Brown pigment formation in heated sugar–protein mixed suspensions containing unmodified and peptically modified whey protein concentrates

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Abstract Commercial whey protein concentrate (WPC) was modified by heating the acidified protein suspensions (pH 2.0) at 80 °C for 30 min and treating with pepsin at 37 °C for 60 min. Prior to spray-drying, such modification did not change the molecular weights (MWs) of whey proteins determined by sodium dodecyl sulfate–polyacrylamide gel electrophoresis (SDS-PAGE). After spray-drying the modified whey protein concentrate with trehalose excipient (MWPC-TH), it was found that the α -lactalbumin (α -La) was the major protein that was further hydrolyzed the most. The reconstituted MWPC-TH contained β -lactoglobulin (β -Lg) as the major protein and small molecular weight (MW) peptides of less than 6.5 kDa. The reconstituted MWPC-TH had higher NH_2 group, Trolox equivalent antioxidant capacity (TEAC), lower exposed aromatic ring and thiol (SH) contents than did the commercial WPC. Kinetic studies revealed that the addition of MWPC-TH in fructose-glycine solution was able to reduce brown pigment formation in the mixtures heated at 80 to 95 °C by increasing the activation energy (E_a) of brown pigment formation due to the retardation of fluoresced advanced glycation end product (AGEs) formation. The addition of MWPC to reducing sugar-glycine/commercial WPC was also able to lower brown pigment formation in the sterilized

(121 °C, 15 min) mixed suspensions containing 0.1 M reducing sugar and 0.5–1.0 % glycine and/or commercial ($P < 0.05$). It was demonstrated that the modification investigated in this study selectively hydrolyzed α -La and retained β -Lg for the production of antibrowning whey protein concentrate.

Keywords AGEs · Browning · Hydrolysate · Maillard reaction · Whey protein

Introduction

Maillard reactions are non-enzymatic browning reactions occurring in both food and biological systems. In foods, Maillard reactions result in changes of color, aroma and taste, depending on temperature, time, pH, reactants, water activity, buffer type and concentration, and the presence of oxygen, light, and metals (Ames 1990; Narayan 1998; Sumaya-Martinez et al. 2005; Kim and Lee 2008). In biological systems, advanced glycation end products (AGEs) and Maillard reaction products (MRPs) accumulation are known to be involved in diabetic and other health disorders such as atherosclerosis, Alzheimer's disease and aging (Peng et al. 2008).

The Maillard reaction is initiated by the glycation of amino groups by carbonyl group of sugar, dicarbonyl group from vitamin C or lipids. Further reactions after Amadori rearrangement involve various pathways and give rise to various products called advanced glycation end products (AGEs) during the intermediate stages of redox pathways. At the final stages of Maillard reactions, the MRPs are usually composed of N-containing polymerized products called melanoidins, which are brown in color (Ames 1990; Friedman and Molnar-Perl 1990; Narayan 1998; Sumaya-Martinez et al. 2005; Kim and Lee 2008; Peng et al. 2008).

Research highlights • Characteristics of WPC modified by pepsin and further drying with trehalose were studied.

- Modification method could selectively hydrolyze α -La.
- Modified WPC showed antibrowning property when used in sterilized milk protein suspensions.

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Maillard browning reactions of sugar–amino acid systems have been extensively studied (Friedman and Molnar-Perl 1990; Ajandouz et al. 2001; Martins and van Boekel 2003; Kim and Lee 2008). However, the investigations of sugar–protein and sugar–peptide systems are quite limited due to the complexity of the reactions. Multi-response modeling of the changes in the contents of reactants and products during Maillard reactions has been demonstrated to be powerful in describing and predicting the pathways and complexities of reactions (Martins et al. 2001; Brands and van Boekel 2002). This is because those reactions occur via different reaction routes depending on temperature, pH, and types and concentrations of sugar. To date, studies on Maillard reactions have been emphasized in a better understanding of the various pathways involved in the chain reactions, influences of food processing parameters used, nutritional changes, bioavailabilities of essential amino acids, as well as toxicological effects of AGEs and MRPs in biological systems (Gerrard 2006). From a practical standpoint, a better understanding of the mechanisms involved in controlling Maillard reactions is also required.

