International Dairy Journal 91 (2019) 55–63

Contents lists available at ScienceDirect

International Dairy Journal

journal homepage: www.elsevier.com/locate/idairyj

Antioxidant activity of Maillard reaction products from a Yak casein-glucose model system

Haixia Wang [a, b], Jitao Yang [a], Min Yang [a, *], Wei Ji [a]

[a] College of Science, Gansu Agricultural University, Lanzhou, 730070, China
[b] College of Food Science and Engineering, Gansu Agricultural University, Lanzhou, 730070, China

ARTICLE INFO

Article history:
Received 17 April 2018
Received in revised form
3 December 2018
Accepted 4 December 2018
Available online 2 January 2019

ABSTRACT

Maillard reaction products (MRPs) were prepared by reacting both Yak and Holstein casein with different ratios of glucose to casein from 0:1 to 2.5:1. The results showed that the content of intermediate products and browning intensity of the Yak casein-glucose system increased with increasing concentrations of glucose, but were lower than for the Holstein system at the same ratio. With glycosylation, the reducing power and the 2,2-diphenyl-1-picrylhydrazyl radical scavenging activity of both Yak and Holstein caseins were enhanced. With increased glucose content, the reducing power of Yak MRPs increased at the same concentration. The metal chelating activity of Holstein MRPs decreased or was eliminated in the ratio range of 0.5:1 to 2.0:1 g glucose g^{-1} casein, while that of Yak MRPs were reduced in general. Improvement of the inhibitory activity for Yak casein was lower than that of Holstein casein after glycosylation by glucose.

1. Introduction

The Maillard reaction is well-known as a very complex reaction between carbonyls of reducing sugars and amino groups of proteins, producing a wide variety of products with odours and colours, with anti-allergenic and antimicrobial properties, and especially antioxidant characteristics (Liu, Yang, Jin, Hsu, & Chen, 2008; Morales & van Boekel, 1998; Rufián-Henares & Morales, 2006).

Casein is one of most important products of the dairy and food industry, which has properties that have been investigated through the Maillard reaction, such as its hypolipidaemic and anti-inflammatory effects (Oh, Koh, Park, Kim, & Kim, 2016a), toxicity to Caco-2 cells (Jing & Kitts, 2002), antiproliferative activity (Jiang, Wang, Wu, & Wang, 2013), angiotensin I converting enzyme inhibitory activity (Hong, Meng, & Lu, 2015; Jiang et al., 2013; Wang et al., 2015), hepatoprotective activity (Oh et al., 2016b), and mutagenicity (Brands, Alink, van Boekel, & Jongen, 2000). Other studies have characterised the kinetics of the casein-sugar Maillard reaction (Naranjo, Pereyra Gonzales, Leiva, & Malec, 2013; Oh, Kim, Hoon Lee, Lee, & Park, 2018; Pereyra Gonzales, Naranjo, Leiva, & Malec, 2010), involving the optimum reaction conditions and the influence of other factors (Akillioglu & Gokmen, 2014; Gu, Abbas, & Zhang, 2009a). It has also been confirmed that the MRPs have the ability to reduce the bitterness of casein (Dong, Wei, Chen, McClements, & Decker, 2011).

Although reducing sugars were the main reagent in the studies of Maillard reaction products (MRPs), some polysaccharides have been used to produce casein conjugates, and their properties have been characterised (Markman & Livney, 2012; Mu, Pan, Yao, & Jiang, 2006; Muhoza et al., 2017).

Among biological activities, the antioxidant activities of MRPs derived from casein have been extensively studied, including reducing activity, radical scavenging activity, metal chelating activity, and inhibition of lipid peroxidation. It was reported that the 2,2-diphenyl-1-picrylhydrazyl (DPPH) scavenging activity of casein peptides improved dramatically after glycosylation with glucose or lactose (Dong et al., 2011; Gu et al., 2010; Oh et al., 2016b). Increases in inhibiting lipid oxidation activity, ferrous chelating, and the reducing power of MRPs from casein-glucose peptides were also observed after short duration heating (Dong et al., 2011). The DPPH scavenging ability and reducing power of casein peptides were increased by glycosylation using lactose, galactose, or ribose (Jiang et al., 2013; Oh et al., 2013). In contrast, both glucose-casein and fructose-casein MRPs showed no DPPH scavenging activity at concentrations of 0.1–0.5 mg mL^{-1}, while a very low DPPH radical scavenging activity was observed for ribose-casein MRPs (Jing & Kitts, 2002). The fermented MRPs of casein-lactose showed lower DPPH radical scavenging activities than intact milk proteins (Oh et al., 2014). The galactose-casein system showed stronger DPPH

* Corresponding author. Tel.: +8613893272871.
E-mail address: yummy12@163.com (M. Yang).

https://doi.org/10.1016/j.idairyj.2018.12.010
0958-6946/

scavenging activity and reducing power than native casein (Wang et al., 2015), and its antioxidant activities depended on molecular weight (Gu et al., 2009b).

Yak milk has a high protein and mineral content, and is particularly rich in α_{S2}-casein (4.80 and 2.68 g L^{-1} in Yak and bovine milk, respectively) and β-casein (18.20 and 9.60 g L^{-1} in yak and bovine milk, respectively) (Cui et al., 2016; Li et al., 2010). It also has a different composition of amino acids and minerals when compared with bovine milk (Cui et al., 2016; Li et al., 2011). It is therefore considered as a unique type of milk.

Yak milk is a crucial resource for herdsmen on the Qinghai-Tibet Plateau. Due to the remote location and poor transportation in this region, Yak milk is usually made into Qula by a traditional method involving defatting, acidifying, and drying in air, resulting in a substance that contains approximately 80% casein. Qula is much easier to collect, store, and transport than fresh Yak milk (Liu et al., 2013). As an important industrial material, Qula is collected from herdsman and manufactured into casein products. However, some properties of casein products from Qula are poorer than those of casein from fresh milk, due to the crude processing of Qula. Several kinds of modifications have therefore been used to improve the properties of Yak casein products (Yang et al., 2014; Yang, Shi, & Liang, 2015). The results of our previous studies confirmed that the modification effect on Yak casein was quite different from bovine casein, such as different modification degree of succinylation and microbial transglutaminase crosslinking at the same condition, and different changes of functional properties with the same modification. Moreover, the antioxidant activity of MRPs from Yak casein was not evaluated.

In this study, the antioxidant activity of MRPs from Yak casein-glucose system was characterised, including DPPH radical scavenging activity, reducing power, ferrous chelating ability, and inhibition of lipid peroxidation. Differences in antioxidant activity between Yak and Holstein casein-glucose systems were also investigated at five different glucose contents. Together, this study provided basic data and reference information for the modification and application of Yak casein.

2. Materials and methods

2.1. Materials

o-Phthaldialdehyde (OPA) was purchased from Aladdin Industrial Company (Shanghai, China). Ferrozine was obtained from Yuanye Biotech Co., Ltd. (Shanghai, China). 2,2-Diphenyl-1-picrylhydrazyl (DPPH) was purchased from Macklin Biotech Co., Ltd. (Shanghai, China). Other chemicals were obtained from Shanghai Chemical Reagent Co., Ltd. (Shanghai, China).

2.2. Preparation of yak and cow caseins

Yak and Holstein acid casein products were purchased from Linxia Huaan Biological products Co. Ltd. (Linxia County, Gansu Province, China). The casein contents were 94.18% and 93.62% (w/w), respectively.

