GELLING CHARACTERISTICS OF LENTIL PROTEINS AND THEIR CONTRIBUTION TO A PORCINE MYOFIBRILLAR SYSTEM

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Abstract – This study investigated the gelling characteristics of lentil legumin (11S) and vicilin (7S) globulins, and their application in a porcine myofibrillar protein (MP) system. Lentil 7S globulin showed better thermal gelation behavior relative to 11S due to its less thermally stable structure. For application of the lentil globulins in a MP system, pre-heat treatment was first necessary in order to unfold some of the globulins to facilitate interactions with the MP during heating. Findings indicated that the 7S globulin could be used to substitute a part of MP in product formulation if pre-heated to temperatures higher than their peak temperature (T_P).

Key Words – legumin and vicilin, pre-heat treatment, rheological properties.

I. INTRODUCTION

In processed meat formulations, various starches and plant proteins are added as binders, fillers or extenders to improve product functional performance while reducing formulation cost. Soy is a major legume protein used in meat processing for these purposes. One inherent disadvantage of legume proteins is their high thermal stability that interferes in the myofibrillar protein (MP) thermal network formation thereby altering rheological properties of the formulation during processing and in the final product [1]. To improve utilization of legume protein sources in a mixed plant protein-meat system, a thorough understanding of the thermal properties and the rheological behavior during heat treatment of each protein and the mixture is necessary.

The legume seed proteins are dominated by 11S and 7S globulins, where the ratio (11S/7S) is believed to play an important role in their thermal gelling abilities [2]. Thermal properties of legume globulins (pea and soy) or the globulin fractions in myofibrillar (MP)-legume globulin mixed systems at varying pH and ionic strength conditions have been reported. The botanical source, component fractionation method as well as plant growth (environmental) conditions affect the performance of legume protein in this mixed system.

Lentil (*Lens culinaris*) is a bland tasting legume, and little is known about its protein functional performance in mixed protein-MP systems. The lentil globulins are composed of legumin (11S) and vicilin (7S) and contain adequate amounts of essential amino acids with lysine and tryptophan particularly in excess [3]. The present study reports thermal gelling properties of lentil globulin fractions under pH and salt levels used in conventional thermally set meat gel product processing (0.3 M NaCl with pH 6.5). Furthermore, the rheological behavior of lentil globulins in porcine MP mixed systems was explored.

II. MATERIALS AND METHODS

Legumin (11S) and vicilin (7S) globulins were extracted and fractionated from dehulled green lentil flour by the method of Suchkov et al. [4]. Pork picnic was purchased at 4 d post-mortem and the MP was extracted by the method of Xiong [5]. Purity of lentil globulin fractions was estimated by SDS-PAGE separation with or without β-mercaptoethanol (ME). To elucidate thermal characteristics, lentil globulins (10%, w/w) were suspended in 50 mM sodium phosphate buffer containing targeted NaCl level (pH 6.5). Thermograms of the protein suspensions were obtained at a heating rate of 10°C/min using a Differential Scanning Calorimeter (DSC, TA Instruments, DE, USA). Onset (T_o) and peak temperature (T_P) was estimated from duplicate determinations. Storage (G') and loss moduli (G'') of protein suspensions were determined using an AR-1000 rheometer (TA Instruments, DE, USA) with 40 mm diameter parallel plate. Protein suspensions were heated from 20 to 95°C at 1.5°C/min, held at 95°C for 10 min and then

cooled to 20°C at 1.5°C/min. Oscillatory measurements were made at a constant frequency (1.0 Hz) and strain amplitude of 0.02. Gel-set temperature (T_G) collected from triplicate determinations was determined by cross-over temperature of G' and G''. To estimate the rheological properties of lentil globulins in MP systems, 4% MP with 1% lentil globulin (total 5% protein) were prepared using 50 mM sodium phosphate buffer (with 0.3 M NaCl, pH 6.5). Each lentil globulin was pre-heated at 4°C (T0, not pre-heated), 75°C (T75), 85°C (T85) and 95°C (T95) for 10 min and cooled in ice/water for 10 min. The lentil globulins were mixed with MP by vortexing vigorously and then centrifuged at 700 ×g for 30 s. Rheological properties of MP and lentil globulin mixtures were compared with those of 4% MP (LC) and 5% MP controls (HC). Oscillatory rheology of the protein suspensions were made at the same conditions above during heating from 20 to 80°C at 1°C/min. For statistical analysis, a completely randomized block design was employed and all treatment effects were tested three times using a different batch of protein isolations as a block (n=3).