The classical methods of controlling Maillard reactions in food systems are: elimination of reactive substances, lowering of pH, chelating of trace minerals, limiting water content, maintaining low temperature, and the use of sulfite as inhibitor and bleaching substances (Peng et al. 2008). However, the inhibition of AGEs and MRPs by inhibiting oxidation mechanisms using natural compounds has been recently investigated. For example, the use of amino acids (Friedman and Molnar-Perl 1990), polyphenolic compounds (Wu and Yen 2005), peptides and proteins (Peng et al. 2008; Hongsprabhas et al. 2011) to inhibit the full intermolecular crosslinking pathways in Maillard reactions have recently been investigated.

Although sulfites have been widely used as antibrowning agent in processed food products, they could be harmful to health (Iyengar and McEvily 1992). Sulfur-containing amino acids and derivatives such as Cys, *N*-acetylcysteine and glutathione also exert antioxidant and antiglycation activities (Friedman 1996). Their antiglycation activities are reported to be due to their abilities to act as reducing agents that scavenge free radicals and destroy lipid hydroperoxides. Moreover, their strong nucleophilic characteristics can trap electrophilic compounds. Antioxidants such as ascorbic acid, α -tocopherol and pyridoxamine could also inhibit the autooxidation of Amadori rearrangement products and subsequently inhibit AGE formation (Alam et al. 2013).

In addition, some amino acids can form complexes with AGEs and prevent the reactions to proceed further to brown pigment formation (Vinson and Howard 1996). Cysteine could react with dicarbonyl compound to give stable *S*-carboxymethylcysteine (Zeng and Davies 2005). Moreover, the α -dicarbonyls could react with Lys and Arg to form stable

AGEs such as *N* ϵ -(carboxymethyl)lysine (Alam et al. 2013). Antioxidative amino acids, such as Cys, Met, Trp, Tyr, Phe and His, have also been shown to inhibit the formation of AGEs due to the donation of H atoms to the radicals generated from autooxidation of AGEs (Kalifah et al. 1999; Goldin et al. 2006; Elias et al. 2008). Amino acid- and peptide-based drugs have also been used as AGE inhibitors due to their low toxicity and good metabolic functions (Shantharam et al. 2013). Carnosine, a dipeptide of β -Ala-His found in chicken extract, was reported to form complexes with AGEs and prevent them from proceeding to full Maillard brown pigment formation (Seidler and Yeorgans 2002; Mariee and Al-Shabanah 2006).

The production of bioactive food peptides and amino acid sequences of antioxidative food peptides and hydrolysates have been investigated extensively (Saadi et al. 2015). However, little is known of the potential use of antioxidative food protein hydrolysates in inhibiting or delaying Maillard reactions in processed foods. Hydrolysis of food proteins could increase NH_2 groups, the main reactant for Maillard reactions. Yet protein hydrolysates, particularly whey protein hydrolysate obtained from tryptic hydrolysis, were reported to have both antioxidant capacity and antibrowning capacity in pasteurized and sterilized whey protein–lactose mixture (Hongsprabhas et al. 2011).

Our previous investigation also revealed that commercial whey protein concentrate (WPC), modified by heating the acidified WPC (pH 2.0) at 80 °C for 30 min and treating with pepsin at 37 °C for 60 min, increased the oxygen radical absorbance capacity from 0.196 μmol Trolox equivalent (TE)/mg protein in the commercial WPC to 0.751 μmol TE/mg protein in the freshly modified whey protein concentrate (Rongsirikul and Hongsprabhas 2011). The modified whey proteins could reduce 50 % of brown pigment formation after re-heating WPC suspensions containing additional lactose at 80 °C for 30 min. The purposes of this study were thus to further explore the influences of such modification and further spray-drying in the presence of the carbohydrate excipient trehalose on antibrowning of modified whey proteins. The nature of the antibrowning activity of modified whey proteins was also explored to achieve a better understanding of the roles of modified whey proteins in inhibiting the cascade of Maillard browning reactions in heated reducing sugar–glycine and sugar–protein suspensions. The results may offer possibilities to optimize food quality with respect to Maillard reactions.

Materials and methods

Materials

Commercial WPC was imported and repacked by a local distributor in Thailand. It contained 75.83 % protein (wet basis

[wb]), 6.5 % moisture content (wb), 2.71 % ash (wb), 1.05 % fat (wb) and 13.91 % carbohydrate (wb) (AOAC 2000). Trehalose (TrehalTM; Hayashibara, Okayama, Japan) was supplied by the East Asiatic Co., Bangkok, Thailand. Trehalose was used to embed whey proteins and hydrolysates in its amorphous matrix upon drying and storage due to its high glass transition temperature of 115–117 °C (Jain and Roy 2008). Porcine gastric mucosa pepsin (E.C.3.4.23.1; 367 U/mg) was purchased from Sigma Chemical Co. (St. Louis, MO, USA). Pepsin was chosen due to its endopeptidase activity and its specificity for the cleavage of peptide linkages at the carboxylic side of phenylalanine, leucine, and glutamic acid. The susceptibility of protein to peptic hydrolysis could be increased in the presence of sulfur-containing amino acid close to the peptide bond, which has aromatic amino acid residue (according to Sigma Chemical Co. datasheet). All chemicals were of reagent grade.

