Food Chemistry 116 (2009) 122–128

Contents lists available at ScienceDirect

Food Chemistry

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

Antioxidant properties of Maillard reaction products obtained by gamma-irradiation of whey proteins

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ARTICLE INFO

Article history:
Received 8 September 2008
Received in revised form 12 January 2009
Accepted 12 January 2009

Keywords:
Maillard reaction
Gamma-irradiation
Antioxidant
Free radicals
Whey protein

ABSTRACT

The radiation processing of sugar–amino acid solutions results in formation of Maillard reaction products (MRPs). In the present study, the efficacy of gamma-irradiation in the formation of MRPs from whey protein powder was investigated. The formation of MRPs in whey protein suspension was studied by monitoring spectrophotometeric and chemical changes. A dose-dependent increase in UV absorbance and development of fluorescence was observed. Formation of brown pigments was established by increased $A_{420\ nm}$ and Hunter colour upon irradiation. These MRPs exhibited antioxidant activity as measured by 1,1-diphenyl-2-picrylhydrazyl and β-carotene bleaching assays. Reducing power and iron-chelating abilities of MRPs also increased upon irradiation. These MRPs were able to scavenge hydroxyl and superoxide anion radicals under *in vitro* conditions. Dose-dependent decrease in free amino groups and lactose suggested the formation of glycated proteins upon irradiation treatment. SDS–PAGE analyses indicated formation of crosslinked proteins upon irradiation.

1. Introduction

The Maillard reaction or non-enzymatic browning corresponds to a set of reactions resulting from the initial condensation between an available amino group and a carbonyl-containing moiety, usually a reducing sugar. This reaction is known to be responsible for the attractive flavour and brown colour of some cooked foods (Jing & Kitts, 2002). It is one of the major reactions taking place during thermal processing, cooking, and storage of foods. A myriad of products are formed, which have direct impact on nutritional and sensory qualities of foods. The Maillard reaction products (MRPs) formed in an amino acid–sugar model system have been known to be associated with the formation of compounds with pronounced antioxidant activity. The development of antioxidant molecules is one of the desirable effects of the Maillard reaction. The antioxidative properties of MRPs produced by heat treatment of amino acid–sugar have been studied in model systems (Jayathilakan & Sharma, 2006).

The majority of studies have been carried out on the sugar–amino acid and sugar–protein model systems. Radiation processing enhances shelf-life and/or improves the microbiological safety of raw and processed food materials without compromising nutritional quality (WHO, 1999). The majority of chemical changes caused due to radiation processing of food are similar to those of other preservation methods (Diehl, 1995). The chemical changes taking place during irradiation are the result of the direct effect of radiation on the food components or by indirect action, through reactive intermediates formed by radiolysis of water (Diehl, 1995). The majority of these chemical changes are similar to those produced by heat treatment. However, information on the formation of MRPs by radiation processing is scanty. Non-enzymatic browning in gamma-irradiated aqueous solutions of different sugars with lysine has been reported (Oh et al., 2006). Formation of antioxidant compounds in sugar–amino acid solutions upon irradiation has also been reported recently from this laboratory (Chawla, Chander, & Sharma, 2007).

Whey is an abundant by-product of the dairy industry. It is the portion of milk left after the manufacture of cheese. It consists of about 96% water but also contains valuable proteins and lactose. The disposal of whey is difficult due to its high biological oxygen demand. In developing countries whey is dumped into streams thereby polluting them. The objective of the present study was to investigate radiation-induced changes in whey protein dispersion in terms of formation of MRPs and to examine their antioxidant activity.

2. Materials and methods

2.1. Chemicals

β-Carotene, 2,2,-diphenyl-1-picryl hydrazyl (DPPH), thiobarbituric acid (TBA), nitroblue tetrazolium (NBT) and linoleic acid were

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doi:10.1016/j.foodchem.2009.01.097

purchased from Sigma Chemical Co. (St. Louis, MO). All other chemicals used were of analytical grade and procured from HiMedia Laboratories (Mumbai, India) or Sisco Research Laboratories (Mumbai, India).

2.2. Preparation of radiation induced MRPs

Whey protein concentrate (WPC) solution was prepared in distilled water to give a final concentration of 1%. The WPC solutions were subjected to different doses of gamma-irradiation (0–100 kGy) in a Gamma-Cell 5000 (BRIT, Mumbai) at a dose rate of 9.87 kGy per hour. Dosimetry was performed by ceric-cerous dosimeter calibrated against Fricke's dosimeter. Dosimetry intercomparison was carried out with National Standards established by the Radiological Physics and Advisory Division (RP&AD), Bhabha Atomic Research Centre (BARC), Mumbai, India.

