Optimization of Maillard reaction products isolated from sugar-amino acid model system and their antioxidant activity

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Optimización de productos de la reacción de Maillard aislados a partir de sistemas modelo azúcar-aminoácido y su actividad antioxidante.

Optimització de productes de la reacció de Maillard aïllats a partir de sistemes model sucre-aminoàcid i la seva activitat antioxidant.

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RESUMEN

Se evaluaron las propiedades funcionales de melanoidinas solubles de sistemas modelo a partir de una combinación simple de monosacáridos (fructosa o glucosa) y aminoácidos. Los aminoácidos seleccionados se encuentran en el zumo de manzana siendo altamente reactivos en la reacción de Maillard, y fueron: asparagina (Asn), ácido aspártico (Asp) y ácido glutámico (Glu). Se aplicó la metodología de superficies de respuesta (RSM) para determinar las condiciones óptimas para maximizar el rendimiento (g de melanoidina obtenida) a partir de la reacción de Maillard. El efecto de estas condiciones sobre la intensidad del color y la absorbancia de las melanoidinas se midió a diferentes longitudes de onda (280, 325, 405 nm). También se determinó el pardeamiento a 420 nm, el color y la intensidad antioxidante. Las condiciones óptimas de la reacción para maximizar la producción de melanoidina para glucosa/Asn fueron de 95ºC durante 109,7 h a una concentración de aminoácido de 0,77 ($g \cdot kg^{-1}$). Las actividades antioxidantes se evaluaron mediante la actividad de captación de radicales libres que incluyeron 1, 1-difenil-2-picril-hidrazil (DPPH) y 2, 20-azinobis (3-etilbenotiazolina-6-ácido sulfónico) sal de diamonio (ABTS). Los resultados mostraron que la absorbancia y la intensidad del pardeamiento de la glucosa/aminoácido fueron mayores que las formadas a partir de la mezcla fructosa/aminoácido. Por otro lado, los MRPs del modelo fructosa/aminoácido mostraron mayor actividad captadora de radicales libres DPPH y ABTS que los del sistema modelo glucosa/aminoácido.

Paraules clau: Melanoidinas, optimización, metodología de superficies de respuesta, actividad antioxidante

SUMMARY

The functional properties of soluble melanoidins from a single combination of monosaccharides (fructose or glucose) and amino acid model systems were evaluated. The selected amino acids, commonly found in apple juice and highly reactive in the Maillard reaction, were asparagine (Asn), aspartic acid (Asp) and glutamic acid (Glu).

The response surface methodology (RSM) was applied to determine the optimum conditions to maximize the yield (g of obtained melanoidin) from the Maillard reaction. The effect of these conditions on the colour intensity and the absorbance of melanoidins was measured at different wavelengths (280, 325, 405 nm). Browning at 420 nm, colour and antioxidant activity were also determined. The optimum reaction conditions to maximize melanoidin production were glucose/Asn 95º C for 109.7 h at 0.77 ($g \cdot kg^{-1}$) amino acid concentration. Antioxidant activities were evaluated through the free radical scavenging activity including 1, 1-diphenyl-2-picryl-hydrazil (DPPH) and 2, 20-azinobis (3-ethylbenothiazoline-6-sulfonic acid), diammonium salt (ABTS). The results showed that the absorbance and browning intensity of the glucose/amino acid were higher than those formed from the fructose/amino acid mixture. On the other hand, the fructose/amino acid model MRPs showed higher DPPH and ABTS radical scavenging activities than the glucose/amino acid model system.

Key words: Melanoidins, optimization, response surface methodology, antioxidant activity.

