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# Effect of glycosylation on structural properties of pale, soft and exudative (PSE)-like chicken breast myofibrillar protein (#337)

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## Introduction

The appearance of PSE-like chicken breast meat has become a major economic concern for production and processing in the global poultry industry. Currently, processors are attempting to identify suitable methods to improve the water holding capacity and textural properties of PSE-like chicken breast meat. Glycosylation, classified as one way of post-translational protein modifications, is a prevailing approach for improving the functional properties of proteins. Recently, significant attention has been given to enzymatic glycosylation catalyzed by microbial transglutaminase (MTGase). The approach shows excellent advantages, including site-specificity, the high efficiency, environmental friendliness and mild reaction conditions. Based on the discussions outlined above, the objective of present study was to conjugate myofibrillar protein (MP) from PSE-like chicken breast meat with glucosamine (GlcN) via glycosylation catalyzed by MTGase. And the spatial structural characterization of the protein was monitored.

## Methods

### *Fourier transform infrared spectroscopy (FT-IR) analysis*

The structural characteristics of all samples were recorded with a NICOLET-380 type Fourier transform infrared spectrometer at 25 °C. The samples were mixed with KBr at a ratio of 1:100 and then pressed into a pellet. The spectra of the samples were collected in a wavenumber range of 400–4000 $cm^{-1}$ with a resolution of 4 $cm^{-1}$ for 32 accumulations.

### *Measurement of surface hydrophobicity*

The surface hydrophobicity of the samples was measured using the fluorescence probe 8-anilino-1-naphthalene sulfonic acid (ANS). First, 20 μl of ANS solution (15 mM ANS in 0.6 mol/L phosphate buffer, pH 7.5) was added to 4 ml of the sample (1 mg/ml). The samples were incubated in the dark at 25 °C for 20 min. Fluorescence was measured with a fluorescence spectrophotometer (SpectraMax M2, Molecular Devices Limited, USA) using an excitation wavelength of 375 nm and an emission wavelength of 460 nm.

### *Determination of turbidity*

Protein dispersions were diluted to 1 mg/ml with 0.6 M KCl phosphate buffer (pH 7.5). The turbidity of the diluted samples was monitored as the increase in optical density at 340 nm in a microplate reader (SpectraMax M2, Molecular Devices Limited, USA).

## Results

### *FT-IR analysis*

FT-IR spectroscopy was used as a rapid and efficient method for identifying interactions between MP and GlcN catalyzed by MTGase. The FT-IR spectra of non-treated MP, cross-linked MP and glycosylated MP were exhibited in Fig. 1. Obviously, it showed that the absorption band at around 1110 $cm^{-1}$ of glycosylated MP was much more enhanced than non-treated MP or cross-linked MP, whereas non-treated MP showed a pattern similar to that of cross-linked MP. It has been reported that absorbance in the 1050-1150 $cm^{-1}$ range is typically generated by -C-O stretching and -OH deforming vibration. Accordingly, the enhanced absorbance at 1110 $cm^{-1}$ suggested that the glycosylated MP contained more C-OH bonds than non-treated MP and cross-linked MP. Hence, the FT-IR spectra result gave an evidence of the modification of GlcN with MP catalyzed by MTGase.

### *Effects of glycosylation on the conformational characteristics of MP*

As shown in Fig. 2, compared with the non-treated MP, the surface hydrophobicity of cross-linked MP increased to 829, while the surface hydrophobicity of glycosylated MP was 563 ($P < 0.05$). This result suggested that crosslinking caused MP molecules to stretch and unfold, which led to the exposure of hydrophobic groups previously buried in the interior regions of MP. ANS had much more access to hydrophobic sites and could bind MP molecules that were previously surrounded by a nonpolar environment, and thus the surface hydrophobicity of cross-linked MP increased. GlcN has good hydrophilicity and is rich in hydroxyl groups (-OH). Thus, the decreased surface hydrophobicity of glycosylated MP might be due to the steric hindrance effect of the covalently bound GlcN molecules.

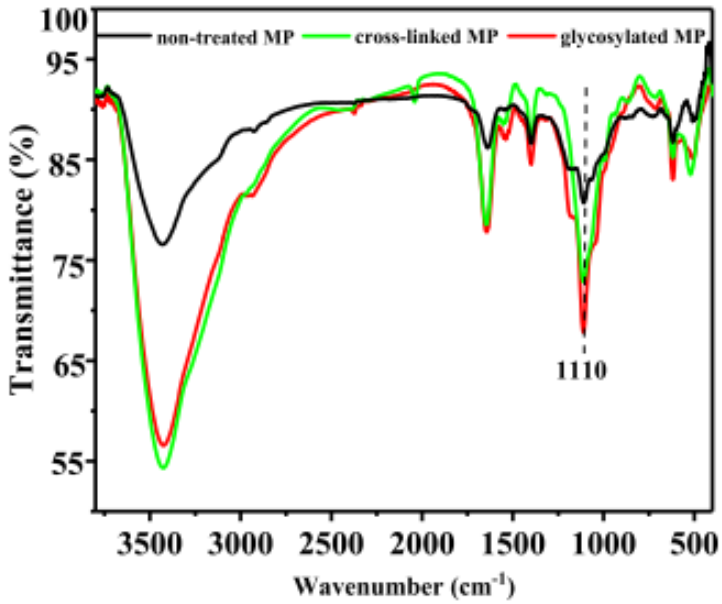
### *Effects of glycosylation on the turbidity of MP*

Compared with the non-treated MP, the turbidity of both crosslinked MP and glycosylated MP increased significantly ($P < 0.05$) (Fig. 3). Moreover, the turbidity of cross-linked MP increased more obviously ($P < 0.05$) than the turbidity of glycosylated MP. Rough estimations of the aggregation of protein molecules could be determined according to the turbidity change. The present result of the turbidity of cross-linked MP clearly confirmed that the MP molecules had undergone unfolding and aggregation in the presence of MTGase. In this regard, the results obtained from turbidity were in line with the conformational changes as described above.