2.3. Spectrophotometric analyses

The radiation-treated whey protein dispersions were appropriately diluted and absorbance at 284 nm (early Maillard reaction products) and 420 nm (late Maillard reaction products) were measured. Fluorescence of samples was determined after 100-fold dilution. The fluorescence intensity was measured at an excitation wavelength of 365 nm and emission wavelength of 440 nm using a fluorescence spectrophotometer (Chawla et al., 2007).

2.4. Measurement of colour

The evaluation of colour of the gamma-irradiated whey protein dispersion was carried out using a colorimeter (MiniScan MS/S-4000S; Associates Laboratory Inc., Reston, VA) according to the CIE Lab scale. The system provides the values of three colour components: L^* (black–white component, luminosity), and the chromaticity coordinates, a^* (+ red to − green component) and b^* (+ yellow to − blue component) (Hunter, 1942). Samples were placed into a cell. The samples were illuminated with D65-Artificial Daylight (10° standard angle), according to conditions provided by the manufacturer. The E index is calculated from the equation:

$$E = (L^{*2} + a^{*2} + b^{*2})^{1/2}$$

and chroma value according to the equation:

$$C = (a^{*2} + b^{*2})^{1/2}$$

Each colour value reported was the mean of four determinations at 25 ± 3 °C.

2.5. Determination of reducing power

Reducing power was determined by the ferricyanide method of Yen and Duh (1993). Appropriately diluted sample (1 ml) was added to 2.5 ml of phosphate buffer (200 mM, pH 6.6) followed by 2.5 ml of 1% potassium ferricyanide. The reaction mixture was incubated for 20 min in a water bath at 50 °C. After incubation, 2.5 ml of 10% trichloroacetic acid (TCA) was added, followed by centrifugation at 3000 rpm for 10 min. The upper layer (5 ml) was mixed with 5 ml distilled water and 1 ml of 0.1% ferric chloride. Absorbance of the resultant solution was measured at 700 nm. A high absorbance was indicative of strong reducing power.

2.6. Determination of DPPH radical-scavenging activity

Electron-donating ability of radiation-induced MRPs was determined by employing DPPH radical-scavenging assay (Blois, 1958). To a 1 ml aliquot of appropriately diluted solution, 1 ml of ethanolic DPPH solution (0.2 mM) was added. The mixture was vortexed and left to stand at ambient temperature for 30 min. A reaction mixture containing 1 ml distilled water and 1 ml of ethanolic DPPH solution (0.2 mM) served as the control. The absorbance of the solution was measured spectrophotometrically at 517 nm. The percentage of DPPH scavenging was calculated from the equation:

$$\text{Radical scavenging activity } (\%) = 100 \times (A_{\text{control}} - A_{\text{sample}})/(A_{\text{control}})$$

where A_{control} is the absorbance of the control and A_{sample} the absorbance of the sample.

2.7. Determination of antioxidant activity by β-Carotene bleaching assay

Antioxidant activity of the aqueous solution was determined by a β-carotene/linoleic acid system, as described by Matthaus (2002). Briefly, 1 ml of β-carotene solution (1 mg/ml in chloroform), 40 μl of linoleic acid, and 400 μl of Tween 80 were transferred into a round-bottomed flask. Chloroform from the sample was evaporated using a stream of nitrogen. Then 100 ml of distilled water was added slowly to the residue and vigorously agitated to give a stable emulsion. To an aliquot of 4.5 ml of this emulsion, 500 μl of appropriately diluted samples were added. To the control reaction mixtures, 500 μl of distilled water were added. Absorbance was measured immediately at 470 nm. The tubes were placed in a water bath at 50 °C and the absorbance was measured after 120 min. Antioxidant activity index (AAI) was calculated as

$$AAI = 100 \times \frac{A_{s(0)} - A_{s(120)}}{A_{b(0)} - A_{b(120)}}$$

where $A_{s(0)}$ is absorbance of the sample at 0 min, $A_{s(120)}$ is the absorbance of the sample at 120 min, $A_{b(0)}$ is the absorbance of the control at 0 min, $A_{b(120)}$ is the absorbance of the control at 120 min.

2.8. Determination of hydroxyl radical-scavenging activity

Hydroxyl radical-scavenging activity of radiation-induced MRPs was determined according to the modified method of Halliwell, Gutteridge and Aruoma (1987). To 1 ml of the appropriately diluted sample, 1 ml phosphate buffer (0.1 M pH 7.4) containing 1 mM ferric chloride, 1 mM EDTA, 1 mM ascorbic acid, 30 mM deoxyribose, and 20 mM hydrogen peroxide were added. After incubation at 37 °C for 90 min, 2 ml of 2% (w/v) TCA and 2 ml of 1% (w/v) TBA was added. The reaction mixture was heated in a boiling water bath for 15 min. The absorbance of the pink colour that developed was measured at 532 nm using a spectrophotometer. The percentage of hydroxyl radical-scavenging activity was calculated as:

$$\%\ \text{inhibition} = 100 \times [(A_{\text{Control}} - A_{\text{Sample}})/A_{\text{Control}}]$$

where A_{Control} is the absorbance of the control and A_{Sample} the absorbance of the sample.