RESUM

Es van avaluar les propietats funcionals de melanoïdines solubles de sistemes model a partir d'una combinació simple de monosacàrids (fructosa o glucosa) i aminoàcids. Els aminoàcids sel·leccionats es troben al suc de poma essent altament reactius en la reacció de Maillard, i foren: asparagina (Asn), àcid aspàrtic (Asp) i àcid glutàmic (Glu). Es va aplicar la metodologia de superfícies de resposta (RSM) per determinar les condicions òptimes per maximitzar el rendiment (g de melanoïdina obtinguda) a partir de la

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reacció de Maillard. L'efecte d'aquestes condicions sobre la intensitat del color y la absorbància de les melanoïdines es va mesurar a diferents longituds d'ona (280, 325, 405 nm). També es va determinar l'embruniment a 420 nm, el color i la intensitat antioxidant. Les condicions òptimes de la reacció per maximitzar la producció de melanoïdina per a glucosa / Asn van ser de 95ºC durant 109,7 h a una concentració d'aminoàcid de 0,77 ($g \cdot kg^{-1}$). Les activitats antioxidants es van avaluar mitjançant l'activitat de captació de radicals lliures que van incloure el 1, 1-difenil-2-picril-hidrazil (DPPH) i el 2, 20-azinobis (3-etilbenotiazolina-6-ácid sulfònic) sal de diamoni (ABTS). Els resultats van mostrar que la absorbància i la intensitat de l'embruniment de la glucosa/aminoàcid van ser més grans que les formades a partir de la barreja fructosa/aminoàcid. Per altra banda, els MRPs del model fructosa/aminoàcid van mostrar activitat captadora de radicals lliures DPPH i ABTS més gran que els del sistema model glucosa/aminoàcid.

Mots clau: Melanoïdines, optimització, metodologia de superfícies de resposta, activitat antioxidant

INTRODUCTION

Non-enzymatic browning reactions between amino acids and reducing sugars are the basis of the Maillard reaction that takes place in thermally processed foods (Cho *et al.*,2010). This reaction improves the desirable sensory characteristics of these foods (baking, cocoa and coffee roasting, cooking of meat), such as colour, aroma and flavour, but it can also have undesirable side effects (milk drying, thermal treatments for stabilizing milk, fruit juices and tomatoes), as the Maillard reaction gives rise to losses in the nutritional value of foods.

Because of the complexity of real food systems, most studies on melanoidins have focused on model browning reactions (Adams *et al*., 2003). In the model systems, reducing sugars or Maillard intermediates were used as starting materials and defined coloured reaction products could be isolated and identified (Hofmann *et al.,*2001; Rufián-Henares *et al*.,2007). Each system has to be studied taking into account its own physical and chemical characteristics and their evolution during the reaction itself. It is very important to find suitable indicators to enable the Maillard reaction to be followed in both its early and final stages. Among the most commonly used indicators of the Maillard reaction are spectrophotometric measurements and colourimetric evaluations (Martins *et al*., 2003). The development of the Maillard reaction is generally monitored by the increase in absorbance of either 280, corresponding to early Maillard Reaction Products (MRPs) for pyrazine compounds (Gu *et al.,* 2010), 320-350 nm (soluble pre-melanoidins, advanced stage), or 420-450 nm (final MRPs), corresponding to the colour intensity of the reaction medium, as well as the formation of specific compounds (Billaud et al.,2004). The formation of heterocyclic derivatives and such intermediate water-soluble compounds as reductones, amino-reductones or pre-melanoidin is detected. Generally, the low absorbance values recorded at 420 nm seemed to reveal a lower proportion of brown pigments or melanoidins (Brands *et al*., 2002). Because most of the melanoidins exert their biological activity in a hydrophilic food matrix, the free radical scavenging capacity of melanoidins in an aqueous medium must be studied (Delgado-Andrade et al., 2005).

