Antioxidant activity of the melanoidin fractions formed from D-Glucose and D-Fructose with L-Asparagine in the Maillard reaction

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Recibido 10 enero 2013; aceptado 26 marzo 2013

Abstract
Melanoidins formed at the last stage of the Maillard reaction have been shown to possess certain functional properties, such as antioxidant activity. In order to gain more insight into these functional properties, soluble model systems melanoidins from L-Asparagine with D-glucose or D-fructose fractionating by ultrafiltration were analyzed. The fractionating/concentration sequence of the melanoidin fraction (1-300 kDa) enabled five fractions to be produced. Additionally, the absorption of melanoidins was measured at different wavelengths (280, 325, 405) and browning at 420 nm. The fractionation effect of melanoidin systems on the color intensity, UV-absorbance scan wavelengths (nm), CIE, L*, a*, b* parameters and antioxidant activity were measured. For this purpose, antioxidant activity was evaluated through the free radical scavenging activity, including 1,1-diphenyl-2-picryl-hydrazil (DPPH) and 2,2'-azinobis (3-ethylbenothiazoline-6-sulfonic acid), diammonium salt (ABTS). The results showed that the absorption of the melanoidins formed from Glucose/L-Asn was higher than for those derived from Fructose/L-Asn. On the other hand, their antioxidant power was lower than that for melanoidins formed from Fructose/L-Asn systems.

Keywords: Maillard reaction, Melanoidins, Model solutions, Fractionation, Antioxidant activity.

1. Introduction
The reaction between reducing sugars and amino acids is known as the Maillard reaction or non-enzymatic browning. The Maillard reaction products (MRPs) form dark brown polymeric compounds named melanoidins. Melanoidins occur extensively in food and biological material (Dolphen and Thiravetyan, 2011) and have significant effects on the quality of food, since colors and flavors (Echavarría et al., 2011) are important food attributes and key factors in consumer acceptance. Food and drink with brown melanoidins may offer substantial health promoting effects (Silvanm et al., 2006). Melanoidins are found in widely consumed dietary components (e.g., coffee, cocoa, bread, malt and honey). In recent years, the study of the different functional properties of melanoidins has increased. The colored products of the Maillard reaction can be divided into two main groups: (a) the low molecular weight compounds (MW < 1000) and (b) the macromolecules, also known as melanoidins (Tehrani et al., 2002). However, the major part of colored Maillard products (MRPs) in food, the melanoidins, are of high molecular weight, up to 300 kDa, and have very complex molecular structures (Gniechwitz et al., 2008).

Until now, due to the complexity of the melanoidin polymers, it has not been fully possible to characterize them from foods, although numerous attempts have been made to isolate and purify melanoidins from food products such as coffee (Morales and Jiménez-Pérez, 2001; Bekedam et al., 2006) and honey.
(Brudzynski and Miotto, 2011a). In model systems, on the other hand, reducing sugars or Maillard intermediates were used as starting materials and defined colored reaction products could be isolated and identified (Hofmann et al., 2001; Rufián-Henares and Morales, 2007). Because the biological activity of most of the melanoidins takes place in a hydrophilic food matrix, the free radical scavenging capacity of melanoidins in an aqueous medium must be studied (Delgado-Andrade and Morales, 2005). In the case of the Maillard reaction, high antioxidant power was generally associated with the formation of brown melanoidins (Borrelli et al., 2002). Although the level of browning has been positively correlated with the levels of radical scavenging activity (Brudzynski and Miotto, 2011b), there is some controversy over the size of the reactive compounds involved (low-versus high-molecular weight melanoidin pigments), their chemical nature and the type of interaction with other macromolecules (Chandra et al., 2008). Little information is available on the chemical structure of the hundreds of brown products which are formed by a series of consecutive and parallel reactions, including oxidations, reductions and aldol condensations among others (Manzocco et al., 2000). Thus, different studies have also demonstrated that the antioxidant power of MRPs and melanoidins substantially contributes to the shelf-life of heat treated foods. Melanoidins exert a significantly lower antiradical activity than classic antioxidant compounds (tannic acid, ferulic acid, caffeic acid, gallic acid, and Trolox) in an aqueous medium. One of the most common approaches to describing the antioxidative properties of MRPs is a scavenging of certain radicals (e.g., DPPH radical cation) in a methanolic or chloroformic medium (Xu et al., 2007). Significant differences have been observed according to the type of amino acid used as reactant during the formation of the melanoidin structure and the antiradical efficiency exerted (Morales and Babbel 2002). It is supposed that its main mechanism act through the ability to trap positively-charged electrophilic metabolites, scavenge oxygen radicals, and chelate metal to form inactive complexes, or synergies (Morales, 2005).