2.9. Measurement of superoxide anion scavenging activity

Superoxide anion scavenging activities of radiation induced MRPs were determined according to the method of Liu, Ooi, and Chang (1997) with some modifications. The reaction mixture consisted of 1 ml of NBT (156 μM in 0.1 M potassium phosphate buffer pH 7.4), 1.0 ml of nicotinamide adenine dinucleotide reduced (NADH 468 μM in 0.1 M potassium phosphate buffer pH 7.4) and

0.5 ml of appropriately diluted sample. The reaction was initiated by addition of 100 μl of phenazine methosulphate (PMS 60 μM in 0.1 M potassium phosphate buffer pH 7.4) to the mixture. The tubes were incubated at ambient temperature for 5 min. and the absorbance was measured at 560 nm. Decreased absorbance of the reaction mixture indicated increased superoxide anion scavenging activity. The percentage inhibition of superoxide anion generation was calculated using the following formula:

$$\% \text{ inhibition} = 100 \times \frac{(A_0 - A_s)}{A_0}$$

where A_0 is the absorbance of the control and A_s is the absorbance of the sample.

2.10. Determination of iron chelation activity

The ferrous ion chelation potentials of radiation-induced MRPs were investigated by estimating the ferrous iron–ferrozine complex at 562 nm (Decker & Welch, 1990). Briefly, the reaction mixture consisted of 1.0 ml of appropriately diluted sample, 3.7 ml distilled water, 0.1 ml ferrous chloride (2 mM) and 0.2 ml ferrozine (5 mM). The reaction mixture containing 1 ml of distilled water instead of sample served as control. Tubes were incubated at ambient temperature for 20 min. The absorbance of the colour developed was measured at 562 nm. A high ferrous chelation ability of sample results in low absorbance at 562 nm. The ability of sample to chelate ferrous ions was calculated using the following equation:

$$\text{Chelation activity } (\%) = 100 \times \frac{(A_0 - A_S)}{A_0}$$

where A_o is absorbance of the control and A_s is the absorbance of the sample.

2.11. Determination of free amino group content

Free amino group content was determined according to the method of Benjakul, Lertittikul, and Bauer (2005). Appropriate volume of sample was mixed with 2 ml of 200 mM phosphate buffer (pH 8.2) and 1 ml of 0.01% TNBS solution was added. The reaction mixture was vortexed and incubated in water bath at 50 °C for 30 min in the dark. The reaction was stopped by adding 2 ml of 100 mM sodium sulfite solution. The tubes were allowed to cool at room temperature. The absorbance was measured at 420 nm.

2.12. SDS–polyacrylamide gel electrophoresis (SDS–PAGE)

SDS–PAGE was performed using 4% stacking gel and 10% running gel, according to the method of Laemmli (1970), with a vertical gel electrophoresis unit (Mini-Kin, Techno Source, Mumbai, India). Protein (25 μg) was applied to the gel. The electrophoresis was carried out at 20 mA. After separation, protein bands were stained using Coomassie Brilliant Blue R-250 (0.2%) in 25% methanol and 10% acetic acid. Destaining was performed using 40% methanol and 10% acetic acid.

2.13. Fractionation of MRPs

The whey protein dispersion was subjected to fractionation using ethanol (Jing & Kitts, 2004). The samples were mixed with absolute ethanol (9:1 v/v) and allowed to stand for 2 h at 4 °C. The MRP ethanol suspension was centrifuged at 5000*g* for 10 mins. The resulting supernatant which contained low molecular weight MRPs was decanted and separated from the precipitate. Both ethanol-soluble supernatant and precipitate were analysed for DPPH radical-scavenging activity.

2.14. Statistical Analysis

All results given in the figures are mean ± standard deviation. Differences between the variables were tested for significance by one-way ANOVA with Tukey's post test using GraphPad InStat version 3.05 for Windows 95, GraphPad Software, San Diego, CA. Differences at $p < 0.05$ were considered to be significant.

3. Results

In the present study an aqueous suspension of whey protein concentrate was used as a representative of a natural food protein/sugar mixture, to investigate the effect of radiation processing. Effect of gamma-irradiation on spectrophotometric analysis of whey protein dispersion is shown in Fig. 1. A sharp increase in UV absorbance at 284 nm of samples was observed with irradiation dose (r^2 = 0.99) (Fig. 1A). The UV absorption of samples irradiated at 100 kGy was corrected for dilution factor and the value was found to be 69.3, suggesting formation of UV-absorbing intermediate products upon irradiation. The Maillard reaction is associated with development of UV-absorbing intermediate compounds, prior to generation of brown pigments. This increased UV absorbance is attributed to decomposition of sugars by dehydration and sugar fragmenation (Hodge, 1953). Dose-dependent formation of UV-absorbing compounds upon irradiation of sugar/amino acid solutions has been reported previously (Chawla et al., 2007; Oh et al., 2006). UV-absorbing intermediate compounds are formed prior to heat-induced MRPs (Ajandouz, Tchiakpe, Ore, Benajiba, & Puigserver, 2001).