In the case of the Maillard reaction, high antioxidant capacity is generally associated with the formation of brown melanoidins (Borrelli *et al.,* 2002). Although the degree of browning has been positively correlated with the levels of radical scavenging activity (Brudzynski et al., 2011), 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 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 capacity of MRPs and melanoidins substantially contribute to the shelf life of heat-treated foods. Melanoidins exert a significantly lower antiradical activity than classical antioxidant compounds (tannic acid, ferrulic acid, caffeic acid, gallic acid) in an aqueous medium. Different approaches have been applied to describe the antioxidative properties of MRPs, such as polarographic methods, Rancimat, and measurement of conjugated dienes, TBARS index, or scavenging of certain radicals (e.g., DPPH radical cation) in a methanolic or chloroformic medium, among others (Xu et al., 2007). Significant differences have been observed depending on the type of amino acid used as a reactant during the formation of the melanoidin structure and the antiradical efficiency exerted (Morales *et al.,* 2002). The main mechanism of action is believed to be the ability to trap positively charged electrophilic metabolites, scavenge oxygen radicals, and chelate metal to form inactive complexes, or synergies (Morales *et al.,* 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 preventing lipid oxidation is limited, but they may act like other antioxidants at different levels in the oxidative sequence, in a similar way to polyphenols (Marti *et al.,* 2009)

The development of the Maillard reaction shows two different and distinct phases: initially slow (lag period), and later relatively fast (post-lag period) (Jousse *et al.,*2006). Many factors influence the velocity of reaction. These include temperature, time, water activity (a_w), pH (Kwaka *et al.,* 2005), the type and ratio of reducing sugar (De Vleeschouwer *et al*., 2001), amino compounds (Morales *et al*., 2001), reactant source and concentration (Matmoroh *et al*., 2006).

Based on preliminary experiments, heating time, temperature and amino acid concentration were found to be the key factors influencing the obtaining of MRPs.

Response Surface Methodology (RSM) was used to design and evaluate experiments to optimize the processing parameters. This methodology is a collection of statistical and mathematical techniques useful for improving and optimizing complex processes. The main advantage of RSM is its ability to reduce the number of experimental trials needed to evaluate multiple parameters and their interactions to provide sufficient information for statistically acceptable results (Gu *et al*., 2009). It has been successfully demonstrated that this technique can be used to optimize process variables (Yang *et al*., 2011).

The overall objective of this work is to optimize the processing conditions of a single combination of sugar (fructose or glucose) and amino acid (Asn) model systems, using RSM to attain the MRPs with the highest yield (Y) under different conditions: heating time, temperature and amino acid concentration. Furthermore, UV-absorbance (A_{280}, A_{325} and browning at A_{420} nm), colour and ºBrix were examined to evaluate the antioxidant activity of melanoidins. Their antioxidant properties were determined by different methodologies; DPPH radical scavenging activity and ABTS were assessed.

MATERIALS AND METHODS

Chemicals and reagents

D-glucose, D-fructose, L-asparagine, L-glutamic acid, L-aspartic acid, potassium persulfate, phosphate buffered saline, l- ascorbic acid and sodium hydroxide (0.25N) were purchased from Panreac Química S.A.U, Barcelona, Spain. Pyridine (Merck, Hohenbrunn, Germany). Active carbon (Probus, S.A. Barcelona, Spain). (±) 6-hydroxy-2,5,7,8-tetramethylchroman-2-carboxylic acid (Trolox) and 2,2-diphenyl-1-picrylhydrazyl hydrate (DPPH) were from Sigma-Aldrich (St. Louis, MO, United States). 2, 2-azinobis (3-ethylbenzthiazoline)-6-sulfonic acid (ABTS) diammonium salt from Fluka Chemicals (Madrid, Spain). The solvents were purchased from Sharlau Chemie, S.A. (Barcelona, Spain). Deionized and distilled water were used throughout.