Characteristics of peptically modified whey protein concentrate (MWPC)

WPC was modified by pre-heating the acidified WPC suspension followed by peptic treatment, as described previously (Rongsirikul and Hongprabhas 2011). Briefly, 6.25 % protein (w/v) WPC suspension (pH 2.0 adjusted by 1.0 M HCl) was pre-heated at 80 °C for 30 min; after cooling down to room temperature, pepsin was added. Hydrolysis was carried out at 37 °C for 1 h, using an enzyme to substrate ratio of 1:17. Pepsin was inactivated by adjusting the protein suspension to pH 8.0 using 1.0 M NaOH.

The protein suspensions, namely commercial WPC and peptically modified WPC (MWPC), were supplemented with trehalose (TH) to obtain a protein to carbohydrate ratio of 0.3:0.7 and a final solid concentration of 20 % (w/v), as previously described (Hongprabhas et al. 2011). The mixed protein–trehalose suspension was spray-dried (Mini Spray Dryer B-190; Buchi, Flawil, Switzerland) at a feed rate of 4.0±0.5 mL/min, inlet temperature of 130 °C and outlet temperature of 100 °C. The spray-dried WPC-TH and MWPC-TH powders were kept in sealed aluminum foil pouches at −20 °C prior to reconstitution with 0.1 M phosphate buffer at pH 8.0, unless stated otherwise, and characterized using the methods described below.

Molecular weight profiles of proteins

Molecular weight profiles of WPC and MWPC before and after spray-drying with TH excipient were characterized by sodium dodecyl sulfate–polyacrylamide gel electrophoresis (SDS-PAGE) (Laemmli 1970).

Trolox Equivalent Antioxidant Capacity (TEAC)

Reconstituted commercial WPC (no TH added), reconstituted WPC-TH, MWPC-TH before spray-drying, and reconstituted MWPC-TH suspensions were measured for Trolox equivalent antioxidant capacity (TEAC) (Re et al. 1999). The absorbance at 734 nm was recorded using a spectrophotometer (Spectronic 20+; Thermo Fisher Scientific, Waltham, MA, USA). The calibration curve was prepared using Trolox concentrations ranging from 0.1 to 2 mM. TEAC values were calculated by plotting the absorbance of decolorized ABTS⁺ radical solution vs. concentration of Trolox. The antioxidant capacity of samples was expressed as μmol TE/mg protein.

Determination of the exposed aromatic ring

The exposed aromatic ring of amino acids of 0.03 % protein samples prepared as described above in a quartz cuvette was measured by UV absorbance at 280 nm using a multifunctional microplate reader (Infinite M200 PRO; Tecan, Männedorf, Switzerland) and reported as Abs_{280} (Aitken and Learmonth 1996).

Determination of reactive amino groups

Reactive amino groups of samples described above were determined by o-phthaldialdehyde (OPA) method (Nielsen et al. 2001) and reported as absorbance measured at 340 nm (Abs_{340}) using a Tecan microplate reader.

Determination of thiol (SH) group content

Free SH group content of samples described above were determined using Ellman's reagent (5,5'-dithiobis(2-nitrobenzoic acid); DTNB) method (Beveridge et al. 1974). The SH contents were calculated according to Eq. (1) and expressed as μM SH/g protein.

$$SH \text{ content}(\mu M/g \text{ protein}) = \frac{73.54 \times Abs_{412} \times 6.04}{\text{Sample concentration}(mg/mL)} \quad (1)$$

Antibrowning activity of peptically modified whey protein concentrate when used in thermally processed glycine–sugar mixed suspensions

Mixed fructose–glycine and fructose–MWPC suspensions containing different glycine and protein concentrations were prepared in 0.1 M phosphate buffer (pH 8.0) and heated in a water bath at 80, 85, 90 and 95 °C for 15, 30, 45 and 60 min. The samples were measured for brown pigment formation and reported as absorbance at 420 nm (Abs_{420}) using a Tecan

microplate reader (Cämmerer et al. 1999). The formation of AGEs was measured as % fluorescence intensity using excitation at 380 nm and emission at 465 nm (Cämmerer et al. 1999). The reaction rate constant (k) of brown pigment formation at different temperatures was determined graphically from a plot of Abs_{420} vs. time, which followed the classical zero-order kinetics. The activation energy (E_a) was determined from an Arrhenius plot of the reaction rate constant (k) vs. inverse absolute temperature.