It can be seen that the browning intensity, as measured by $A_{420\ 'nm}$ for whey protein dispersion increased (r^2 = 0.99) with the radiation dose (Fig. 1A). Dose-dependent increase in browning in sugar/amino acid solution has been reported previously (Chawla et al., 2007; Oh et al., 2006). These findings are in concurrence with other studies where browning of protein/sugar solutions due to heat-induced Maillard reaction is reported (Benjakul et al., 2005; Jing & Kitts 2002). Development of brown colour, due to the formation of chromophores, has been widely studied in different model systems, and studies on melanoidin formation have been summarised (Rizzi, 1997).

The extent of browning due to Maillard reaction is regularly measured as a single wavelength absorbance. Wavelengths between 360 and 470 nm are frequently used for the purpose. However, it may not be a reliable way to describe visual colour changes, in terms of the visual properties of the coloured compounds. We attempted to correlate visual colour with browning, by applying the colour parameters provided by a tristimulus colourimeter. The CIE Lab system established a system of numerical coordinates to locate individual colour in uniform visual colour spacing. The results of the evaluation of colour of the gamma-irradiated whey protein dispersion are shown in Table 1. It can be seen that there was no significant change in a^* value whereas b^* value increased with irradiation dose. These findings suggest a net increase in yellow–brown colour as a result of radiation treatment of whey protein dispersion. The colour index (E), which is influenced by L^*, a^* and b^* values. It was observed that L^* values decreased upon irradiation due to loss of lightness whereas b^* values increased upon irradiation, due to formation of yellow–brown colour. The E index could describe how far apart two colours are in the colour space. It was seen that there was a slow increase in E index upon irradiation, as a net effect of reduced lightness and increased yellow–brown colour. The parameter C^* indicates the degree of saturation,

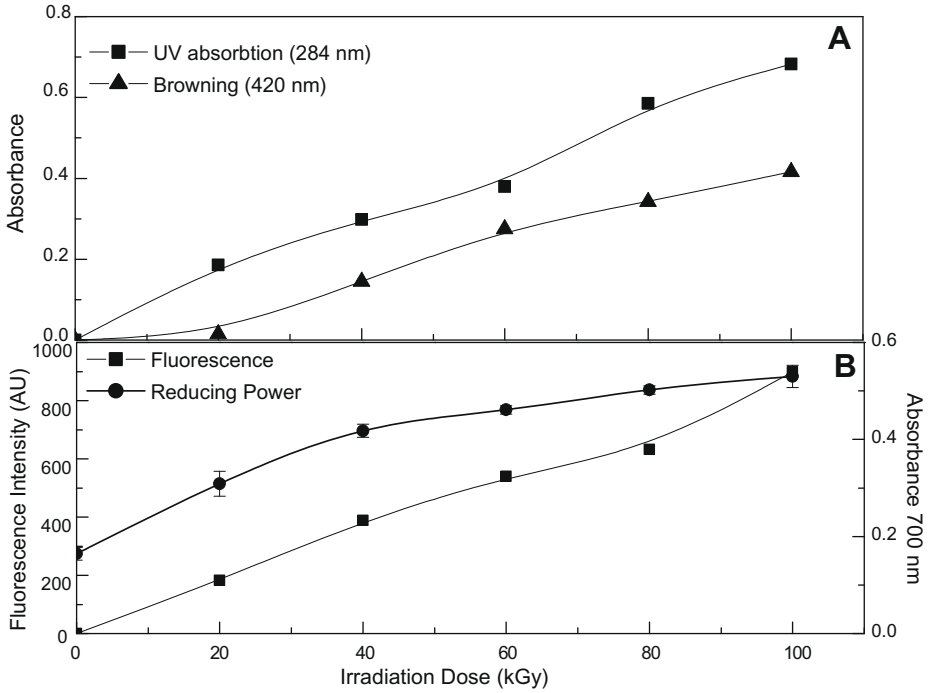

Fig. 1. Effect of gamma-irradiation on (A) browning (A_{420}) and UV absorbance (A_{284}) and (B) fluorescence and reducing power of whey protein dispersion.

Table 1
Effect of gamma-irradiation on Hunter colour values of whey protein dispersion.

