing = a time sweep at 10°C for 1 h to observe the maturation of the gelling samples; heating = a temperature sweep from 10 to 30°C at a heating rate of 1°C/min to observe melting of the gelatin gels, which relates to the unique "melt-in-mouth" property of gelatin in yogurt.

The gelling and melting temperatures were calculated when there were appreciable increases and decreases, respectively, in complex viscosity (η^*). The complex viscosity, η^*, was defined as in Eq. [1]:

$$\eta^* = \sqrt{G'^2 + G''^2}\big/\omega, \qquad [1]$$

where G' = storage modulus, G'' = loss modulus, and ω = frequency. The crossover temperature was defined as when G'' equals G' (or the loss tangent, which is the ratio of G'' to G', was equal to 1); and the point of inflection was defined as the temperature of maximum or minimum change in complex viscosity per unit change in temperature. It was obtained by differentiating the complex viscosity with respect to temperature, T (first derivative, $d\eta^*/dT$) and finding the temperature at which the derivative was zero. All rheological measurements were performed in duplicate and the samples were randomized for the analysis (Pang et al., 2014).

Evaluation of TSG in Stirred Acid Milk Gel

Preparation of the Stirred Acid Milk Gels. Skim milk powder (**SMP**; protein 33%, moisture 3.6%, fat 0.9%, lactose 54.7%, and ash 7.8%) was obtained from Murray Goulburn Co-Operative Ltd. (Melbourne, Australia). Reconstituted milk was prepared by dispersing the required amount of SMP in distilled water under continuous stirring for 30 min to obtain a milk protein concentration of 4.5% (wt/wt). Three concentrations of TSG (0.4, 1.0, and 2.5% wt/wt) were added to the milk. All solutions were stored at 4°C overnight before use. The solutions were heated in a 95°C water bath for 10 min at their natural unadjusted pH and then cooled to 45°C immediately using cold water. Gluconoδ-lactone was added to the solutions at 1.5% (wt/wt) to decrease the pH to 4.6 in 4 h at 45°C. A sample (~600 μL) was drawn to perform dynamic oscillatory measurements in a 4-stage process (acidification, cooling, annealing, and heating stage) as described below. The remaining sample was immediately transferred to a water bath at 45°C for acidification. After 4 h (at pH 4.6), the samples were stirred at 1,200 rpm for 2 min and stored at 10°C in an incubator.

Rheology. Samples were loaded onto a stress-controlled rheometer (model AR-G2, TA Instruments) and measurement parameters similar to those used for pure TSG gels were applied. The samples were held at 45°C for 4 h (acidification); the temperature was then decreased to 10°C at a constant rate of 1°C/min (cooling), maintained at 10°C for 2.5 h (annealing), and then increased to 45°C at 1°C/min (heating). Preliminary experiments for strain sweep showed that a strain of 0.5% was within the linear viscoelastic region for all samples at a frequency of 1 Hz. The gelation point was