Mixed fructose–glycine, glucose–glycine and lactose–glycine suspensions containing 0.1 M of sugars, with or without commercial WPC and MWPC-TH at different glycine and protein concentrations, were prepared in 0.1 M phosphate buffer (pH 8.0) and sterilized at 121 °C for 15 min, cooled down to room temperature, and measured for brown pigment formation by measuring the absorbance at 420 nm (Abs_{420}) (Cämmerer et al. 1999) using a Tecan microplate reader.

Statistical analysis

Experiments were carried out in two separate trials of spray-dried MWPC-TH preparation. The determinations were run in triplicate. The data were analyzed by analysis of variance (ANOVA) with significance at $P < 0.05$. Significant differences among mean values were determined by Duncan's multiple range test. All statistical analyses were performed using SPSS software version 12 (SPSS Inc., Chicago, IL, USA).

Results and discussion

Characteristics of reconstituted peptically modified whey protein concentrate (MWPC)

The major whey proteins found in commercial WPC used in this study were β -lactoglobulin (β -Lg; 18.3 kDa) and α -lactalbumin (α -La; 14.2 kDa) (Fig. 1). However, commercial WPC also showed protein bands having molecular weights (MW) between 45 and 65 kDa and above 65 kDa, which were likely be polymerized proteins generated during commercial production of commercial WPC (Fig. 1a). Some high MW polymerized proteins, particularly those having MW above 65 kDa, were reduced in the presence of β -mercaptoethanol (Fig. 1b). This suggested that polymerization of proteins through disulfide bond formation was involved during the production of commercial WPC prior to modification investigated in this study.

Reconstitution of commercial WPC and spray-drying in the presence of TH did not change the MW profiles of whey proteins (Fig. 1, lane 3). Peptic treatment of acidified and pre-heated WPC (Fig. 1, lane 4) did not result in apparent MW changes, as observed by SDS-PAGE. This was probably due to the compact structures of whey proteins, particularly β -Lg

Fig. 1 Molecular weight profiles of proteins and peptides in whey protein products in the (a) absence or (b) presence of β -mercaptoethanol. Lane 1: low-range molecular weight marker; lane 2: commercial WPC; lane 3: WPC-TH; lane 4: MWPC-TH suspension before spray-drying; and lane 5: MWPC-TH after spray-drying

and α -La (Bolder et al. 2006), that were quite stable to peptic hydrolysis for 1 h at 37 °C, although the commercial WPC was pre-heated at 80 °C for 30 min under acidic conditions prior to peptic treatment. Longer hydrolysis time may be needed to evidently hydrolyse both proteins acidically and enzymatically.

However, further hydrolysis of whey proteins, particularly α -La, occurred during spray-drying, which involved high shear force at the nozzle. The α -La, of which the tertiary structure was held by intramolecular disulfide bonds and Ca^{2+} -crosslinking, was prone to further hydrolysis during spray-drying of MWPC and was entirely hydrolyzed (Fig. 1, lane 5), although the pH of the protein suspension was adjusted to 7.0 prior to spray-drying. The polymerized proteins of high MWs were further hydrolyzed during spray-drying, since all bands disappeared. Nonetheless, the β -Lg was retained after spray-drying. After reconstitution, the MWPC-TH contained only residual β -Lg and low MW peptides having MWs below 6.5 kDa.

Although peptic modification prior to spray-drying (Fig. 1, lane 4) did not show apparent alterations in the MWs of whey proteins, further spray-drying of WPC-TH altered the chemical characteristics of whey proteins after spray-drying (Table 1). Exposed aromatic amino acid of WPC-TH slightly increased after spray-drying ($P < 0.05$). Modification of whey proteins by acidifying, pre-heating and peptic treatment, as well as spray-drying, however, drastically lowered the exposed aromatic amino acid in reconstituted MWPC-TH.