2.3. Preparation of Maillard reaction products

Dried casein was dispersed in distilled water with magnetic stirring at 45 °C. The pH of the dispersion was adjusted to 9.0 using 1 M NaOH. The dispersion was stirred until caseins were completely dispersed with a final casein concentration of 10% (w/v). Glucose was mixed with the casein dispersion according to the following ratios: glucose/casein (w/w) at 0:1, 0.5:1, 1:1, 1.5:1, 2:1, 2.5:1. Eighty millilitres of dispersion was transferred to a 100 mL hydrothermal synthesis reactor (Sineo Microwave Chemistry Technology Co., Ltd., Shanghai, China) and heated at 100 °C for 3 h without pH control. After heating, the sample was placed in an ice bath to cool down immediately. The heated casein–glucose mixture was termed as Maillard reaction products (MRPs). The pH of each sample was determined by a pH metre (PHS-3C, INESA Scientific Instrument Co., Ltd., Shanghai, China). Five millilitre samples of MRPs were then removed to measure absorbance at 294 and 420 nm and the content of free amino group. The rest of sample was dialysed (cut off 1.0 kDa) for 48 h at 4 °C, and freeze dried.

2.4. Measurement of absorbance

The absorbance of the MRPs solutions was measured using a TU-1901 spectrophotometer (Beijing Persee instrument company, Beijing, China) at 294 and 420 nm, absorbance was the indicator of intermediate and browning intensity of the reactions, respectively (Ajandouz, Tchiakpe, Ore, Benajiba, & Puigserver, 2001). Samples were diluted 360 and 60-fold with distilled water to obtain absorbance value at 294 and 420 nm, respectively.

2.5. Measurement of free amino group

OPA assay was used to analyse for free amino groups of casein by calculating the decrease of the free amino groups in the protein after the Maillard reaction (Jiang et al., 2013). The OPA solution was prepared daily as described by Yang et al. (2014) with some modifications. The following compounds were diluted with distilled water to 100 mL:80 mg OPA (dissolve in 2 mL methanol), 50 mL 0.1 M sodium tetraborate buffer solution, pH 9.5, 5 mL 20% (w/v) sodium dodecyl sulphate (SDS), 0.2 mL β-mercaptoethanol. One hundred microlitres of samples (18-fold dilution in pH 7.0 phosphate buffer) were mixed with 3 mL of OPA solution. After vortexing and a minimal 5 min delay in the dark at room temperature, the absorbance was recorded at 340 nm using a TU-1901 spectrophotometer. A blank reading was determined in the same manner, except that distilled water was used instead of the samples. A calibration curve was obtained by using 0–400 μg mL^{-1} lysine as a standard, by which sample absorbance values were converted into free amino group content.

2.6. Determination of reducing power

The reducing power of MRPs was determined according to the method of Gu et al. (2009b) with some modifications. One millilitre of MRPs samples at a concentration of 1, 2, 4, 8, 16 mg mL^{-1} were mixed with 1.0 mL 0.2 M sodium phosphate buffer (pH 6.6) and 1.0 mL 1% potassium ferricyanide ($K_3Fe(CN)_6$), respectively. Mixtures were incubated in a temperature-controlled water bath at 50 °C for 20 min, followed by addition of 1.0 mL 10% trichloroacetic acid after cooling to room temperature. Mixtures were then centrifuged at 4000×*g* for 10 min at room temperature. One millilitre of supernatant was mixed with 1.0 mL distilled water and 200 μL 0.1% ferric chloride. Absorbance of the mixture was measured at 700 nm with TU-1901 spectrophotometer, the lowest value of the tests was used as the initial reference value, and an increase in absorbance was used as the measure of the increased reducing power. Reducing power was expressed as absorbance at 700 nm. For the comparison, the assay was conducted in the same manner but 1 mg mL^{-1} butylhydroxytoluene (BHT) was added instead of MRPs solution.

2.7. Determination of DPPH radical scavenging activity

DPPH radical scavenging activity was determined according to the method of Benjakul, Visessanguan, Phongkanpai, and Tanaka (2005) with some modifications. An aliquot solution (1.0 mL)

with MRPs concentrations of 1, 2, 4, 8, 16 mg mL^{-1} were added to 2.0 mL 0.1 mM DPPH in ethanol. The solution was then mixed vigorously and allowed to stand at room temperature in the dark for 20 min. The mixture was centrifuged for 10 min at 4000×*g*. The absorbance of supernatant was measured at 517 nm using the TU-1901 spectrophotometer. A blank was prepared in the same manner, except that distilled water was used instead of MRPs samples. For a control, the assay was conducted in the same manner but ethanol was added instead of DPPH solution. For comparison, the assay was conducted in the same manner but 1 mg mL^{-1} BHT was added instead of sample solution. The DPPH radical scavenging activity of MRPs was calculated as follows:

$$\text{Radical scavenging activity } (\%) = [1 - (A_S - A_C)/A_B] * 100 \quad (1)$$

where A_S is the absorbance of sample, A_C is the absorbance of the control and A_B is the absorbance of the blank.

2.8. Determination of chelating activity on Fe^{2+}

Chelating activity on ferrous ion (Fe^{2+}) of MRPs was determined by the method of Gu et al. (2009b) with modifications. One millilitre MRPs solution at concentrations of 1, 2, 4, 8, 16 mg mL^{-1} were mixed with 1.85 mL distilled water and 0.05 mL 2.0 mM $FeCl_2$. The mixture was allowed to rest at room temperature for 30 s. The mixture was then added to 0.1 mL 5 mM ferrozine and mixed. Absorbance of mixture at 562 nm was determined with the TU-1901 spectrophotometer after 10 min resting time at room temperature and 5 min centrifugation at 4000×*g*. For the blank, the assay was conducted in the same manner but distilled water was added instead of MRPs solution. The chelating activity was calculated as follows,

$$\text{Chelating activity } (\%) = (1 - A_S/A_B) * 100 \quad (2)$$

where A_S is the absorbance of sample and A_B is the absorbance of the blank.

2.9. Inhibition of lipid peroxidation induced by iron

Lecithin was suspended in phosphate buffered saline (0.01 M, pH 7.4) at a concentration of 10.0 mg mL^{-1} with magnetic stirring (Yi, Meyer, & Frankel, 1997). The solution was labelled as LLS. Fifteen grammes of trichloroacetic acid (TCA), 0.37 g thiobarbituric acid (TBA) and 2 mL concentrated hydrochloric acid (HCl) were added to distilled water, and the volume was adjusted to 100 mL. The solution was labelled as TCA–TBA–HCl. One millilitre LLS, 1.0 mL 400 μM $FeCl_3$, 1.0 mL 400 μM ascorbic acid and 1.0 mL MPRs solution were mixed in the tubes in this order. The solution was placed in water bath at 37 °C in dark for 60 min, then 2.0 mL TCA–TBA–HCl was added. The mixture was cooled down with ice water after heating in water bath to 95 °C for 15 min. The solution was centrifuged at 4000×*g* for 10 min, and the absorbance of the supernatant was read at 532 nm using a TU-1901 spectrophotometer. A blank was made with 1.0 mL distilled water substitution for 1.0 mL sample. The inhibition percentage was calculated as formula,

$$\text{Inhibition percentage } (\%) = (A_B - A_S)/A_B * 100 \quad (3)$$

where A_S is the absorbance of sample and A_B is the absorbance of the blank.