METHODS

Obtaining water-soluble melanoidins

Six model systems were prepared from a single combination of sugar (glucose/G or fructose/Fru and amino acid asparagine/Asn, glutamic acid/Glu, aspartic acid/Asp). Water-soluble melanoidins were obtained from different Maillard model systems by dissolving 50 g of each sugar (G and Fru), in 250 mL of distilled water with different amounts amino acid which gave a final concentration of (0.2, 0.7 or 1.2 $g \cdot kg^{-1}$). This solution in a sealed container was treated for different times (72, 96, 120 h) in a stove at different temperatures (50, 75, 100 ºC). The melanoidins formed during the thermal treatment were isolated as, described by Ibarz *et al.* (2008). The 500 mL glass columns were used, the bases of which contained a glass-fibre plate. A 10 g layer of active carbon was placed in these columns. The solution that contained the melanoidins was diluted with distilled water and made to pass 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 et al.,1987). Once it had been checked that the active carbon in the column contained no sugars, the melanoidins are extracted by passing an aqueous solution of pyridine (Merck, Hohenbrunn, Germany) at 25% through the active carbon. This pyridine solution, together with the melanoidins extracted, may contain small particles of active carbon, so the solution was filtered in a vacuum through a 3-5 µm pore size filter. Once the solution was free of active carbon particles, the solvent was removed through evaporation in a Labo Rota C-311 rotavapor (Resona Technics, Gossau, Switzerland) that operates under a vacuum at a temperature of 45º C. To quantify the melanoidins extracted, these were lyophilized (Cryodos-50/230V 50 Hz Telstar, Madrid, Spain) and weighed. The solid residue represented the extracted melanoidins and was expressed as g of melanoidins obtained from 250 mL of initial solution.

Analytical measurement

After measuring the soluble solid content of each sample with an Atago RX-1000 digital refractometer, these were diluted to 12 °Brix with doubly 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.

UV-absorbance and browning intensity

The UV-absorbance of the melanoidin fraction was determined with absorbance spectrum detection between 280 nm to 420 nm using a Helios gamma spectrophotometer (Thermo Fisher Scientific Inc., Waltham, MA, USA), with a 1 cm-path length cell. The samples were dissolved in deionized water at a concentration of 0.1 mg/mL.

Colour determination at optimum conditions

The colour of the melanoidin fractionations were directly measured using the CIE (Commission Internationale de l'Eclairage) values L^* (lightness), a^* (redness), b^* (yellowness). These values were determined with a light source C, with a colour-meter (Minolta ChromaMeter Model CR-400, Konica Minolta, Tokyo, Japan). The equipment was set up for illuminant D65 and 10º observer angle and calibrated using a standard white reflector plate.

Antioxidant capacity

ABTS Radical cation decolourization assay

The antioxidant capacity of fractionation melanoidins was estimated in terms of the radical scavenging activity in water media, following the procedure described by Millar & Sampson, (Miller *et al*., 1996) with slight modifications. The $ABTS^{\bullet+}$ radical was produce by reacting 7 mM ABTS (2,2-azinobis (3-ethylbenzthiazoline)-6-sulfonic acid (ABTS), stock solution with 2.45 mM potassium persulphate. The reaction mixture was left to stand at room temperature overnight (12 to 16 h) in the dark before use. The resulting intensely coloured $ABTS^{\bullet+}$ radical cation was diluted with 5 mM PBS (phosphate buffered saline), pH 7.4, to give an absorbance value of 0.70 (±0.02) at 734 nm and equilibrated at 30 ºC. After addition of 30 µL of the test compound (melanoidins) or Trolox (6-hydroxy-2,5,7,8-tetramethylchroman-2-carboxylic acid) was diluted with to 3 mL of diluted $ABTS^{\bullet+}$ solution were mixed for 45s and measured after 5 min (absorbance did not change after significantly up to 10 min). The assay was performed at least in triplicate with a Helios gamma spectrophotometer (Thermo Fisher Scientific Inc., Waltham, MA, USA). Aqueous solutions of Trolox at various concentrations were used to perform the calibration curves (0.15-1.15 mM).