III. RESULTS AND DISCUSSION

Under non-reducing condition (-ME), lentil 11S globulin showed 50-65 kDa polypeptide bands (Fig. 1) which were dissociated to acidic (Lα, 39-44 kDa) and basic subunits (Lβ, ~24 kDa) upon providing reducing conditions (+ME).

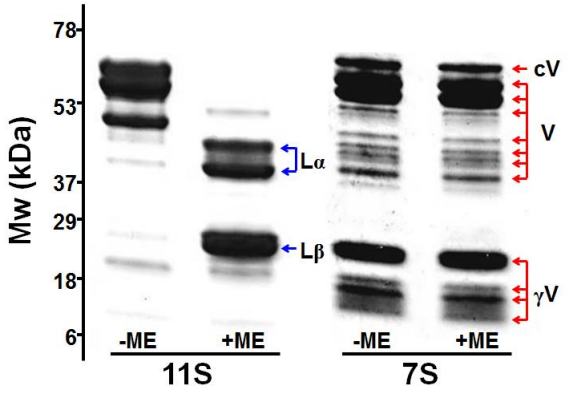

Figure 1. SDS-PAGE patterns of fractionated lentil 11S and 7S globulins under non-reducing (-ME) and reducing conditions (+ME).

Lentil 7S globulin was resolved into at least 12 polypeptides with molecular weights ranging from 11 to 74 kDa and showed absence of disulfide bonds (Fig. 1). These 7S globulin bands can be grouped as convicilin (cV, α-vicilin, 63 kDa), vicilin (V, 38-60 kDa) and γ-vicilin (γV, 11-22 kDa) [6].

The T_P of lentil globulins ranged from 89°C (7S) to 93°C (11S) at 0.1 M NaCl medium (Fig. 2). Both T_o and T_P values increased with increasing NaCl concentration, reflecting that NaCl stabilized the structure of both fractions. Although limited information is available on thermal properties of lentil proteins, the thermal transition values of lentil globulins estimated in the present study were comparable to other legume sources, in particular, lentil 7S globulin showed slightly higher T_P than soy β-conglycinin, whereas that of 11S was lower than soy glycinin reported by Jiang et al. [7].

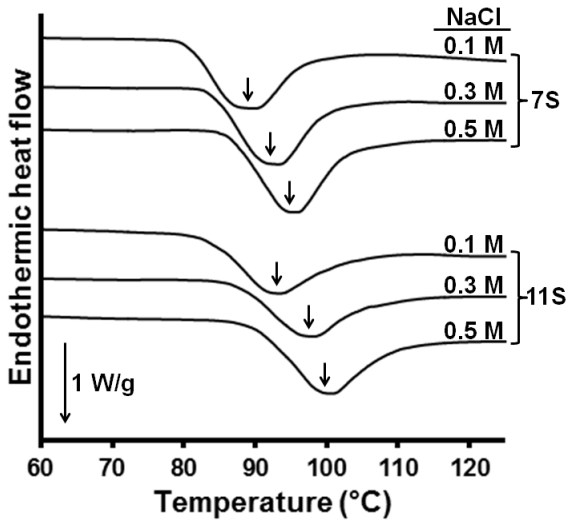

Figure 2. DSC thermograms of lentil 11S and 7S globulins (10% protein, w/w) at varying NaCl concentrations. Small arrows indicate peak temperature (T_P).