2.10. Statistical analysis

Collected data were expressed as mean ± standard deviation (SD) from at least three independent trials. Analysis of variance (ANOVA) was performed and means comparisons were carried out by Duncan tests. A value of $P < 0.05$ was considered significant. PASW Statistics 18.0 software (SPSS Inc., Chicago, IL, USA) and Origin 8.0 (OriginLab Corporation, Northampton, MA, USA) were used to analyse the data.

3. Results and discussion

3.1. Changes of pH

The pH of Yak and Holstein casein-glucose systems decreased as glucose concentration increased ($P < 0.05$), except for the ratio of 1:1 (glucose:casein, w/w) for Yak casein (Fig. 1A). The pH of the Yak casein-glucose model was similar to the pH of the Holstein casein-glucose model at the same concentrations of glucose. Compared with a heated casein solution without glucose, the pH of the casein-glucose system at a glucose concentration of 0.5 g g^{-1} of casein was dramatically reduced in both the Yak and Holstein systems ($P < 0.05$), which suggested the occurrence of the Maillard reaction and formation of organic acids, such as formic acid and acetic acid (Wang et al., 2015). Concerning the Yak and Holstein casein solutions without glucose, the pH decreased after heating, and the Yak pH was much lower than that of the Holstein pH. It was assumed that casein degrades to peptides or free amino acids when heated at high pH and high temperature, and heating also resulted in cross-linking and aggregation of caseins (Gu et al., 2009b; Wang et al., 2016). The pH decreases of the casein solution without glucose after heating were possibly due to its degradation or aggregation. The pH difference between Yak and Holstein caseins after heating may be due to the different casein monomers of the different species (Wang et al., 2013).

3.2. Changes in intermediate products and the intensity of browning

Absorbances at 294 nm and 420 nm were used to investigate the formation of intermediate products and the extent of the Maillard reaction, respectively (Morales & van Boekel, 1998; Wang et al., 2015). The effect of glucose concentrations on the absorbance at 294 nm for Yak and Holstein casein-glucose systems are shown in Fig. 1B. With increasing glucose concentrations, the absorbance at 294 nm of the Yak casein-glucose system increased sharply at the ratio of 0.5:1 (glucose:casein, w/w; $P < 0.05$). Afterwards, it rose slightly ($P > 0.05$) at glucose:casein ratios between 0.5:1–1.5:1, and dropped at ratios > 1.5:1. Absorbance at 294 nm of the Holstein casein-glucose system steadily increased with increasing glucose concentrations ($P < 0.05$), except for the ratio of 1.5:1. The increase of absorbance at 294 nm of the Holstein casein-glucose system with increasing glucose concentrations was similar to a bovine casein peptide-glucose system (Dong et al., 2011).

Although intermediate products were produced to a great extent after glucose was added to casein, the amount of intermediate products in the Yak casein-glucose system was higher than that in the Holstein casein-glucose system at the same ratio. In addition, changes in intermediate products of the Yak casein-glucose system were quite different from those of the Holstein casein-glucose system. Past studies have reported that some intermediate products polymerized to form melanoproteins during extended heating, resulting in the formation of a small amount of intermediate products (Gu et al., 2009b; Lertittikul, Benjakul, & Tanaka, 2007). It was suggested that the intermediate products of the Yak casein-glucose system polymerised when the added glucose exceeded 1.5 g g^{-1} casein.

The browning intensity of the Holstein casein-glucose system (shown as the absorbance at 420 nm in Fig. 1C) increased with increasing glucose concentrations ($P < 0.05$), in a similar manner as the absorbance at 294 nm. However, a slight change of browning

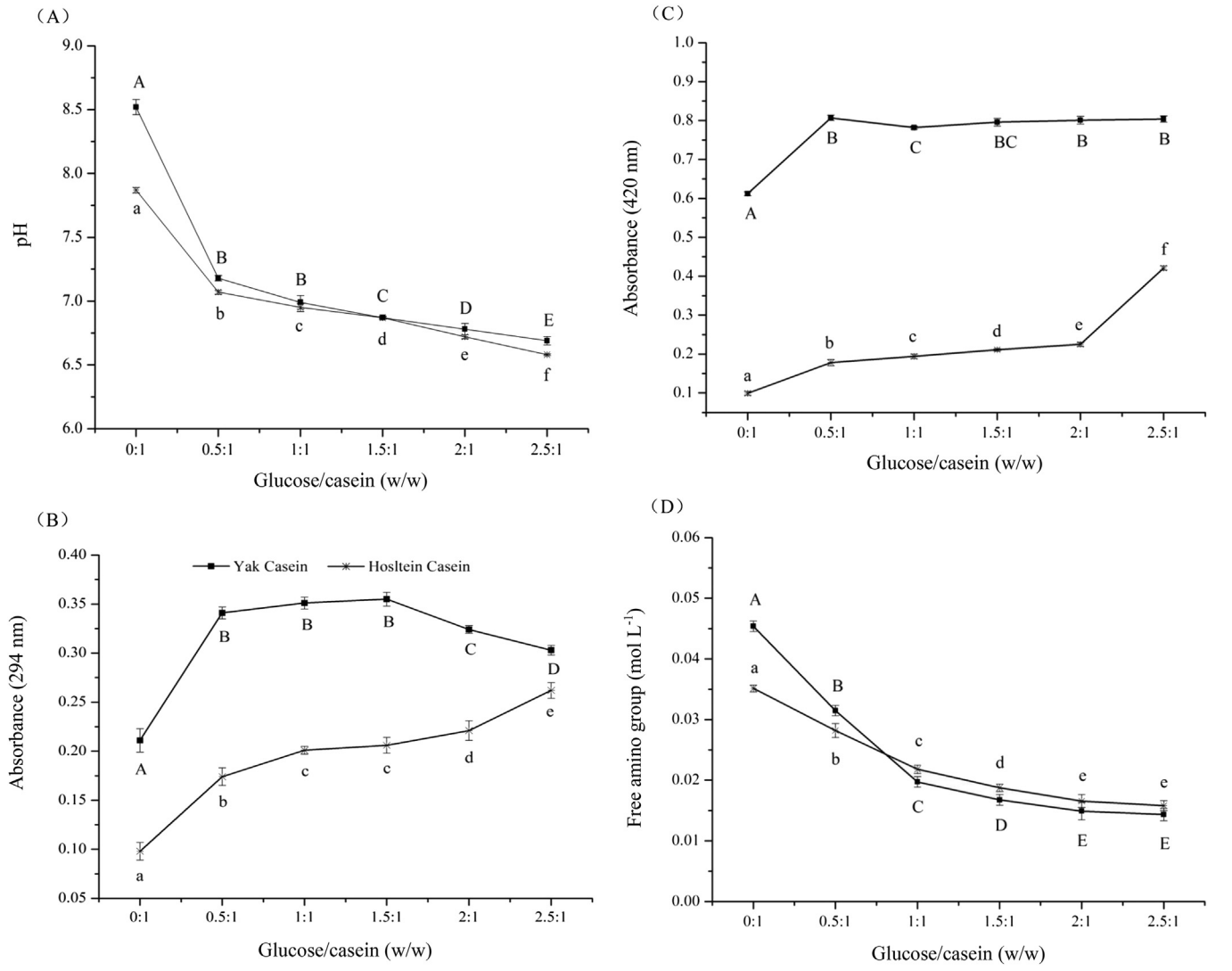

Fig. 1. The pH (A), absorbance at 294 nm (B), browning intensity at 420 nm (C) and free amino group (D) of Maillard reaction products from Yak (■) and Holstein (✳) casein–glucose model systems. Values are mean ± standard deviation; means with different lowercase letters for Holstein casein and capital letters for Yak casein are significantly different ($P < 0.05$).

intensity in the Yak system was observed as the concentration of glucose increased. Similar to the absorbance at 294 nm, that at 420 nm for the Yak system was higher than that of the Holstein system at each ratio. It has been verified that the content of lysine in Yak caseins, which involved in the Maillard reaction, was higher than that in bovine caseins (Li et al., 2010). In addition, the amino acid sequences and composition of Yak caseins were different from cow caseins (Li et al., 2011; Wang et al., 2013). Therefore, the absorbance at 294 nm and 420 nm of the Yak casein-glucose system were higher than those of the Holstein casein-glucose system.