Hydrolysis of whey proteins was further enhanced during spray-drying, observed as a drastic increase in reactive NH_2 of reconstituted MWPC-TH, which agreed with the MW profiles of proteins evaluated by SDS-PAGE (Fig. 1). Free SH groups were also drastically decreased after spray-drying of MWPC-TH mixture (Table 1). However, the antioxidant capacity of MWPC-TH, measured as TEAC, was drastically increased after modification by acidifying, pre-heating, peptic treatment and spray-drying, which was in good agreement with the

Table 1 Effect of spray-drying on reactive groups of whey protein products

Treatment	Exposed aromatic amino acid (Abs_{280})	Reactive amino groups (Abs_{340})	Free SH group content ($\mu\text{M SH/g protein}$)	TEAC ($\mu\text{mol TE/mg protein}$)
Commercial WPC	$0.510^b \pm 0.004$	$0.437^b \pm 0.066$	$12.79^a \pm 1.40$	$0.144^c \pm 0.004$
Commercial WPC-TH	$0.540^a \pm 0.007$	$0.467^b \pm 0.075$	$14.45^a \pm 1.55$	$0.264^b \pm 0.027$
MWPC-TH before spray-drying	$0.470^c \pm 0.013$	$0.472^b \pm 0.064$	$12.94^a \pm 1.49$	$0.234^b \pm 0.015$
MWPC-TH after spray-drying	$0.396^d \pm 0.014$	$0.754^a \pm 0.136$	$6.17^b \pm 0.17$	$0.464^a \pm 0.007$

Means $\pm$ s.d. followed by different superscripts are significantly different ($P < 0.05$)

antioxidant capacity (measured as oxygen radical absorbance capacity) previously reported (Rongsirikul and Hongprabhas 2011).

Antibrowning activity of peptically modified whey protein concentrate when used in thermally processed glycine–sugar mixed suspensions

The modification procedure used in the current study altered the NH_2 content, SH content and TEAC of reconstituted MWPC-TH, which could make MWPC prone to Maillard reactions. This was due to the increase in reactant NH_2 groups and a decrease in the potential reducing power of SH groups and inhibition of AGE formation by Cys (Vinson and Howard 1996; Zeng and Davies 2005; Alam et al. 2013). This method of modification thus offered opportunities to investigate the role of the antioxidant capacity of MWPC-TH, regardless of the role of Cys, in understanding the antibrowning activity of proteins and peptides.

The reaction rates of AGEs and brown pigment formation were accelerated when the temperature was increased from 85 to 95 °C (Fig. 2). In the absence of N-containing compound

(glycine in this case), brown pigment formation still occurred due to caramelization (Ajandouz et al. 2001), observed as low fluorescence intensity (Fig. 2a) and high absorbance values at 420 nm (Fig. 2f) as heating temperature increased. In heated mixed 0.1 M fructose+0.5 % glycine solution, the formation of AGEs (Fig. 2b) and brown pigment (Fig. 2g) increased when the mixtures were heated at 80 and 85 °C for 60 min. However, at higher heating temperature of 90 and 95 °C, AGEs increased and their contents dropped after 30 min of heating. This coincided with a drastic increase of brown pigment formation at high temperature (Fig. 2g). These results suggested that prolonging heating time at high temperature favored full Maillard reactions, observed as the formation of brown-colored N-containing melanoidins in addition to brown pigment formed by caramelization.

Both AGEs and brown pigment formed to a lesser extent and at a slower rate when MWPC-TH was present in the heated mixed solutions. The low content of AGEs in the presence of MWPC-TH may result from the inhibition of AGE formation or the progress of AGEs to brown pigment formation via a cascade of redox reactions. Figure 3f, however, indicates that the heated fructose–MWPC-TH exhibited the

Fig. 2 Effect of heating temperature and time on the formation of AGEs, measured as % fluorescence intensity (a–e), and the formation of brown pigment, measured as absorbance at 420 nm (f–j). a, f: heated 0.1 M fructose; b, g: heated 0.1 M fructose+0.5 % glycine; c, h: heated 0.1 M

fructose+0.5 % MWPC-TH; d, i: heated 0.1 M fructose+0.25 % MWPC-TH+0.25 % glycine; and e, j: heated 0.1 M fructose+0.5 % MWPC-TH+0.5 % glycine. Bars represent standard deviations (s.d.)

Fig. 3 Effect of modified whey protein concentrate in trehalose excipient (MWPC-TH) addition on brown pigment formation of glycine/protein WPC mixtures containing different types and concentrations of reducing

sugar, presented as **a** Arrhenius plot and **b** calculated activation energy (E_a). Bars represent standard deviation (s.d.)

least brown pigment formation and the least accumulation of AGEs. Results suggested that MWPC-TH likely retarded full Maillard browning reactions of fructose–glycine mixtures by slowing down AGE formation.