Radical-scavenging activity using the DPPH method

DPPH is one of the chromogen-radical-containing compounds that can directly react with antioxidants

(Ozcelik). This activity was measured by the procedure described by Molyneux (Molyneux, 2004). In its radical form, $DPPH^{\bullet+}$ absorbs at 517 nm. Briefly, 0.12 mM solution of DPPH in methanol was prepared daily and protected from light. An aliquot of 3 mL of this solution was added to 80 µL of melanoidin at (1.2 $g \cdot kg^{-1}$) concentration dissolved in 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 colour. Radical-scavenging activity was expressed as the inhibition percentage and calculated with the following equation:

$$\%RSA = \frac{ControlAbs - SampleAbs}{ControlAbs} \times 100 \quad (1)$$

Experimental design and statistical analysis

Response surface methodology (RSM) was used to study optimize the Maillard reaction conditions with special reference to yield (Y). Conditions of time, temperature and amino acid concentration of melanoidins were performed by applying the Statgraphics v.2.3 statistical package (Statistical Graphics Corp., Rockville, MD). All the statistical procedures were performed at a significance level of 95%. In this design, 15 different experiments were completed for each type of combination of sugar (G and Fru) with the amino acid Asn. The coded values of the independent variables were, -1, 0 and 1. The three independent variables (reaction conditions) were reaction time (X_1), reaction temperature (X_2) and concentration of amino acid (X_3). The response functions *Y* were related to the coded variables (X_i =1, 2, 3) by a second-order response function was determinated by the Equation (2).

$$Y = \beta_0 + \sum_{i=1}^{4} \beta_i X_i + \sum_{i=1}^{4} \beta_{ii} X_i^2 + \sum \sum_{i<j=1} \beta_{ii} X_i X_j \quad (2)$$

where Y is the dependent variables, β_o is a constant β_i, β_{ij} and β_{ii} represent the coefficient of the linear, quadratic and interactive effects, respectively; X_1, X_2 and X_3 factors on the response.

Analysis of variance (ANOVA) was performed. ANOVA tables were generated and the effect and regression coefficients of individual linear, quadratic and interaction terms were determined. The statistical significance of the regression coefficients was determined by using the Durbin-Watson (DW) test and the applicability of the model was checked with significance coefficients of determination (R^2) and the coefficient of variation (CV) values with a $p \leq 0.05$. The optimum processing conditions were obtained by using graphic and numerical analysis based on the criterion of desirability.

All of the analyses were performed at least in duplicate.

RESULTS AND DISCUSSION

Statistical analysis

The actual values, chosen from the preliminary studies, and the corresponding values of three independent variables for G/Asn and Fru/Asn model system are given in Table 1. Values obtained gave a higher yield (g) of melanoidin formed from G/Asn model system.

The ANOVA confirms the adequacy for the statistical models since their Prob>F values, six effects have p-values less than 0.05, indicating that they are statistically significant at the 95 % confidence level. The models present high determination coefficients (low coefficients of variation (CV) and their corresponding coefficients of determination (R^2) obtained by fitting the experimental data to the second order response model. The combined effect of treatment temperature and amino acid concentration on extraction *yield* (Y, g) is shown in Table 2 described by the Equation (2) for glucose and fructose combined with amino acids.

The treatment conditions to reach the highest extraction yield for G/Asn (85.79 g) and for Fru/Asn and (62.8 g). The adjusted R-squared statistic, which is more suitable for comparing models with different variables, is 98.4% for the G/Asn and 96.7% for the Fru/Asn model system.