Irradiation dose	L^*	a^*	b^*	c	E
Control	86.7	1.25	9.11	9.2	87.4
20 kGy	85.6	0.09	12.7	12.7	87.0
40 kGy	84.3	−0.88	22.1	22.1	87.1
60 kGy	82.3	−0.97	30.1	30.1	87.6
80 kGy	81.4	−0.98	35.9	35.9	88.9
100 kGy	80.9	−0.98	37.6	37.6	89.2

L^*(black–white component, luminosity), chromaticity coordinates,
a^*(+ red to − green component),
b^*(+ yellow to − blue component),
$C = (a^{*2} + b^{*2})^{1/2}$,
$E = (L^{*2} + a^{*2} + b^{*2})^{1/2}$.
Each colour value reported was the mean of four determinations at 25 ± 3 °C.

purity or intensity of visual colour and is defined as the degree of departure from grey (a^* and $b^* = 0$) towards pure chromatic colour. The increased values of C^* signify that the irradiated model system shows more red or yellow characteristics and no saturation was observed at the doses investigated in the present study. In the heat-induced Maillard reaction of a sugar/amino acid model system it was reported that E index decreases significantly with heating time, whereas, the C^* value increases with heating time to a maximum after which the colour of the system becomes more complicated (Morales & Jimenez-Perez, 2001).

Development of fluorescent compounds has been reported to be associated with heat-induced Maillard reaction (Jing & Kitts, 2000, 2002). In the present study dose-dependent formation of fluorescent compounds was observed in irradiated whey protein dispersion (Fig. 1B). These fluorescent compounds are known to be precursors of brown pigments formed during the Maillard reaction. Similar results in heat-induced the Maillard reaction in a model system consisting of bovine serum albumin and sugar have been reported, where the fluorescence intensity of the system increased with time (Yeboah, Alli, & Yaylayan, 1999). However, in a number of other studies it has been reported that fluorescence intensity reached a maximum during heat treatment and further heating resulted in a gradual decrease in intensity (Benjakul et al., 2005; Jing & Kitts, 2002).

Iron reducing power of the whey protein dispersion before and after subjecting to gamma-irradiation is shown in Fig. 1B. It was seen that non-irradiated whey protein dispersion had negligible reducing power, which increased significantly ($p < 0.05$) upon irradiation treatment. It has been reported that compounds responsible for reducing activity are formed during thermolysis of Amadori products in the primary phase of Maillard reactions (Hwang, Shue, & Chang, 2001) or could be heterocyclic products of Maillard reaction or caramelisation of sugars (Charurin, Ames, & Castiello, 2002). Possibly, gamma-irradiation induces similar changes in whey protein dispersion, resulting in the formation of products which contribute towards the reducing power. Heat-induced MRPs from xylose/lysine (Yen & Hsieh, 1995), glucose/glycine (Yoshimura, Ujima, Watanabe, & Nakazawa, 1997) and porcine plasma protein/glucose models (Benjakul et al., 2005; Lertittikul, Benjakul, & Tanaka, 2007) possessed reducing power.

We estimated the effect of radiation dose on free amino groups in whey protein dispersion. In the present study a dose-dependent reduction in free amino groups was observed in irradiated whey protein dispersion (Fig. 2A). The decrease in free amino groups was coincidental with increase in the browning intensity (Fig. 1A). A reduction of free amino groups, which are major reactants of the Maillard reaction is also reported in other studies in sugar/amino acid systems (Sun, Hayakawa, & Izumori, 2004).

The changes in sugar content of whey protein dispersion, as a function of irradiation dose, are also shown in Fig. 2A. In the present study a significant dose-dependent decrease in the reducing sugar content was observed in irradiated whey protein dispersion. Reduction in reducing sugar content during heat-induced Maillard reaction in fructose/lysine (Ajandouz et al., 2001) and porcine protein/glucose (Lertittikul et al., 2007) model systems has been reported.