The specific role of melanoidins in the overall antioxidant activity of the MRPs has not been addressed. Knowledge of the role of melanoidins in the prevention of lipid oxidation is limited, but they may act like other antioxidants at different levels in the oxidative sequence, similarly to polyphenols (Marti et al., 2009). The best way to investigate the Maillard reactions in heated foods is the use of model systems in which sugars and amino acids react under simplified conditions.

The aim of this research was to evaluate the antioxidant activity of melanoidins, obtained from D-glucose/L-asparagine and D-fructose/L-asparagine in model systems and the influence of their molecular weight on this activity. In accordance with their relative molecular weights, high (HMW) or low (LMW) fractions of the melanoidins were separated by ultrafiltration/concentration system. Their antioxidant properties were determined by different methodologies; the DPPH radical scavenging activity and ABTS were assessed. The UV-absorbance (scan at $A_{280\text{-}A_{405\text{nm}}}$), browning intensity at $A_{420\text{nm}}$, CIE, $L^*$, $a^*$, $b^*$ parameters and °Brix were also examined.

2. Materials and Methods

2.1 Reagents

D-Glucose, D-Fructose, L-Asparagine, sodium hydroxide (0.25N) potassium persulfate, phosphate buffered saline, L-Ascorbic acid were purchased from Panreac Química, S.A. (Barcelona, Spain). Active carbon from Probus, S.A. (Barcelona, Spain) Pyridine from Merck (Hohenbrunn, Germany). ($\pm$) 6-Hydroxy-2,5,7,8-tetramethylchroman-2-carboxylic acid
(Trolox), 2,2-Diphenyl-1-picrylhydrazyl hydrate (DPPH) were supplied by Sigma-Aldrich (St. Louis, MO, USA), 2, 2-azinobis (3-ethylbenzthiazoline)-6-sulfonic acid (ABTS) diammonium salt (Fluka Chemicals, Madrid, Spain). The solvents were purchased from Sharlau Chemie, S.A. (Barcelona, Spain). Deionized and distilled water were used throughout.

2.2 Equipment

2.2.1 The Ultra-filtration unit (UF)

The laboratory UF system (Figure 1) was used as described in a previous work (Echavarria et al., 2011).

Figure 1. Scheme of the membrane ultrafiltration system employed in experiments (1). Feed tank; (2). Peristaltic pump; (3). Damping tank; (4). Regulation valves; (5). Manometers; (6). Thermostatic Bath 50ºC; (7). Membrane module; (8). Permeate tank; (9). Digital balance.

Ceramic tubular membranes with diameter of 10 mm, 25 cm in length 300, 150, 50, 15, 8 and 1 kDa molecular weight cut-off (MWCO) (Tami Industries, Nyons, France) were used. In this way melanoidin extraction solutions could be divided into six parts of simple solutions in accordance with the relative molecular weights, HMW or LMW. This solution circulates tangentially to the surface of the membrane channels. The feed solution (3000 mL) in a constant-temperature recirculation bath (50º C) was introduced into the system from a feed tank. During circulation, the liquid was separated into two phases: one that passed through the membranes (permeate or filtrate) which was collected in a tank and measured in grams, with a digital scale and the other that did not pass through the membranes (retentate or melanoidin concentrate) which was circulated by the peristaltic pump (Prominent, Heidelberg, Germany).