Table 1 *Experimental design used in RSM studies by three independent variables for glucose/Asn and fructose/Asn model system*

Experiments	Variables						Response **Y(g)**	
	Coded			Uncoded				
	X_1	X_2	X_3	t (h)	T (ºC)	[a]C (g/kg)	Glu/Asn	Fru/Asn
1	1	1	0	120	100	0.7	52.2	48.7
2	1	-1	0	96	75	0.7	62.6	56.2
3	0	0	0	96	50	0.2	23.8	21.5
4	0	1	-1	96	75	0.7	62.6	56.2
5	0	0	0	96	100	1.2	48.3	42.3
6	0	1	1	96	75	0.7	62.6	56.2
7	1	0	1	120	75	0.2	28.5	20.2
8	-1	-1	0	72	100	0.7	49.6	42.6
9	0	-1	-1	72	75	1.2	39.8	35.2
10	1	0	-1	96	50	1.2	29.5	26.6
11	0	-1	1	120	50	0.7	25.8	23.6
12	-1	0	1	72	75	0.2	30.6	25.8
13	0	0	0	120	75	1.2	40.7	31.2
14	-1	1	0	72	50	0.7	23.6	22.6
15	-1	0	-1	96	100	0.2	43.6	35.8

[a] *amino acid concentration*

Effects of treatment conditions on the extraction yield

The extraction yield of melanoidin from G/Asn depended significantly ($p<0.001$) on the treatment temperature, as well as the amino acid concentration. However, the treatment time presented no significant effect ($p>0.05$) on the extraction yield of the melanoidins. As shown in Table 2, it can be observed that yield (g) obtained from of glucose or fructose with asparagine combination, their ability is positively related to the linear effect of heating temperature, time and initial concentration ($p< 0.01$). Figure 1 shows the dependence of yield (Y) with temperature (T) and concentration (C) at a fixed heating time for G/Asn. This means that the linear effects of initial pH ($p < 0.05$) and heating time ($p < 0.05$) were dominant over the quadratic and interaction terms. The interaction effects between heating time, temperature and concentration were significant, influenced the Y.

Table 2 *Analysis of variance (ANOVA) for regression equation of melanoidins from glucose/ Asn and fructose /Asn model system*

Source	Estimate Std.		DF	Std error		F-Value		Pr (>[F])	
	G/Asn	F/Asn		G/Asn	F/Asn	G/Asn	F/Asn	G/Asn	F/Asn
intercept	-3.224e+02	-2.915e+02	1	2.337e+01	3.096e+01	-13.794	-9.415	7.37e-07***	1.33e-05 ***
T	3.263e+00	2.580e+00	1	3.013e-01	3.992e-01	10.829	6.465	4.67e-06 ***	0.000195 ***
t	4.385e+00	4.199e+00	1	4.173e-01	5.529e-01	10.508	7.596	5.86e-06***	6.33e-05
c	8.971e+01	9.459e+01	1	7.189e+00	9.523e+00	12.479	9.933	1.59e-06***	8.93e-06 ***
T^2	-1.871e-02	-1.470e-02	1	1.996e-03	2.644e-03	-9.379	-5.559	1.37e-05***	0.000535 ***
t^2	-2.274e-02	-2.194e-02	1	2.166e-03	2.869e-03	-10.501	-7.647	5.89e-06***	6.03e-05 ***
c^2	-5.840e+01	-6.185e+01	1	4.990e+00	6.611e+00	-11.703	-9.356	2.59e-06***	2.59e-06***

Melanoidins from G/Asn
Residual standard error:2.397 on 8 degrees of freedom
Multiple R-squared:0.984, Adjusted R-squared:0.9721
F-statistic:82.25 on 6 and 8 DF, p-value:9.466e-07
*Signif.codes: 0***,0.001 ****
Melanoidins from F/Asn
Residual standard error:3.176 on 8 degrees of freedom
Multiple R-squared:0.674, Adjusted R-squared:0.43
F-statistic:39.6 on 6 and 8 DF, p-value:1.602e-05
*Signif.codes: 0***,0.001 ****