3.3. Changes in content of free amino groups

Fig. 1D shows changes in the free amino group contents of the Yak and Holstein casein-glucose systems. As expected, the content of free amino groups of the casein-glucose system decreased with increasing glucose concentrations ($P < 0.05$), except for a glucose concentration of 2.5 g g^{-1} casein. The change of amino groups with increasing glucose content was in agreement with a previous report (Dong et al., 2011). The content of amino groups in Yak casein was higher than in Holstein casein, which was also consistent with a previous report (Li et al., 2011). At a ratio of 0.5:1 (glucose:casein, w/w), the concentration of free amino groups of Yak casein was higher than for Holstein casein, but it was lower than that of Holstein casein with high contents of glucose, indicating a higher level of Yak casein compared with Holstein casein.

3.4. Changes in reducing power

The ferrous reducing power of Yak and Holstein casein-glucose systems as a function of the concentrations of glucose and MRPs is shown in Fig. 2. Regarding the Yak casein-glucose system (Fig. 2A), the higher the concentration of glucose, the greater the reducing activity at the same concentration ($P < 0.05$), especially at MRP concentrations > 4 mg mL^{-1}. At concentrations <2 mg mL^{-1}, the reducing power of Yak MRPs increased significantly as the amount of glucose increased from 0.5 g g^{-1} to 1 g g^{-1} casein ($P < 0.05$), but it changed little as the content of glucose increased from 1.5 to 2.5 g g^{-1} casein ($P > 0.05$).

The Yak and Holstein MRPs showed increasing reducing power with increasing concentration ranges of 1 mg mL^{-1} to 8 mg mL^{-1}. Although the reducing power of Holstein MRPs was enhanced by glycosylation, it did not show a consistent pattern with increases in glucose concentration (Fig. 2B). Heated Yak

casein alone showed higher reducing power than heated Holstein casein at the same concentrations, due to its higher content of lysine (Fig. 1D). Glycosylation improved the reducing power of Holstein casein at concentrations >4 mg mL^{-1}, which was much higher than that of Yak casein at the same concentration with different ratios.

It was confirmed that glycosylation improved the reducing power of both Yak and Holstein casein, though they were still lower than the reducing power of 1 mg mL^{-1} butylated hydroxytoluene (BHT) (3.219 ± 0.082, data not shown). The same results were reported in previous studies of casein-sugar (Gu et al., 2009b, 2010) or casein peptides-sugar systems (Jiang et al., 2013). It was suggested that hydroxyl and pyrrole groups of MRPs might play a role in reducing activity (Yoshimura, Iijima, Watanabe, & Nakazawa, 1997). It was also reported that intermediate compounds of MRPs could break the radical chain by donation of a hydrogen atom (Gu et al., 2009b). However, steric obstruction, S-S covalent combination, and interaction among proteins might affect the reducing power of MRPs.

Because of different compositions and amino acid sequences, Yak casein has a different reducing power when compared with bovine casein (Li et al., 2010, 2011; Wang et al., 2013). It also shows a different behaviour during chemical or enzymatic modification (Yang et al., 2014, 2015), which was also shown by absorbance at 294 nm and 420 nm and the content of free amino groups (Fig. 1). Hence, Yak MRPs showed different reducing activity when compared with Holstein MRPs at the same glycosylation conditions and concentrations.

3.5. Changes in DPPH radical scavenging activity

As shown in Fig. 3, free radical scavenging activity of both Yak and Holstein caseins were dramatically enhanced with glycosylation at a ratio of 0.5:1 (glucose:casein, w/w) ($P < 0.05$), and

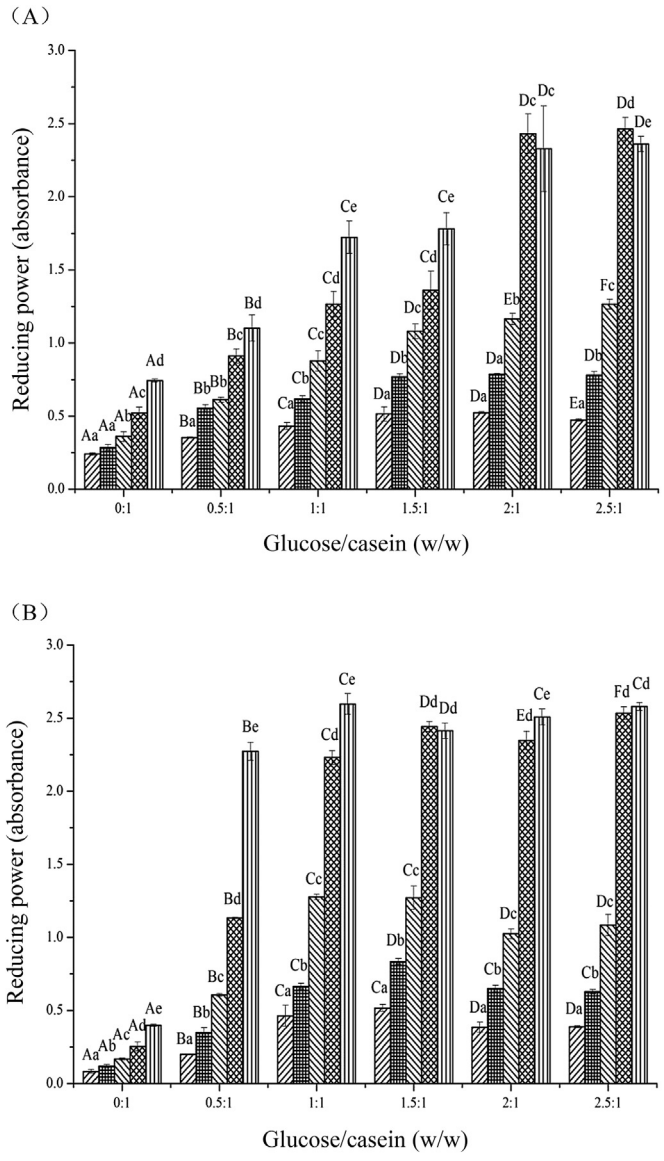

Fig. 2. The reducing power of Yak (A) and Holstein (B) Maillard reaction products from casein–glucose model system (▨, 1 mg mL^{-1}; ▦, 2 mg mL^{-1}; ▧, 4 mg mL^{-1}; ▩, 8 mg mL^{-1}; ▥, 16 mg mL^{-1}). Values are mean ± standard deviation; means with different lowercase letters are significantly different among different concentration at the same ratio of glucose and casein ($P < 0.05$); means with different capital letters are significantly different among different ratio of glucose and casein at the same concentration ($P < 0.05$).