The G' of 7S globulin was higher than 11S (Fig. 3). The gel-set temperature (T_G) of 7S globulin was observed at 82°C compared to that of 11S with a T_G of 92°C. The T_G estimated by viscoelastic moduli was similar to T_o obtained from DSC, although the values between two parameters were not exactly in agreement, possibly due to different heating rates (1.5°C/min versus 10°C/min). As shown in Fig. 3, the rheological behavior of 7S

globulin demonstrated a comparative advantage over 11S globulin in producing an elastic thermal gel. This can be attributed to the different gelling nature of these two globulins. Thermal gel of 7S globulin is mainly established by hydrophobic interactions compared to 11S globulin in which the disulfide bonds, electrostatic interaction as well as hydrophobic interactions are involved in gel formation [8]. Higher thermal stability of 11S globulin would give rise to low degree of unfolding during heating, causing less hydrophobic contribution for elastic gel network formation in the presence of 0.3 M NaCl. In addition, shielding of side chain polar groups by NaCl reduces electrostatic contribution which is another factor involved in 11S globulin thermal gelation [8]. Consequently, low contribution of electrostatic interactions in the presence of NaCl and intra-molecular disulfide bonds possibly led to less elastic gel formation by 11S globulin.

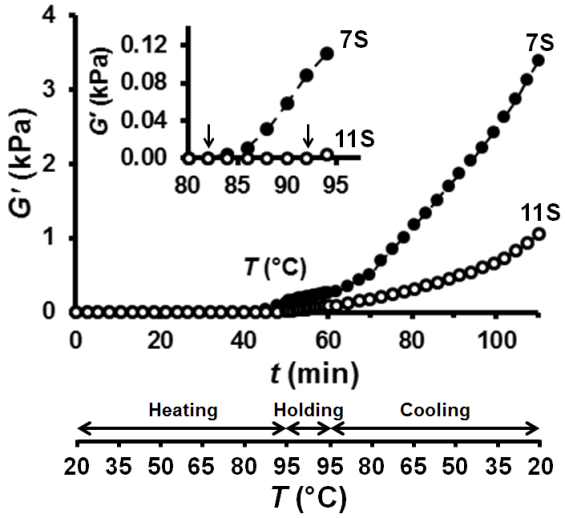

Figure 3. Storage modulus (G') of lentil 7S and 11S globulins (10% protein, w/w) at 0.3 M NaCl (pH 6.5). Inner figure depicts G' during heating step and arrows indicate gel-set temperature (T_G).

According to the above results, one can expect that 7S globulins may provide better rheological properties in mixed MP system than 11S. However, the thermal stability of 7S globulin is still too high to interact adequately with a MP network at normal meat processing temperatures (75~80°C). It was identified that pre-heat treatment of legume globulins is one strategy that can reduce the thermal denaturation temperature of globulins and improve thermal gel network formation [1], which was attempted in the present study.

At 0.3 M NaCl concentration, the addition of native 7S globulin in MP system (T0) with 1% 7S protein in 4% meat protein exhibited a hindering effect on thermal MP gel network formation showing lower G' than LC (4% meat protein) (Fig. 4). Increasing pre-heat temperature enhanced the elastic nature of mixed MP system, nonetheless the G' of pre-heated samples up to 85°C was still lesser than LC. Meanwhile, pre-heated lentil 7S globulin at 95°C (T95) improved elastic MP network formation and showed even higher final G' than HC (5 % meat protein). It should be noted that T_P of 7S globulin at 0.3 M NaCl was 92°C (Fig. 2). To achieve elastic gel network formation in mixed MP/lentil 7S system, the 7S globulin had to be heated to a higher temperature than its T_P.