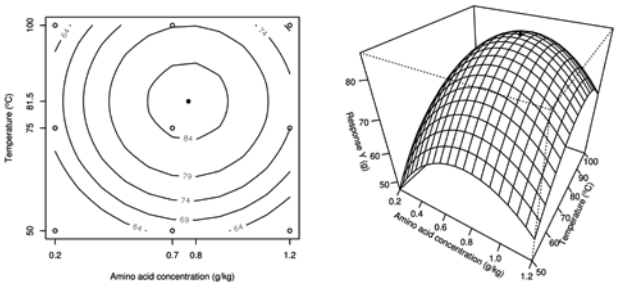

Figure. 1 *Response surfaces for the effects of variables on yield (g) of melanoidins from Glucose/Asn model system*

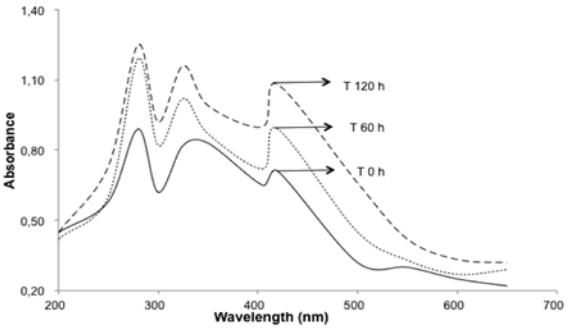

Figure .2 *UV-Visible spectra of the melanoidin obtained from the Glucose/Asn model system at optimum conditions of amino acid concentration, temperature at different times*

The optimum processing parameters were determined to yield of melanoidins from glucose/Asn MRPs with 0.7 g·kg^{-1} concentration. Y (g) can be optimized from the contour. The model describes the optimum conditions of *yield* for glucose/Asn as: heating time 109.7 hours at 81.5°C and concentration of 0.77 g·kg^{-1}and for melanoidins from fructose/Asn the optimum temperature was 89.6 ºC , 105 hours and 0.76 g·kg^{-1} of concentration.

UV-absorbance and colour for optimum conditions of melanoidins model systems

Absorbance values at four wavelengths of melanoidins from the glucose and fructose model systems were recorded. Melanoidins showed their maximum absorbance, at 280 nm and 325nm, except for the Fru/Glu 1.2 (g·kg^{-1}) that showed absorbance at 275 nm.

The final stage of the browning reaction was monitoring by the increase in absorbance at 420nm. The G/Asn system showed the highest degree of browning among all the model systems (Figure 2). The development of browning in the G/Asn systems was higher than in the Fru/Asn system. This may be associated with glucose which is an aldose reacts easily with amino compounds as fructose.

Asparagine melanoidins produce more colouration, while fructose and glutamic acid produce fewer colours. Melanoidins are classified in decreasing order of colour as glucose melanoidins: glutamic acid (G), aspartic acid (Asp) and asparagine (Asn), fructose melanoidins: Glu, Asp and Asn.

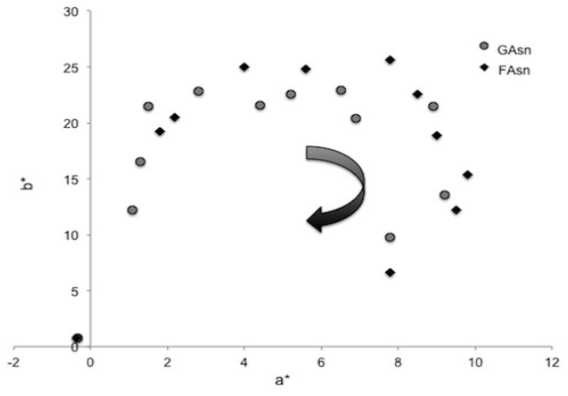

Figure 3 *CIELAB, chroma (a*, b*) plots of colour development of melanoidins from Glucose/ Asn and Fructose/Asn at optimum conditions*