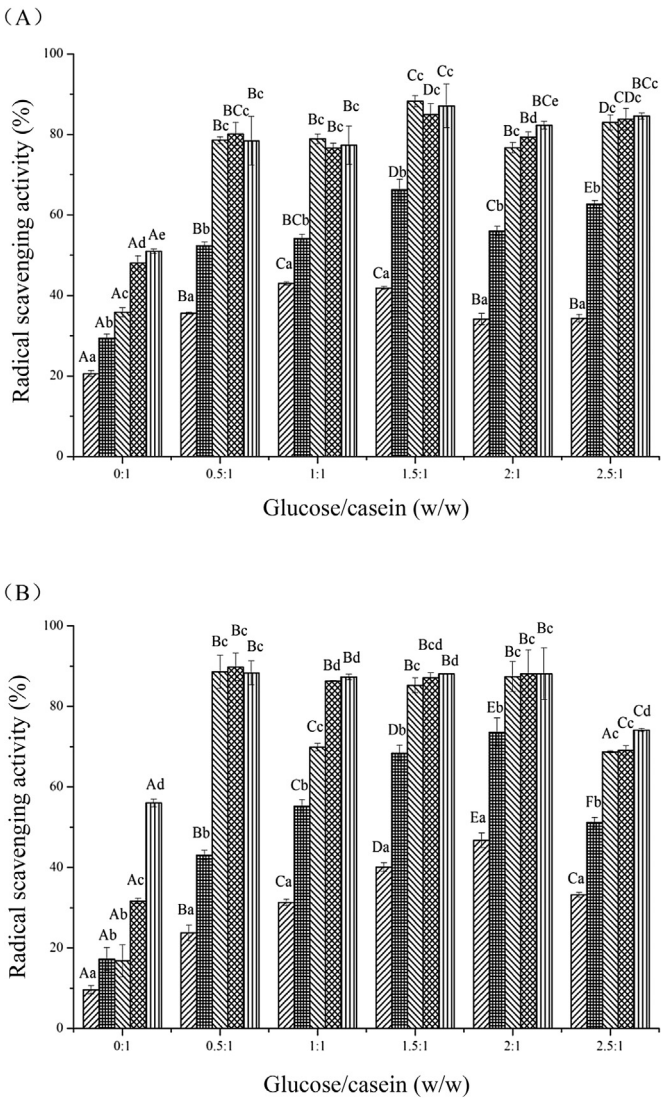

Fig. 3. The DPPH radical scavenging activity of Yak (A) and Holstein (B) Maillard reaction products from casein–glucose model system (▨, 1 mg mL^{-1}; ▦, 2 mg mL^{-1}; ▧, 4 mg mL^{-1}; ▩, 8 mg mL^{-1}; ▥, 16 mg mL^{-1}). Values are mean ± standard deviation; means with different lowercase letters are significantly different among different concentration at the same ratio of glucose and casein ($P < 0.05$); means with different capital letters are significantly different among different ratio of glucose and casein at the same concentration ($P < 0.05$).

the increase of Holstein MRPs at the same ratio was much higher than that of Yak MRPs ($P < 0.05$). Free radical scavenging activity of Yak MRPs reached a maximum at the ratio of 1.5:1 (glucose:casein, w/w) at different concentrations (Fig. 3A). After glycosylation, the free radical scavenging activity of Yak casein showed no significant difference at concentrations >4 mg mL^{-1} ($P > 0.05$), except for a glucose:casein ratio of 2:1 (w/w). This indicated that the free radical scavenging activity of Yak MRPs did not depend on concentration, when the concentration was >4 mg mL^{-1}.

Compared with unmodified casein, glycosylation of 1 g of casein by 0.5 g glucose greatly increased the scavenging activity of Holstein MRPs ($P < 0.05$), which led to its higher radical scavenging activity at a concentration > 4 mg mL^{-1} when compared with that of Yak MRPs. Free radical scavenging activity of Holstein MRPs increased when the concentration of glucose increased from 0.5 g g^{-1} to 2 g g^{-1} casein at concentrations <2 mg mL^{-1}. When the ratio of glucose:casein increased to 2.5 (w/w), the free radical scavenging activity of Holstein MRPs was sharply reduced ($P < 0.05$). As the content of Holstein MRPs increased to >8 mg mL^{-1}, its free radical scavenging activity did not depend on the glucose content in the ratio range of 0.5–2.0 w/w (glucose:casein).

The DPPH free radical scavenging activity of heated Yak casein in the absence of glucose was higher than that of Holstein casein at the same concentrations, except for 16 mg mL^{-1}. Holstein MRPs had a stronger free radical scavenging activity than Yak MRPs at a ratio of 2:1 (glucose:casein, w/w), and when the ratio reached 2.5:1, the Holstein activity was lower than that of the Yak activity.