Notes

## Conclusion

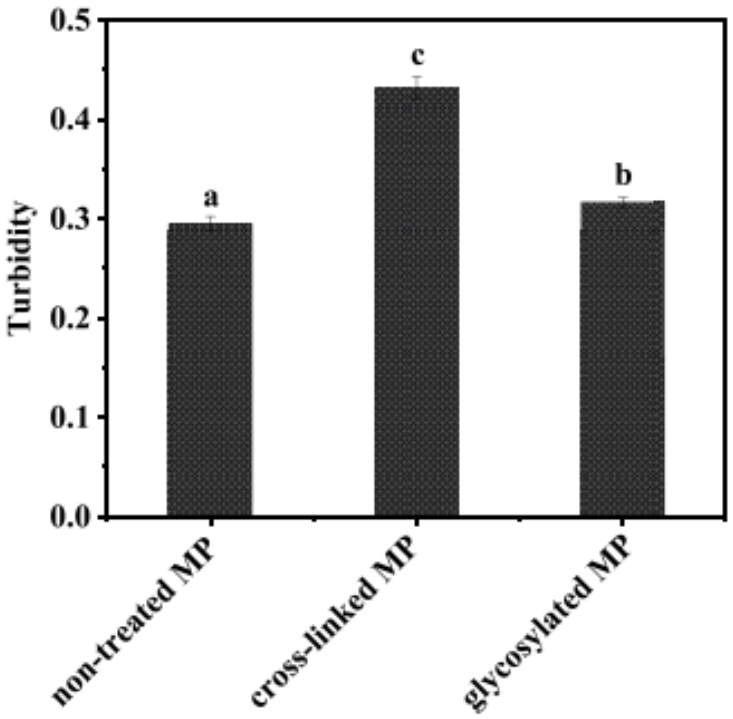
In the current work, MTGase was effective in catalyzing MP chains grafting onto the GlcN backbone. FT-IR confirmed the covalent attachment of MP to GlcN. Glycosylated MP showed a significantly different structures from MP. The conformational changes in MP might result in increased turbidity. Further investigations into MTGase-catalyzed glycosylation reactions between MP and GlcN should be carried out to quantify the effect on other functional properties to increase the economic value of PSE-like chicken breast meat.




**FT-IR spectra**

**Fig. 1.** FT-IR spectra of non-treated MP, cross-linked MP and glycosylated MP.

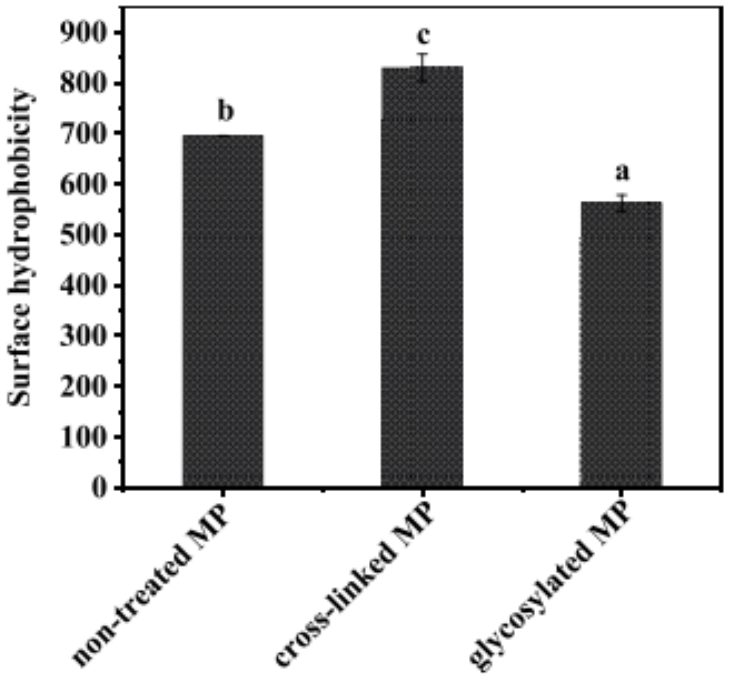
Notes




**Turbidity**

**Fig. 3.** The turbidity of non-treated MP, cross-linked MP and glycosylated MP. Values are given as means ± standard deviation (n = 3). Different letters (a–c) above columns indicate significant differences ($P < 0.05$) between the samples.

Notes




**Surface hydrophobicity**

**Fig. 2.** Surface hydrophobicity of non-treated MP, cross-linked MP and glycosylated MP (samples were prepared under the optimum condition). Values are given as means ± standard deviation (n = 3). Different letters (a–c) above columns indicate significant differences ($P < 0.05$) between the samples.

Notes