2.3 Methods

2.3.1 Water-soluble melanoidins

The melanoidins were obtained from a sugary solution containing asparagine (Asn). For this, the glucose/Asn and fructose/Asn solutions as described by Ibarz et al. (2008) were prepared, dissolving 300 g of each sugar and 2 g of Asn in 1L of distilled water. This solution was treated in a sealed container for 13 days in an oven at 95º C. Five-cm diameter and 500 mL capacity glass columns, the bases of which contained a glass-fibre plate, were used to extract the melanoidins formed during the thermal treatment. A 10 g layer of active carbon was placed in this column. The solution that contained the melanoidins was diluted with distilled water and passed through the active carbon, where the melanoidins were adsorbed. Distilled water continued to be passed through the active carbon bed until the solution coming out of the column gave no positive reaction for reducing sugars (Waffenschmidt and Jaenicke, 1987). Once it had been checked that the active carbon in the column contained no sugars, the melanoidins were extracted by passing an aqueous solution of pyridine (Merck, Hohenbrunn, Germany) at 25% through the active carbon. This pyridine solution, together with the extracted melanoidins, may contain small particles of active carbon, so the solution was filtered in a vacuum through a 3 to 5-μm pore size filter. Once the solution was free of active carbon particles, the solvent was removed by evaporation in a Labo Rota C-311 rotavapor (Resona Technics, Gossau, Switzerland) that operated under a vacuum at a temperature of 45º C.
2.3.2 Fractionating and concentration of the melanoidin solution by ultra-filtration

Water-soluble melanoidins were isolated from the model system by ultra-filtration. The fractionating/concentration sequence for the samples made it possible to produce five fractions, to obtain the suitable operation conditions: ultrafiltration membrane, trans-membrane flow pressure (350 kPa), temperature (50ºC) were determined.

In accordance with the conditions obtained, a membrane module with a molecular weight of 300 kDa was first installed, and the melanoidin solution was isolated via the cycling pump. The permeate solution after the ultrafiltration isolation flowed from the output end of the outside of the membrane module to obtain the melanoidin solution with a relative molecular weight under 300 kDa. The retentate solution was returned to the flasks for repeated ultra-filtration cycles. The melanoidin solution with the relative molecular weight less than 300 kDa was treated via a MWCO 150 ultrafiltration membrane to obtain the melanoidin sample solution with a relative molecular weight of 300-150 kDa. For the same reason, the molecular weights of the 50, 15, 8 and 1 kDa ultrafiltration membrane modules were used in their respective orders to obtain sample pectin solutions with their relative molecular weights of 150-50 kDa, 50-15 kDa, 15-8 kDa, 8-1 kDa and below 1kDa. Thus, the melanoidin extraction solutions could be divided into 6 parts of sample solutions in accordance with their relative molecular weights. The retentate for each fraction was filled up to 200 mL with water and washed again to reduce the concentration of the contaminating low molecular weight melanoidins fraction.

The washing step (diafiltration) was repeated at least three times. Figure 2 illustrates the flowchart of the obtaining each of the different melanoidin fractions. Each fraction was then utilized for the in-vitro digestion procedure and for measuring and analyzing the browning intensity and antioxidant activity. To quantify the melanoidins extracted from filtration, in some experiments the retentate was lyophilized (Cryodos - 50 / 230V 50 Hz Telstar, Madrid, Spain) and the solid residue was weighed. The solid residue represented the extracted melanoidins and was expressed as g of melanoidins/100 g of dry matter.

![Figure 2. Sequence fractionating/concentration of melanoidins.](image-url)

2.4 Analytical determinations

After measuring the soluble solid content of each sample with an Atago RX-1000 digital refractometer, they were diluted to 12º Brix with bi-distilled water, according to Carabasa-Giribet and Ibarz-Ribas (2000). The pH of the melanoidin model was determined with a Horiba F-14 pH-meter, and the sample was adjusted to 7.5 with 1 mol/L NaOH.
2.4.1. Measurement of colour
The color of the melanoidin fractionations was measured directly using the CIE (Commission Internationale de l’Eclairage) values $L^*$ (lightness), $a^*$ (redness), $b^*$ (yellowness) and $\Delta E^*$ (colour difference, which was expressed as in Equation 1):

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

These values were determined with a light source C, with a color meter (Minolta ChromaMeter Model CR-400, Konica Minolta, Tokyo, Japan). The equipment was set up for illuminant D65 and 10° observer angle and calibrated using a standard white reflector plate.

2.4.2 UV-absorbance and browning intensity
The UV-absorbance of the melanoidin fraction was determined with absorbance spectrum detection between 200 nm to 420 nm, using a Helios gamma spectrophotometer (Thermo Fisher Scientific Inc., Waltham, MA, USA). Using a 1 cm-path width cell. The samples were dissolved in deionized water at a concentration of 0.1 mg/mL.