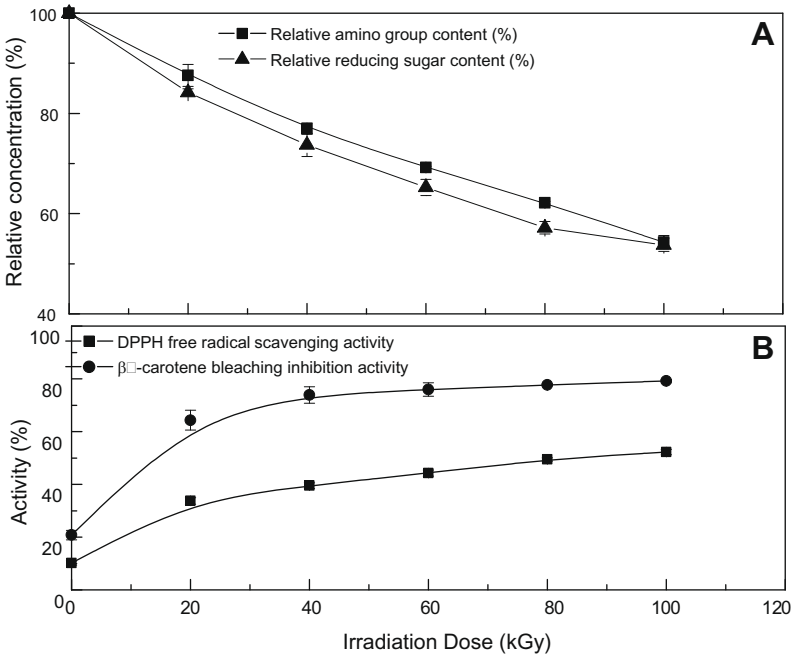

Fig. 2. (A) Changes in free amino groups and reducing sugars and (B) DPPH radical-scavenging activity and β-carotene bleaching inhibition activity in whey protein dispersion, as a function of irradiation dose.

These results indicated the involvement of amino group and reducing sugar in formation of MRPs during irradiation treatment, as substantiated by the lower free amino groups and reducing sugars remaining upon irradiation treatment.

To evaluate the free radical-scavenging, whey protein dispersions subjected to different doses of gamma-irradiation were allowed to react with stable DPPH free radical. The scavenging of DPPH free radical, indicating a positive antiradical activity, was followed by monitoring reduction in the absorbance at 517 nm. DPPH free radical-scavenging activity increased with the irradiation dose in whey protein dispersion ($r^2 = 0.90$). In whey protein dispersion irradiated at 40 kGy it was observed to be 33.8% (Fig. 2B). Upon further irradiation to 80 kGy, the increase was not linear and tended to plateau at a higher irradiation dose. Capability of heat-induced MRPs to scavenge DPPH radical have been reported in a number of studies (Benjakul et al., 2005; Jing & Kitts, 2002; Lertittikul et al., 2007; Morales & Jimenez-Perez, 2001). In our previous studies, on radiation-induced Maillard reaction in sugar/amino acid model system, similar findings were observed, where the radical-scavenging activity saturated with an irradiation dose of 40 kGy (Chawla et al., 2007). These finding are also in agreement with previous studies on heat-induced MRPs, where radical-scavenging activities tend to saturate after a certain heating time (Jing & Kitts, 2002; Lertittikul et al., 2007; Morales & Jimenez-Perez, 2001).

It was observed that β-carotene bleaching was significantly ($p < 0.05$) inhibited in the presence of irradiated whey protein dispersion, compared to that offered by its non-irradiated counterpart (Fig. 2B). These findings indicated that irradiation of whey protein dispersion resulted in formation of compounds having significant antioxidant potential. These findings are similar to our previous results on radiation induced Maillard reaction in sugar/amino acid model system (Chawla et al., 2007). As in the case of DPPH radical-scavenging, inhibition of β-carotene bleaching tended to maximize at a higher irradiation dose. Synthesis of antioxidative compounds upon heat treatment of sugar/amino acid solutions is reported in a number of studies (Jayathilakan & Sharma, 2006; Jing & Kitts, 2002; Morales & Jimenez-Perez, 2001; Yoshimura et al., 1997).

The hydroxyl radical is the most reactive of species and induces most severe damage to adjacent biomolecules, resulting in lipid peroxidation in biological systems. In the present study the Fenton reaction system was used in deoxyribose degradation by generating hydroxyl radicals. The treatment of deoxyribose with Fenton reaction reagent resulted in a high rate of deoxyribose degradation. Hydroxyl radical-scavenging activity of irradiated whey protein dispersion was significantly higher than that of its non-irradiated counterpart (Fig. 3A). These findings revealed that compounds formed upon irradiation treatment of whey protein dispersion have the potential of being antioxidants in biological systems. Development of compounds capable of scavenging hydroxyl radicals and the utility of this test in studies to demonstrate *in vitro* hydroxyl radical-scavenging activity of heat-induced MRPs has been reported (Jing & Kitts, 2000, 2002).

The superoxide radicals are generated by a number of biological reactions. Although they do not directly initiate lipid oxidation, superoxide radical anions are precursors of highly reactive hydroxyl radical, which contributes towards lipid peroxidation in biological systems. Thus superoxide anion scavenging activity indirectly contributes towards antioxidant potential. Irradiated whey protein dispersion showed dose-dependent superoxide anion radical-scavenging activity (Fig. 3A). Our results are in agreement with a previous study on heat induced MRPs formed from glucose/glycine that were reported to scavenge superoxide anion radical (Yoshimura et al., 1997). Formation of compounds that are capable of scavenging hydroxyl and superoxide anion radicals, as result of radiation-induced Maillard reaction in sugar/amino acid model systems, has been reported (Chawla et al., 2007).