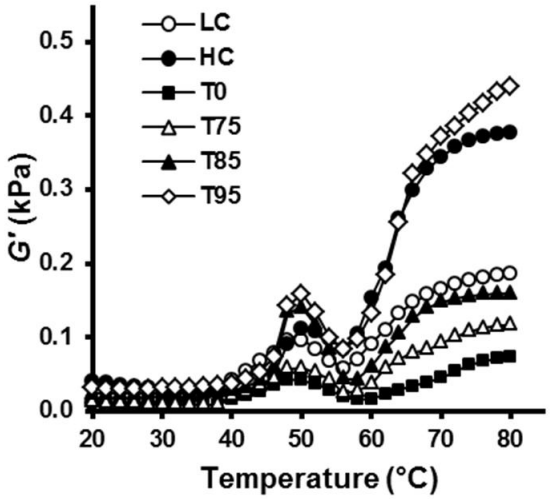

Figure 4. Storage modulus (G') of myofibrillar protein (MP) combined with lentil 7S globulin at 0.3 M NaCl (pH 6.5). Treatments indicate 4% MP (LC), 5% MP (HC) and 4% MP with 1% lentil globulin without (T0) and with pre-heating at 75 (T75), 85 (T85) and 95°C (T95) for 10 min, respectively.

In contrast, pre-heat treatment at temperatures up to 95°C was of no use for improving rheological properties of 11S globulin in mixed MP system and showed lower final G' than LC irrespective of the pre-heating temperature (Fig. 5). With a T_P of 11S at 0.3 M NaCl of 97°C (Fig. 2), a higher pre-heating temperature (> 100°C) may be necessary

to promote thermal unfolding of lentil 11S globulin, limiting practical application of native 11S globulin in actual meat processing. It is worth noting that the basic subunit of 11S globulin was associated with acidic subunit through sulfide bonds (Fig. 1). The basic subunit of 11S globulin is a key factor responsible to thermal aggregation of 11S gel formation [9], therefore strategies to modify structure (easy release of basic subunit) and to lower thermal stability of 11S globulin warrant further exploration.

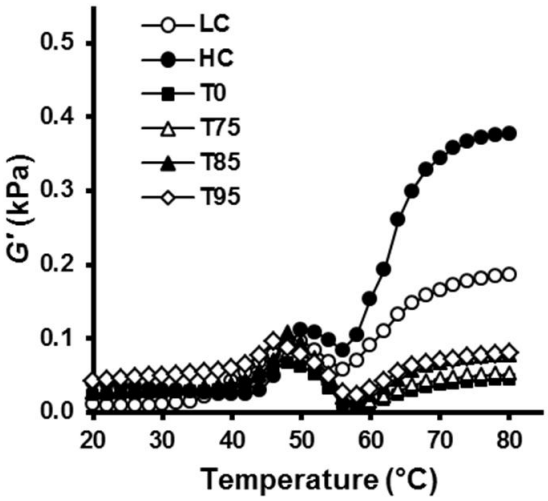

Figure 5. Storage modulus (*G'*) of myofibrillar protein (MP) combined with lentil 11S globulin at 0.3 M NaCl (pH 6.5). Treatments indicate 4% MP (LC), 5% MP (HC) and 4% MP with 1% lentil globulin without (T0) and with pre-heating at 75 (T75), 85 (T85) and 95°C (T95) for 10 min, respectively.

IV. CONCLUSION

This study shows a more suitable thermal performance of lentil 7S globulin for incorporation in a mixed myofibrillar-legume protein system than with 11S globulin. The elastic nature of the 7S gel reflects its potential in various meat product formulations. A strategic approach to reduce the thermal stability of lentil globulins by pre-heat treatment at temperatures higher than their T_P would enable the improvement of rheological properties of mixed MP/lentil globulin systems.

ACKNOWLEDGEMENTS

Financial support for this research was from Saskatchewan Pulse Growers, the Natural Sciences and Engineering Research Council and the Agriculture Bioproducts Innovation Program – PureNet Project. The authors acknowledge Dr. Linda Malcolmson (Canadian International Grains Institute, Winnipeg, MB, Canada) for providing lentil flour and Dr. Peter Chang (Agriculture and Agri-Food Canada, Saskatoon, SK, Canada) for permitting the DSC determination.

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