2.5 Antioxidant capacity
2.5.1 ABTS Radical Cation Decolorization Assay
The antioxidant capacity of fractionation melanoidins was estimated in terms of radical scavenging activity in water media, following the procedure described by Miller et al. (1996) with slight modifications. ABTS$^{•+}$ radical was produced by reacting 7 mM of ABTS (2,2-azinobis (3-ethylbenzthiazoline)-6-sulfonic acid (ABTS), stock solution with 2.45 mM of potassium persulphate. The reaction mixture was left to stand at room temperature overnight (12 to 16 h) in the dark before usage. The resulting intensely coloured ABTS$^{•+}$ radical cation was diluted with 5 mM of PBS (phosphate buffered saline), pH 7.4, to give an absorbance value of 0.70 (±0.02) at 734 nm using a spectrophotometer and equilibrated at 30º C. After the addition of 30 µL of the test compound (melanoidins) or Trolox (6-Hydroxy - 2,5,7,8 - tetramethylchroman - 2 - carboxylic acid) was diluted with 3 mL of ABTS$^{•+}$. These solutions were vortex-mixed for 45s and measured immediately after 5 min (absorbance did not then changes significantly up to 10 min). The assay was performed at least in triplicate. Aqueous solutions of Trolox at various concentrations were used to perform the calibration curves (0.15-1.15 mM).

2.5.2 Radical-scavenging activity using the DPPH method
DPPH is one of the chromogen-radical-containing compounds that can react directly with antioxidants (Ozcelik et al., 2003). The bleaching rate of a stable free radical, DPPH was monitored at a characteristic wavelength in the presence of the sample. This activity was measured following the procedure described by Molyneux (2004). In its radical form, DPPH$^{•+}$ absorbs at 517 nm. Briefly, the 0.12 mM solution of DPPH in methanol was prepared daily and protected from the light. An aliquot of 3 mL of this solution was added to 80 µL of melanoidin solution in water at 500 mg/mL concentration and 320 mL of distilled water. The solution was then mixed vigorously and allowed to stand at room temperature in the dark for 30 min. The antiradical activity of the sample was expressed as the percentage of disappearance of the initial purple color. Radical-scavenging activity was expressed as the inhibition percentage and was calculated using the following equation (Equation 2):

$$\% RSA = \frac{\text{Control Abs} - \text{Sample Abs}}{\text{Control Abs}} \times 100$$ (2)

Where Control and Sample Abs refers to the absorbance of control and sample, respectively.

2.6 Statistical analysis
Was performed using the one-way analysis of variance (ANOVA), where $p < 0.05$, and the means separated by Duncan’s
multiple-range test, were performed by applying the Statgraphics v.2.3 statistical package (Statistical Graphics Corp., Rockville, MD). All of the statistical procedures were performed at a significance level of 95%. All the analyses were carried out at least in duplicate.

3. Results and discussion
Continuous ultrafiltration is a faster process than the classical dialysis for fractionating and determining the preliminary melanoidin structure formed without additional aggregation or reorganization. The molecular weight distribution of the MRPs formed in the course of heating D-glucose and D-fructose in the presence of L-Asparagine was further researched by separating the water-soluble MRPs into five fractions via ultrafiltration, using membranes with cutoff of 300, 150, 50, 15, 8, and 1 kDa. Ultrafiltration of the Glu /L-Asn water-soluble MRPs showed nearly 53.68% of the water-soluble products of > 300 kDa, demonstrating that this fractionation technique is very suitable for quantitative measurements (Figure 3).

![Figure 3. Proportion of yield fraction of melanoidins with different molecular weights](image)

These data clearly indicate that the majority of the water-soluble compounds formed are of high molecular weight (HMW). MRPs with molecular weights between 8 and 150 kDa were found in comparatively low yields in the total reaction mixture. Comparing these data with those obtained by ultrafiltration of a Glu/L-Asn and Fru/L-Asn mixture, which was heated in an aqueous solution, revealed that the processing conditions significantly influenced the oligomerisation of low-molecular weight MRPs.

The use of ultrafiltration fractions allowed the radical scavenging activity to be assigned to high molecular weight, brown fractions, which absorbed at 420 nm as well as at 280, 325 and 405 nm.