In contrast to DPPH radical-scavenging and inhibition of β-carotene bleaching activity, scavenging of hydroxyl and superoxide anion radical did not stabilise at higher irradiation dose. These findings probably suggest involvement of different compounds in various radical-scavenging reactions.

Metal chelation activity plays an important role in antioxidant action as it results in reduction in the concentration of the transition metals required for lipid peroxidation. The Fe^{2+} ion is the most powerful pro-oxidant amongst various species of metal ions. In the

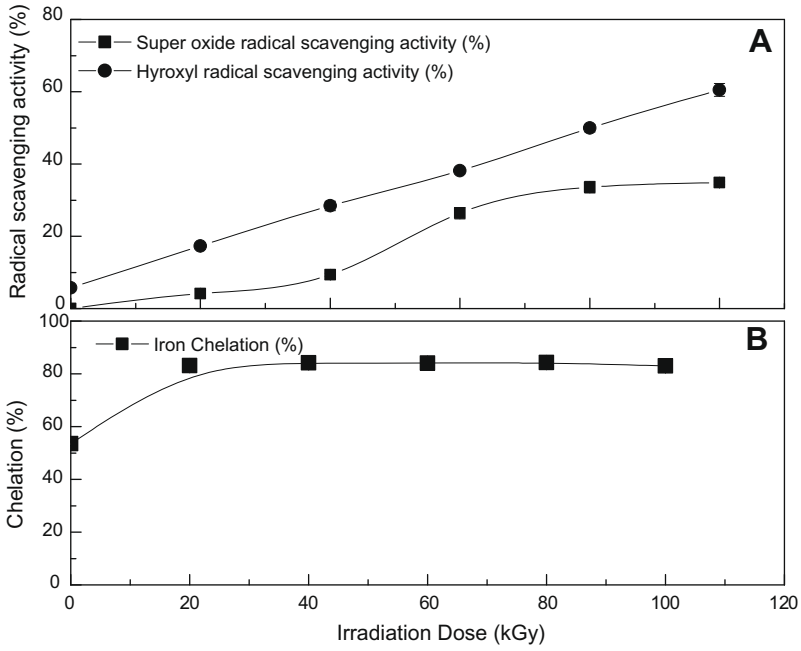

Fig. 3. Effect of gamma-irradiation on (A) hydroxyl and superoxide anion radical-scavenging activity and (B) iron chelation activity of whey protein dispersion.

present study we studied the effect of gamma-irradiation on ferrous ion chelating activities of whey protein dispersion (Fig. 3B). It can be seen that iron chelation activity of whey protein dispersion significantly ($p < 0.05$) increased upon irradiation at 20 kGy. However, further irradiation did not result in any significant increase in iron chelation activity. Formation of compounds that are able to chelate ferrous ions as a result of radiation-induced Maillard reaction in sugar/amino acid model systems, has been reported in our previous study (Chawla et al., 2007). Our findings are in concurrence with an earlier report where iron chelation activity was observed in glucose/glycine model system, as a result of MRPs formed due to heat treatment (Yoshimura et al., 1997).

The SDS–PAGE pattern of the whey protein dispersion subjected to different doses of gamma-irradiation is shown in Fig. 4. The non-irradiated whey protein dispersion consisted of a number of proteins with molecular weight in the range of 14–97 kDa. Low molecular weight proteins (≡14 and 20 kDa) were highly predominant (Fig. 4, lane 2). Upon irradiation there was a dose-dependent reduction in low molecular weight bands with coincidental formation of high molecular weight bands (Fig. 4, lanes 3–7). At irradiation doses of 60 kGy and above a high molecular weight band, which was unable to move in gel, was formed. These results suggested that there was a dose-dependent polymerisation of whey proteins upon irradiation. The formation of high molecular weight proteins during heat-induced Maillard reaction in β-lactoglobulin/chitosan (Miralles, Martinez-Rodriguez, Santiago, Lagemaat, & Heras, 2007) and porcine plasma protein/glucose model (Lertittikul et al., 2007) has been reported. The formation of cross-linked protein chains during the advanced and final stages of Maillard reaction, and the types of cross-link involved have been widely studied in different model systems (Oliver, Melton, & Stanley, 2006).