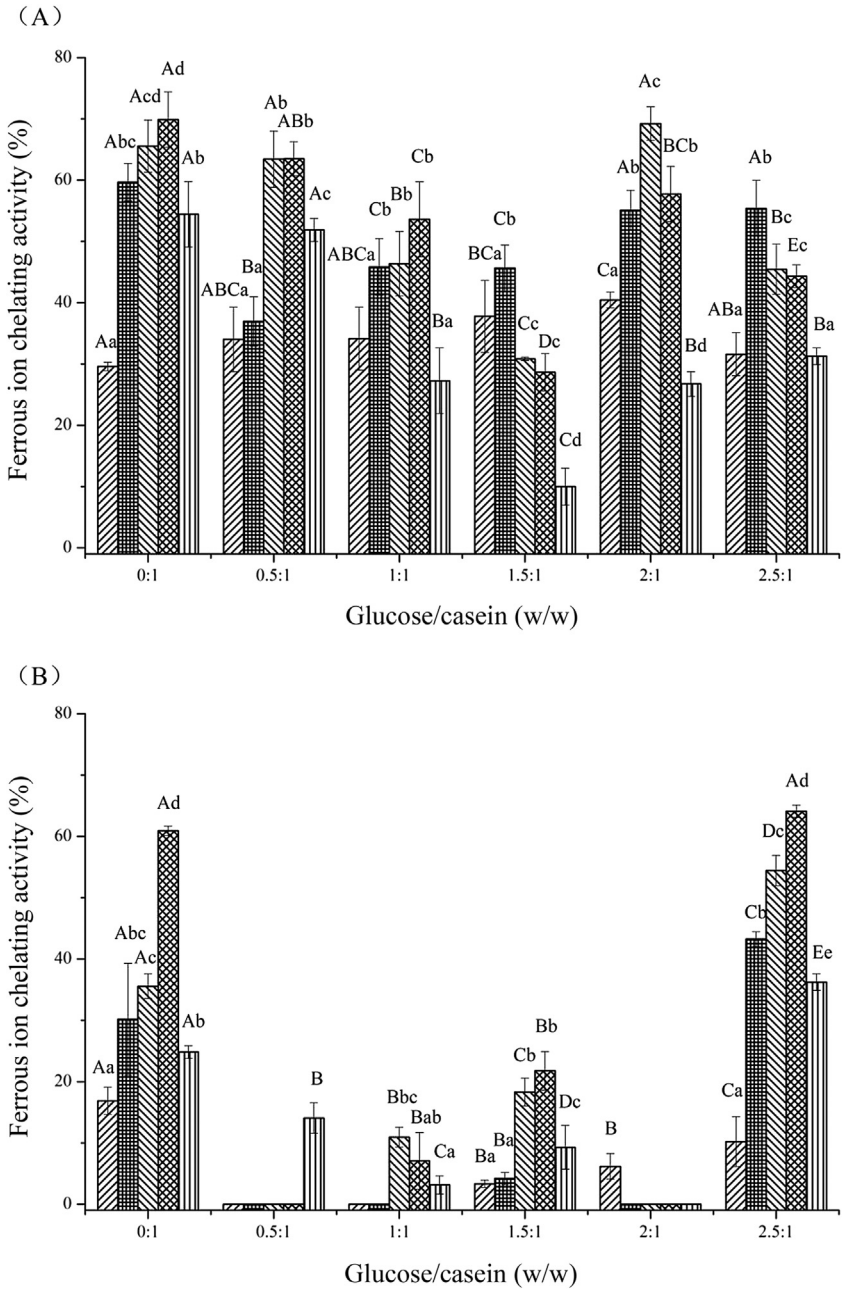

Fig. 4. Ferrous ion chelating activity of Yak (A) and Holstein (B) Maillard reaction products from casein–glucose model system (▨, 1 mg mL^{-1}; ▦, 2 mg mL^{-1}; ▧, 4 mg mL^{-1}; ▩, 8 mg mL^{-1}; ▥, 16 mg mL^{-1}). Values are mean ± standard deviation; means with different lowercase letters are significantly different among different concentration at the same ratio of glucose and casein ($P < 0.05$); means with different capital letters are significantly different among different ratio of glucose and casein at the same concentration ($P < 0.05$).

The free radical scavenging activity of BHT at 1 mg mL^{-1} was 82.07% ± 0.91% (data not shown), which was lower than that of Yak MRPs at 4 mg mL^{-1} with a glucose:casein ratio of 1.5:1 (w/w) and that of Holstein MRPs at 4 mg mL^{-1} with a glucose:-casein ratio of 0.5:1 (w/w). The MRPs of Yak and Holstein caseins had good DPPH free radical scavenging abilities and reached a plateau at concentrations >4 mg mL^{-1} at different ratios, which were consistent with a previous report (Gu et al., 2009b). However, the free radical scavenging abilities of Yak and Holstein MRPs in our study were greater than those of reports at the same MRPs and glucose concentrations (Dong et al., 2011; Gu et al., 2009b).

Either the final brown polymer or intermediates can function as hydrogen donors and contribute to the anti-free radical activity measured by the DPPH test (Benjakul et al., 2005). Changes in free radical scavenging activities in our system were not consistent with that of the reducing activity, because of the complexity of the MRPs. MRPs have been reported to quench hydrophilic free radicals more efficiently than hydrophobic free radicals (Jing & Kitts, 2002). Similar to reducing power, Yak MRPs showed a different free radical

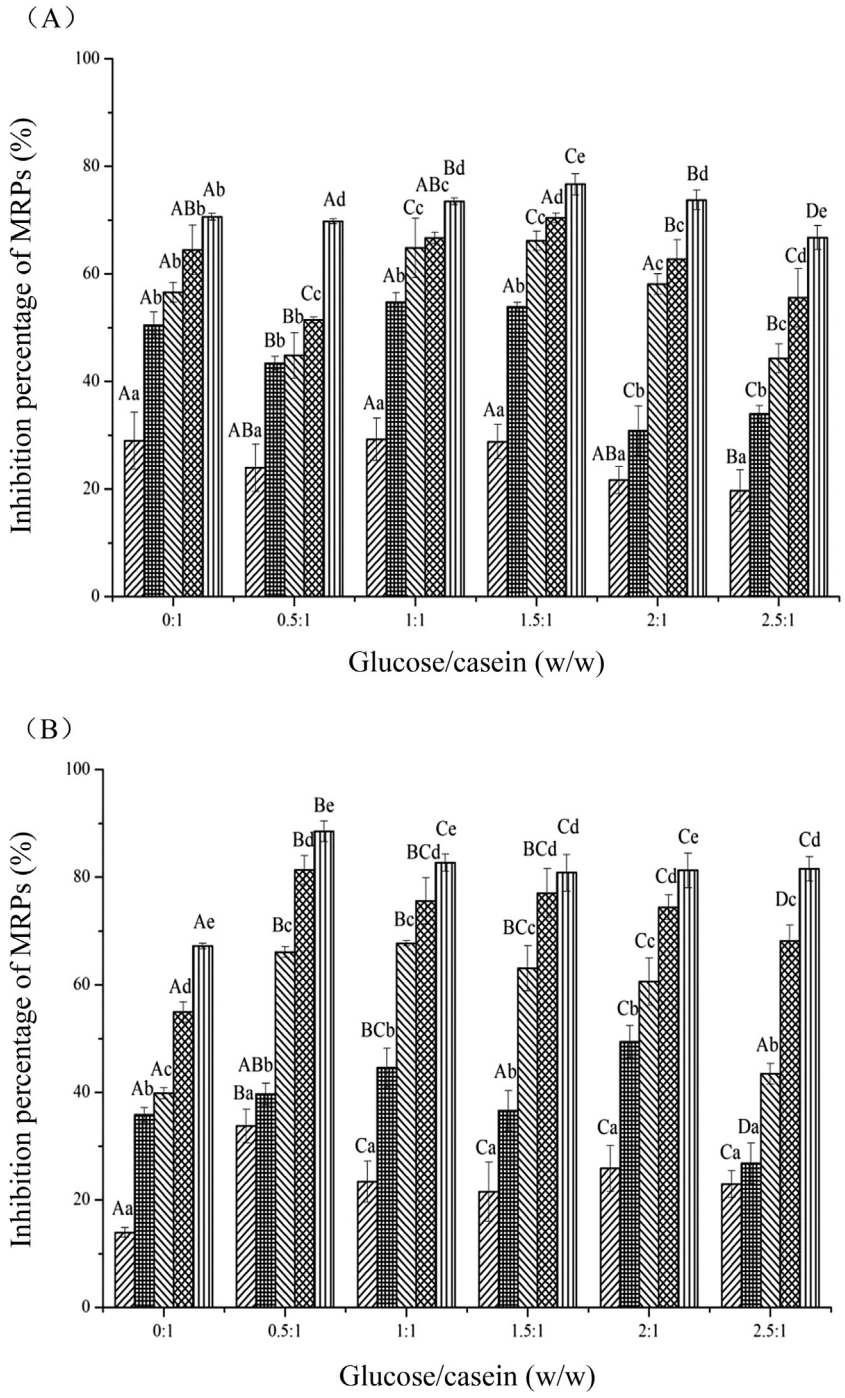

Fig. 5. The inhibition of lipid peroxidation of Yak (A) and Holstein (B) Maillard reaction products from casein–glucose model system (▨, 1 mg mL^{-1}; ▦, 2 mg mL^{-1}; ▧, 4 mg mL^{-1}; ▩, 8 mg mL^{-1}; ▥, 16 mg mL^{-1}). Values are mean ± standard deviation; means with different lowercase letters are significantly different among different concentration at the same ratio of glucose and casein ($P < 0.05$); means with different capital letters are significantly different among different ratio of glucose and casein at the same concentration ($P < 0.05$).

scavenging ability when compared with Holstein MRPs, which resulted from different degrees of glycosylation and the different amounts of brown polymers or intermediates (Fig. 1).