The absorption values of melanoidins from Glucose/L-Asn and Fructose/L-Asn fractions at four wavelengths were recorded (Table 1). It appeared that the Gluc/L-Asn melanoidins showed the maximum absorption, at 280 nm and 325nm. These absorbances are characteristic of melanoidins (López-Galilea et al., 2008). They correspond to colourless browning intermediates produced during the initial induction period. Simple structure compounds produced during the early stages of the Maillard reaction are related to absorbance in the UV region and the analysis of the spectra of the browned fraction solutions produced allowed changes related to subsequent stages of the Maillard reaction to be detected. The first stages of the browning reaction in the systems used are characterized by the appearance of absorption around 280 nm (A280). This increased linearly with time, and can be related to heterocyclic derivatives (Bekedam et al., 2008). As the reaction proceeds, this absorbing material is progressively reduced due to the appearance of more complex compounds absorbing at 325 nm (A325). It is generally accepted that melanoidins contain conjugated systems which result in light absorption across the whole spectrum. On the basis of these data, it can be stated that measurement of the absorption at 280, 325, and 405 nm provides useful information on the relative amount of melanoidins fraction.
<table>
<thead>
<tr>
<th>Fraction</th>
<th>MW (kDa)</th>
<th>Yield (%)</th>
<th>$A_{420\text{nm}}$ (GA)</th>
<th>$A_{405\text{nm}}$ (FA)</th>
<th>$A_{325\text{nm}}$ (GA)</th>
<th>$A_{280\text{nm}}$ (FA)</th>
<th>Color GA</th>
<th>Color FA</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td>GA</td>
<td>FA</td>
<td>GA</td>
<td>FA</td>
<td>GA</td>
<td>FA</td>
</tr>
<tr>
<td>1</td>
<td>&gt;300</td>
<td>53.68</td>
<td>51.06</td>
<td>1.24</td>
<td>1.18</td>
<td>0.98</td>
<td>0.92</td>
<td>0.93</td>
</tr>
<tr>
<td>2</td>
<td>300-150</td>
<td>28.90</td>
<td>27.92</td>
<td>1.09</td>
<td>1.02</td>
<td>0.91</td>
<td>0.83</td>
<td>0.86</td>
</tr>
<tr>
<td>3</td>
<td>150-50</td>
<td>10.60</td>
<td>12.28</td>
<td>0.98</td>
<td>0.95</td>
<td>0.84</td>
<td>0.70</td>
<td>0.74</td>
</tr>
<tr>
<td>4</td>
<td>50-15</td>
<td>7.02</td>
<td>8.52</td>
<td>0.92</td>
<td>0.86</td>
<td>0.80</td>
<td>0.71</td>
<td>0.47</td>
</tr>
<tr>
<td>5</td>
<td>15-8</td>
<td>5.75</td>
<td>6.56</td>
<td>0.73</td>
<td>0.82</td>
<td>0.65</td>
<td>0.56</td>
<td>0.40</td>
</tr>
<tr>
<td>6</td>
<td>8-1</td>
<td>4.05</td>
<td>3.66</td>
<td>0.57</td>
<td>0.49</td>
<td>0.40</td>
<td>0.34</td>
<td>0.28</td>
</tr>
</tbody>
</table>

**Note:** Averaged results of their determinations performed on diluted samples (1:10) with water (lyophilized melanoidin).
Absorbance at 420 nm ($A_{420}$) allows the browning due to the presence of soluble premelanoids to be measured. The majority of the colored compounds after filtration were not retained in the high-molecular-weight fraction (300-150 kDa) but below it. This result is in line with the literature describing browning in sugar–amino acid systems. A much higher percentage of color was detected in the high-molecular-weight fraction (Wagner et al., 2007).

**Color measurements by colorimeter**

The changes in color parameters of the melanoid fractions formed from Gluc/L-Asn and Fru/L-Asn according to the different molecular weight cut off (MWCO) are shown in Table 1. The $L^*$ value from the Lab color scale was used to measure the color differences indicating melanoids produced as a result of Maillard browning (Nursten, 2005). The $L^*$ value represents the luminosity of the melanoids, from the 100 (lightness) to 0 (darkness) scale. The $L^*$ value of the melanoids formed from the Glu/L-Asn was higher than that of the melanoids formed from the Fru/L-Asn, at high molecular weight (HMW).

The $a^*$ value is a measure of redness or greenness and the $b^*$ value is a measure of yellowness or blueness, respectively. The $a^*$ value of the melanoids formed from the Gluc/L-Asn were higher than those of the melanoids formed from the Fru/L-Asn. The highest $b^*$ value of the melanoids formed from the Glu/L-Asn system was at 8-1 kDa fraction. These results suggest that the red-blue pigment of nondialysable melanoids results from low molecular weight (MW < 50 kDa) compounds. It is thought that low molecular weight compounds are polymerized by many intermediate Maillard reactions and generate higher molecular weight compound of yellow-red pigment by the color lightness ($L^*$). A decrease in the $\Delta E^*$ index is related to a loss of lightness.