The mixed 0.1 M fructose+0.25 % MWPC+0.25 % glycine solution, however, showed a drop in AGE formation but an increase in brown pigment formation when it was heated at 95 °C (Fig. 2d). At lower heating temperature, between 80 and 90 °C, AGEs accumulated. The formation of brown pigment in the heated mixed suspension was most likely due to the caramelization of fructose. A similar trend was observed at a higher glycine concentration of 0.5 % (Fig. 2e). The presence of MWPC-TH, however, had no effect in inhibiting the formation of caramelized brown pigments.

The Arrhenius plot and E_a illustrated in Fig. 3 show that full Maillard reactions to brown pigment formation occurred the most in heated 0.1 M fructose+0.5 % glycine solution due to its having the lowest E_a . However, the heated fructose+0.5 % MWPC had the highest E_a , which corroborated the

results on the lowest formation of AGEs (Fig. 2c) and the lowest brown pigment formation (Fig. 2h). This was likely due to the inhibition of AGE formation prior to the final stages of melanoidin formation.

The role of MWPC-TH in inhibiting brown pigment formation in sterilized mixed sugar–glycine and mixed sugar–WPC are illustrated in Table 2. In the absence of commercial WPC and MWPC-TH, brown pigment formation was maximized in sterilized sugar–glycine solution. Unlike commercial WPC, which had higher SH content, lower NH₂ group content and lower TEAC, MWPC prepared in the current study could retard brown pigment formation in sterilized sugar–glycine solution by delaying the formation of fluoresced AGEs due to the antioxidant capacity of MWPC-TH rather than the actions of SH groups per se (Friedman 1996; Zeng and Davies 2005). Although an increase in NH₂ groups after the modification process could increase the susceptibility of whey proteins in MWPC-TH to glycation compared with commercial WPC (by providing more reactive NH₂ groups for Maillard

Table 2 Influences of NH₂ group source on brown pigment formation in sterilized (121 °C, 15 min) glycine/protein protein mixtures containing different types of reducing sugar

Sources of NH ₂ group (% w/v)			Browning pigment formation (measured as Abs ₄₂₀)		
Glycine	Commercial WPC	MWPC-TH	Types of reducing sugar		
			0.1M fructose	0.1M glucose	0.1M lactose
0.00	0.00	0.00	0.185 ^e ±0.009	0.172 ^{de} ±0.001	0.095 ^d ±0.023
0.50	0.00	0.00	0.448 ^a ±0.027	0.384 ^a ±0.005	0.295 ^a ±0.030
0.00	0.50	0.00	0.215 ^{de} ±0.023	0.171 ^{de} ±0.005	0.122 ^d ±0.002
0.00	0.00	0.50	0.248 ^{cd} ±0.011	0.189 ^{de} ±0.002	0.137 ^d ±0.011
0.25	0.00	0.25	0.286 ^c ±0.000	0.225 ^c ±0.010	0.181 ^c ±0.017
0.00	0.25	0.25	0.213 ^{de} ±0.004	0.160 ^e ±0.000	0.107 ^d ±0.007
0.50	0.00	0.50	0.364 ^b ±0.002	0.307 ^b ±0.004	0.234 ^b ±0.010
0.00	0.50	0.50	0.171 ^e ±0.045	0.131 ^f ±0.025	0.120 ^d ±0.031

Means±s.d. in the same column followed by different superscripts are significantly different ($P<0.05$)

reactions to occur), the MWPC-TH was effectively able to inhibit brown pigment formation under the sterilization scheme used in the present study.

Conclusions

Modification of WPC by pre-heating the acidified proteins, treating with pepsin, and spray-drying in the presence of TH excipient was able to increase the antioxidant and antibrowning capacities of MWPC-TH. With respect to this modification and spray-drying in the presence of carbohydrate excipient, β -Lg was the protein that was retained the most. Further hydrolysis of whey proteins generated small MW peptides of less than 6.5 kDa decreased free SH groups and aromatic groups. It was shown, for the first time, that α -La in WPC could be selectively modified by method described in this study. The reconstituted MWPC-TH could also serve as an antioxidative and antibrowning agent for whey protein products when used in sterilized amino acid/protein suspensions containing fructose, glucose or lactose.

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