3.6. Changes in the chelating activity of Fe^{2+}

The ferrous ion chelating activities of MRPs from Yak and Holstein casein-glucose systems are listed in Fig. 4. After glycosylation at a glucose concentration of 0.5 g g^{-1} casein, the Fe^{2+} chelating activity of Yak MRPs decreased at concentrations between 2 and 16 mg mL^{-1}. Compared with unmodified Yak casein, the slight increase of chelating ability of Yak MRPs at 1 mg mL^{-1} after glycosylation with each ratio was probably due to an increase in its solubility. With increasing concentrations of MRPs, the chelating ability first increased and then decreased.

Holstein MRPs had weaker chelating ability than Yak MRPs, which was eliminated after glycosylation at a glucose concentration of 0.5 g g^{-1} of casein at concentrations of 1–8 mg mL^{-1}. The chelating ability of Holstein MRPs increased at a ratio of 2.5:1 from a concentration of 2–16 mg mL^{-1}, when compared with unmodified casein.

According to previous studies, the chelating activity could be attributed to hydroxyl and pyrrole groups originating from MRPs (Yoshimura et al., 1997). Gu et al. (2009b) reported that the chelating activity of MRPs from the glucose-casein system increased as its concentration increased, consistent with the results of the Yak casein at the same ratios at low concentrations. An opposite trend has been reported in the glucose-glycine model system (Yoshimura et al., 1997). An increase in the chelating capacity of the glucose-casein system was found within 3 h after heating at low concentrations (Dong et al., 2011). In conclusion, the chelating mechanism of MRPs was complicated and affected not only by hydroxyl and pyrroline groups, but also by net effects, steric effects, and the interactions among proteins.

3.7. Changes in inhibition of lipid peroxidation

Fig. 5 shows the ability of Yak and Holstein MRPs to inhibit lecithin oxidation. The inhibitory activity of lipid peroxidation of the two types of caseins and MRPs increased with increasing concentrations, which was consistent with a previous report (Gu et al., 2009b). Unglycosylated Yak casein showed stronger inhibition than unglycosylated Holstein casein at the same concentrations, in agreement with the other two antioxidant test results. Notably, glycosylation did not enhance the inhibition of lipid peroxidation of Yak casein at certain concentrations. Overall, the results showed that Yak casein was a good natural inhibitor of lipid peroxidation.

However, glycosylation improved the inhibition ability of Holstein casein significantly at a glucose:casein ratio of 0.5:1 (w/w) ($P < 0.05$), which was consistent with the results of previous studies (Dong et al., 2011; Gu et al., 2009b; Jing & Kitts, 2002). At the glucose casein ratio of 0.5:1 (w/w) the inhibition of Holstein MRPs was stronger than the inhibition of Yak MRPs at each concentration except for 2 mg mL^{-1}. At 16 mg mL^{-1} of Holstein MRPs, the content of glucose affected the inhibition activity insignificantly as it increased from 1 to 2.5 (g g^{-1} of casein; $P > 0.05$). However, the inhibitory ability of Holstein MRPs decreased at each concentration as the content of glucose increased from 2 g to 2.5 g. The results of the two types of caseins indicated the complexity of MRPs. Although forming MRPs from casein increased their free radical scavenging activities, it decreased their ability to chelate metals, resulting in no dramatic changes in the inhibitory activities of lipid peroxidation.

4. Conclusion

Since there is composition difference between Yak casein and Holstein casein, the degree of Maillard reaction, the absorbance at 294 nm and the browning intensity of the Yak casein-glucose system were higher than those of the Holstein casein-glucose system at the same conditions. In addition, the native Yak casein had better antioxidant activity than native Holstein casein. Although glycosylation enhanced the reducing power and DPPH radical scavenging activity of both caseins ($P < 0.05$), the changes of Yak casein are different from those of Holstein casein. The reducing power of Yak MRPs increased at the same concentration as the content of glucose increased, but Holstein MRPs had the highest reducing power at a ratio of 1.5:1 (glucose:casein, w/w) with different concentrations, except for 16 mg mL^{-1}. At concentrations of 1–8 mg mL^{-1}, the reducing power of Yak and Holstein MRPs increased as the concentration increased at the same ratio. After glycosylation by glucose, the DPPH radical scavenging activity of Yak casein showed a significant difference at concentrations >4 mg mL^{-1} ($P > 0.05$) except for a glucose:casein ratio of 2:1 (w/w). The DPPH radical scavenging activity of Holstein MRPs increased as the content of glucose increased from 0.5 g to 2 g g^{-1} casein at concentrations <2 mg mL^{-1}, and then was sharply reduced thereafter.

The concentration changes of both MRPs and glucose were not consistent regarding their abilities to chelate Fe^{2+}. Overall, the Fe^{2+} chelating activity of Yak MRPs decreased at concentration between 2 and 16 mg mL^{-1} after glycosylation with 0.5 g glucose g^{-1} casein, while that of Holstein MRPs decreased or was completely eliminated after glycosylation with 0.5–2.0 g glucose g^{-1} casein. In addition, a slight increase of metal chelating ability of Yak MRPs at 1 mg mL^{-1} was observed with each ratio compared with unmodified Yak casein, because of increased solubility.

Native Yak casein could be used as a lipid peroxidation inhibitor, with much higher inhibitory activity than that of Holstein native casein. The improvement in the inhibitory activity of Yak casein was lower than that of Holstein casein after glycosylation by glucose. The inhibition activity of both Yak and Holstein MRPs at the same ratio of glucose and casein increased as the concentration increased.

Acknowledgements

This work was financially supported by the National Natural Science Foundation of China (No. 31560430), the Fuxi Foundation of Gansu Agricultural University (No. Gaufx-02J02), and Special Funds for Discipline Construction (No. GAU-XKJS-2018-118).

References

Ajandouz, E. H., Tchiakpe, L. S., Ore, F. D., Benajiba, A., & Puigserver, A. (2001). Effects of pH on caramelization and Maillard reaction kinetics in fructose-lysine model systems. *Journal of Food Science, 66*, 926–931.

Akillioglu, H. G., & Gokmen, V. (2014). Effects of hydrophobic and ionic interactions on glycation of casein during Maillard reaction. *Journal of Agricultural and Food Chemistry, 62*, 11289–11295.

Benjakul, S., Visessanguan, W., Phongkanpai, V., & Tanaka, M. (2005). Antioxidative activity of caramelisation products and their preventive effect on lipid oxidation in fish mince. *Food Chemistry, 90*, 231–239.

Brands, C. M. J., Alink, G. M., van Boekel, M. A. J. S., & Jongen, W. M. F. (2000). Mutagenicity of heated sugar-casein systems: Effect of the Maillard reaction. *Journal of Agricultural and Food Chemistry, 48*, 2271–2275.

Cui, G. X., Yuan, F., Degen, A. A., Liu, S. M., Zhou, J. W., Shang, Z. H., et al. (2016). Composition of the milk of yaks raised at different altitudes on the Qinghai-Tibetan Plateau. *International Dairy Journal, 59*, 29–35.

Dong, S., Wei, B., Chen, B., McClements, D. J., & Decker, E. A. (2011). Chemical and antioxidant properties of casein peptide and its glucose Maillard reaction products in fish oil-in-water emulsions. *Journal of Agricultural and Food Chemistry, 59*, 13311–13317.