The color value of the melanoids formed from all the model systems was located at a dominant wavelength of 420 nm, the blue zone of the diagram. In addition, $L^*$, $a^*$, $b^*$, $C^*$ value, and the $\Delta E^*$ index on the basis of the different sugars (Glu, Fru) with L-Asn, shown differences at HMW and low color from LMW fractions.

**DPPH Radical Scavenging Activity**

The results obtained from glucose and fructose with L-Asparagine fraction systems are shown in Figure 4. The DPPH radical scavenging activity of the melanoids formed from the Fru systems was higher than that of the melanoids formed from the Glu systems, with exceptions in the Fru/L-Asn systems <50 (FA3).

![Figure 4. DPPH radical scavenging activity of melanoidins from Glucose-Asparagine (GA; MW > 300 kDa) and individual Glucose-Asparagine fractions (GA1: MW = 300-150 kDa; GA2: MW = 150-50 kDa, GA3: MW < 50 kDa) and Fructose-Asparagine (F-A > 300 kDa) and individual Fructose-Asparagine fractions (FA1: MW > 300 kDa; FA2: MW = 150-50 kDa, FA3: MW < 50 kDa). Ascorbic acid (AA) was used as positive controls. All values are presented as the mean ± SD (n = 3).](image)

**Antioxidant activity (ABTS)**

Table 2 shows the antioxidant activity of the melanoid fractions measured by means of the ABTS$^+$ assay and expressed as $\mu$mol equivalents of Trolox per g of dry melanoid. The antioxidant activity of the sample obtained from the Fruc/L-Asn was significantly higher than that from the Glu/L-Asn samples.

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-52-
The ABTS radical scavenging activity of the melanoidins formed from the Fru and Glu systems showed the following order: > 300 kDa > 300-150 kDa > 150-50 kDa. The < 50 kDa was lower than that of the melanoidins formed from the 300-150 kDa. The ABTS radical scavenging activity of the melanoidins formed from the Fru/L-Asn system was the highest, while the melanoidins formed from the Glu/L-Asn system showed lower activity in all fractions. It is assumed that the difference in radical scavenging activity is due to different reaction media; these being aqueous for ABTS and methanolic for DPPH.

**Table 2**

Antioxidant activity determined by the ABTS method

<table>
<thead>
<tr>
<th>Fractions</th>
<th>D-Glucose/L-Asn</th>
<th>D-Fructose/L-Asn</th>
</tr>
</thead>
<tbody>
<tr>
<td>MW &gt;300</td>
<td>576.2 ± 19.0</td>
<td>635 ± 28</td>
</tr>
<tr>
<td>300-150 kDa</td>
<td>407.4 ± 25.3</td>
<td>609 ± 4</td>
</tr>
<tr>
<td>150-50 kDa</td>
<td>317.3 ± 3.2</td>
<td>364.2 ± 1.9</td>
</tr>
<tr>
<td>MW &lt;50</td>
<td>284.4 ± 2.2</td>
<td>341 ± 4</td>
</tr>
</tbody>
</table>

**Note:** Data expressed as μmol equivalents of trolox/melanoidins released from 1 g of melanoidins. Values are mean ± standard deviation of three experiments.

**4. Conclusions**

The high molecular weight (HMW) ultrafiltration fraction of glucose and amino acid water-soluble MRPs showed a yield of 53.68%. These data indicate that the majority of the water-soluble compounds formed have a HMW. Also, the use of ultrafiltration fractions allowed the radical scavenging activity to be assigned to brown HMW fractions that absorbed at 420 nm ($A_{420}$) as well as at 280 and 325 nm. The browning of glucose-amino acid was higher than in fructose-amino acid systems. The antioxidant activity of the melanoidins decreased with the decrease in the molecular weight fraction. It can also be noted that the Glu/L-Asn fraction showed the lowest antioxidant activity values.

**Acknowledgements**

Echavarria A.P., wishes to thank the support of the Commission for Universities and Research of the DIUE in the Generalitat de Catalunya and the European Social Fund for financing a predoctoral grant.

**References**