The results of DPPH-scavenging activity of ethanol-fractionated radiation-induced MRPs in whey protein dispersion as a function of irradiation dose are shown in Fig. 5. It can be seen that both ethanol-soluble supernatant and precipitate from non-irradiated whey protein dispersion did not yield any DPPH-scavenging activity. However, DPPH radical-scavenging activity was evident in both the fractions of irradiated samples. When comparison was made between the supernatant and precipitate from samples subjected to the same irradiation dose, it was observed that the radical-scavenging activity was significantly higher in the precipitate fraction. These findings suggested that the high molecular weight fraction contained more potent antioxidants than the low molecular weight fraction. These results are in agreement with a previous study on

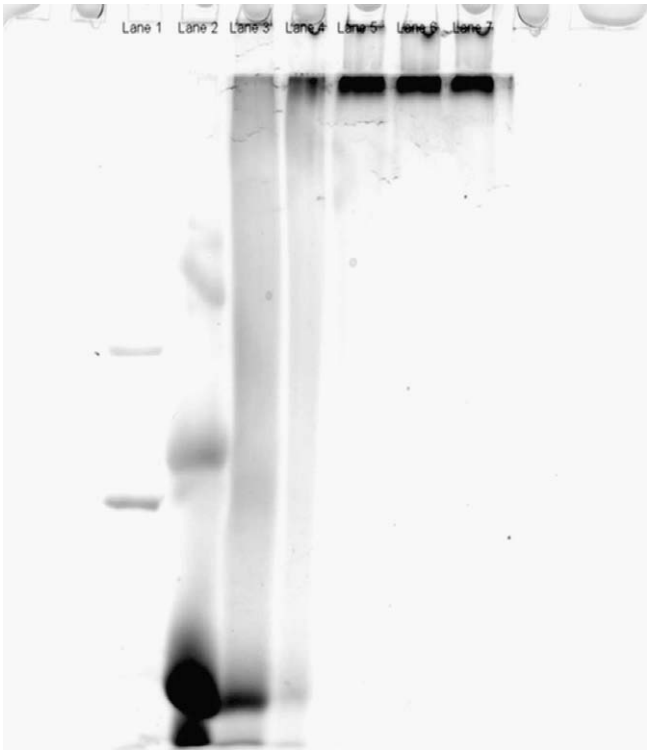

Fig. 4. SDS–PAGE protein pattern of gamma-irradiated whey protein dispersion. Lane 1 Molecular weight markers; lanes 2–7, whey protein dispersion subjected to 0, 20, 40, 60, 80 and 100 kGy, respectively.

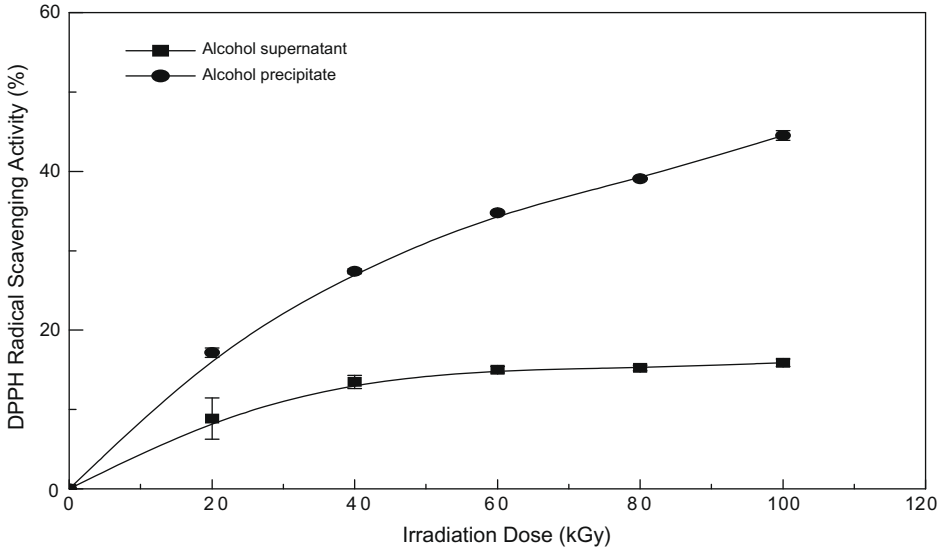

Fig. 5. DPPH radical-scavenging activity of gamma-irradiated whey protein dispersion subjected to ethanol fractionation.

heat-induced MRPs where both DPPH as well as hydroxyl radical-scavenging activity were greater in the high molecular weight fraction compared to the low molecular weight fraction (Jing & Kitts, 2004).

4. Conclusion

Ionizing radiation, such as gamma rays, can be successfully employed to produce MRPs in whey protein dispersion. The MRPs are probably produced because of a carbonyl-amine reaction, similar to heat-induced Maillard reaction. This study confirms our earlier report that compounds of antioxidant potential are formed upon irradiation of glucose/amino acid solutions (Chawla et al., 2007). Covalent bonding of proteins to reducing sugars has been shown to alter their functional properties. Maillard reaction is one of the technologically feasible methods to prepare conjugates (Oliver et al., 2006). The results of the present study demonstrated the formation of MRPs with antioxidant potential when whey protein dispersion was irradiated. The formation of protein conjugates is evident from the results. Further studies are needed to elucidate the mechanism and explore radiation as a tool to form protein conjugates with novel functional properties.

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