Gu, F. L., Abbas, S., & Zhang, X. M. (2009a). Optimization of Maillard reaction products from casein-glucose using response surface methodology. *LWT Food Science and Technology, 42*, 1374–1379.
Gu, F. L., Kim, J. M., Abbas, S., Zhang, X. M., Xia, S. Q., & Chen, Z. X. (2010). Structure and antioxidant activity of high molecular weight Maillard reaction products from casein-glucose. *Food Chemistry, 120*, 505–511.
Gu, F., Kim, J. M., Hayat, K., Xia, S., Feng, B., & Zhang, X. (2009b). Characteristics and antioxidant activity of ultrafiltrated Maillard reaction products from a casein–glucose model system. *Food Chemistry, 117*, 48–54.
Hong, X., Meng, J., & Lu, R. R. (2015). Improvement of ACE inhibitory activity of casein hydrolysate by Maillard reaction with xylose. *Journal of the Science of Food and Agriculture, 95*, 66–71.
Jiang, Z., Wang, L., Wu, W., & Wang, Y. (2013). Biological activities and physico-chemical properties of Maillard reaction products in sugar-bovine casein peptide model systems. *Food Chemistry, 141*, 3837–3845.
Jing, H., & Kitts, D. D. (2002). Chemical and biochemical properties of casein-sugar Maillard reaction products. *Food and Chemical Toxicology, 40*, 1007–1015.
Lertittikul, W., Benjakul, S., & Tanaka, M. (2007). Characteristics and antioxidative activity of Maillard reaction products from a porcine plasma protein–glucose model system as influenced by pH. *Food Chemistry, 100*, 669–677.
Li, H., Ma, Y., Dong, A. J., Wang, J. Q., Li, Q. M., He, S. H., et al. (2010). Protein composition of yak milk. *Dairy Science and Technology, 90*, 111–117.
Li, H., Ma, Y., Li, Q., Wang, J., Cheng, J., Xue, J., et al. (2011). The chemical composition and nitrogen distribution of Chinese yak (Maiwa) milk. *International Journal of Molecular Sciences, 12*, 4885–4895.
Liu, S. C., Yang, D. J., Jin, S. Y., Hsu, C. H., & Chen, S. L. (2008). Kinetics of color development, pH decreasing, and anti-oxidative activity reduction of Maillard reaction in galactose/glycine model systems. *Food Chemistry, 108*, 533–541.
Liu, H. N., Zhang, C., Zhang, H., Guo, H. Y., Wang, P. J., Zhu, Y. B., et al. (2013). pH treatment as an effective tool to select the functional and structural properties of yak milk caseins. *Journal of Dairy Science, 96*, 5494–5500.
Markman, G., & Livney, Y. D. (2012). Maillard-conjugate based core-shell co-assemblies for nanoencapsulation of hydrophobic nutraceuticals in clear beverages. *Food and Function, 3*, 262–270.
Morales, F. J., & van Boekel, M. A. J. S. (1998). A study on advanced Maillard reaction in heated casein/sugar solutions: Colour formation. *International Dairy Journal, 8*, 907–915.
Muhoza, B., Xia, S., Cai, J., Zhang, X., Su, J., & Li, L. (2017). Time effect on coenzyme Q 10 loading and stability of micelles based on glycosylated casein via Maillard reaction. *Food Hydrocolloids, 72*, 271–280.
Mu, M., Pan, X., Yao, P., & Jiang, M. (2006). Acidic solution properties of beta-casein-graft-dextran copolymer prepared through Maillard reaction. *Journal of Colloid and Interface Science, 301*, 98–106.
Naranjo, G. B., Pereyra Gonzales, A. S., Leiva, G. E., & Malec, L. S. (2013). The kinetics of Maillard reaction in lactose-hydrolysed milk powder and related systems containing carbohydrate mixtures. *Food Chemistry, 141*, 3790–3795.
Oh, M. J., Kim, Y., Hoon Lee, S., Lee, K., & Park, H. Y. (2018). Prediction of CML contents in the Maillard reaction products for casein-monosaccharides model. *Food Chemistry, 267*, 271–276.
Oh, N. S., Koh, J. H., Park, M. R., Kim, Y., & Kim, S. H. (2016a). Short communication: Hypolipidemic and antiinflammatory effects of fermented Maillard reaction products by *Lactobacillus fermentum* H9 in an animal model. *Journal of Dairy Science, 99*, 9415–9423.
Oh, N. S., Kwon, H. S., Lee, H. A., Joung, J. Y., Lee, J. Y., Lee, K. B., et al. (2014). Preventive effect of fermented Maillard reaction products from milk proteins in cardiovascular health. *Journal of Dairy Science, 97*, 3300–3313.
Oh, N. S., Lee, H. A., Lee, J. Y., Joung, J. Y., Lee, K. B., Kim, Y., et al. (2013). The dual effects of Maillard reaction and enzymatic hydrolysis on the antioxidant activity of milk proteins. *Journal of Dairy Science, 96*, 4899–4911.
Oh, N. S., Young Lee, J., Lee, H. A., Joung, J. Y., Shin, Y. K., Kim, S. H., et al. (2016b). Chemical characteristics and enhanced hepatoprotective activities of Maillard reaction products derived from milk protein-sugar system. *Journal of Dairy Science, 99*, 947–958.
Pereyra Gonzales, A. S., Naranjo, G. B., Leiva, G. E., & Malec, L. S. (2010). Maillard reaction kinetics in milk powder: Effect of water activity at mild temperatures. *International Dairy Journal, 20*, 40–45.
Rufián-Henares, J. A., & Morales, F. J. (2006). A new application of a commercial microtiter plate-based assay for assessing the antimicrobial activity of Maillard reaction products. *Food Research International, 39*, 33–39.
Wang, T. T., Guo, Z. W., Liu, Z. P., Feng, Q. Y., Wang, X. L., Tian, Q., et al. (2016). The aggregation behavior and interactions of yak milk protein under thermal treatment. *Journal of Dairy Science, 99*, 6137–6143.
Wang, L., Jiang, Z., Tian, B., Bai, L., Shi, X., & Zhang, X. (2015). Effects of Galactose concentration on characteristics of angiotensin-I-converting enzyme inhibitory peptides derived from bovine casein in Maillard reaction. *International Journal of Food Properties, 19*, 2238–2250.
Wang, P., Liu, H., Wen, P., Zhang, H., Guo, H., & Ren, F. (2013). The composition, size and hydration of yak casein micelles. *International Dairy Journal, 31*, 107–110.
Yang, M., Shi, Y., & Liang, Q. (2015). Effect of microbial transglutaminase crosslinking on the functional properties of yak caseins: A comparison with cow caseins. *Dairy Science and Technology, 96*, 39–51.
Yang, M., Shi, Y., Wang, P., Liu, H., Wen, P., & Ren, F. (2014). Effect of succinylation on the functional properties of yak caseins: A comparison with cow caseins. *Dairy Science and Technology, 94*, 359–372.
Yi, O. S., Meyer, A. S., & Frankel, E. N. (1997). Antioxidant activity of grape extracts in a lecithin liposome system. *Journal of American Oil Chemical Society, 74*, 1301–1307.
Yoshimura, Y., Iijima, T., Watanabe, T., & Nakazawa, H. (1997). Antioxidative effect of Maillard reaction products using glucose-glycine model system. *Journal of Agricultural and Food Chemistry, 45*, 4106–4109.