The redness (a*) and yellowness (b*) colour parameters were measured and plotted for all amino acids with glucose and fructose at different temperatures. The curves of G/Asn and Fru/Asn proceed with the clockwise as well as heating time. The net increase in brown-red is observed during the first hours of heating reaching a maximum in this zone. Then the curve of G/Asn changing from the brown-yellow to the brown-red zone shows a decrease in the a^* and b^* values with treatment time, which is accentuated by the optimum conditions of treatment time and temperature (Figure 3). Depending on the amino acid group and temperature, the coordinate values decreased progressively towards brownish colour. This indicates that there is a precipitation of particles of melanoidin with time at high temperature. The results agree with those previously found in lemon juice by Ibarz-Martínez et al. (2010). However, The Fru/Asn from the brown-red zone b^* decreased with high treatment in the red-brown and the a^* decreased significantly with time.

Effects of reaction conditions on the antioxidant activity of melanoidins

DPPH is a chromogen-radical-containing compound that can directly react with antioxidants. When the DPPH radical is scavenged by antioxidants through the donation of hydrogen to form a stable DPPH molecule, the colour changes from purple to yellow. Stable radical DPPH has been widely used to determine primary antioxidant activity, that is, the free radical scavenging activities of pure antioxidant compounds. The results obtained from the DPPH assay using different melanoidins formed from glucose and fructose with asparagine, glutamic acid and aspartic acid are shown in Figure 4. In the fructose systems, the DPPH radical scavenging activity of melanoidins formed from the Fru/Asn (0.7 and 1.2 $g{\cdot}kg^{-1}$) and Fru/Asp (1.2 $g{\cdot}kg^{-1}$) systems increased with the concentration. The DPPH radical scavenging activity of the melanoidins formed from the glucose system was the lowest.

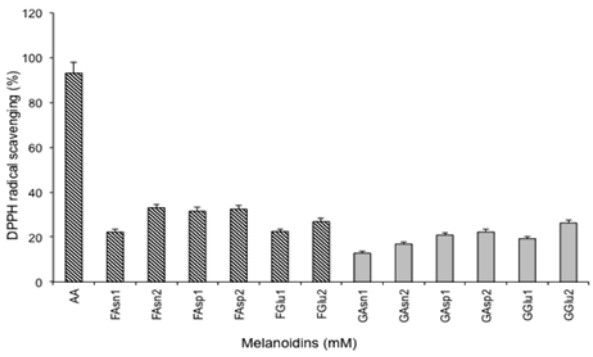

Figure 4 *DPPH radical scavenging activity of melanoidins from Glucose/amino acids and Fructose/amino acids*

The results for the antioxidant capacity of these samples were compared to ABTS at the end point of 10 min as determined by spectrophotometric measurement. The ABTS radical scavenging activities of the melanoidins formed from glucose and fructose with Asn, Glu and Asp are shown in Figure 5. The results showed that the inhibitory effect of ABTS increased with time for all samples. The Fru/Asn (1.2 $g{\cdot}kg^{-1}$) had the highest percentage of inhibition, as well as the highest antioxidant capacity (20.6 mmol TE/g). G/Asp showed the lowest antioxidant capacity (6.9 mmol TE/g).

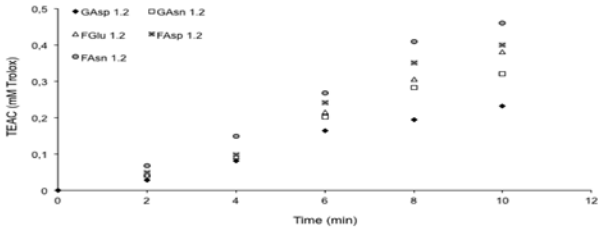

Figure 5 *Kinetics of 2, 2-azinobis (3-ethylbenzthiazoline)-6-sulfonic acid (ABTS) diammonium salt scavenging effect of melanoidins. FAsn:fructose/asparagine; FAsp (fructose/aspartic acid); FGlu(fructose/glutamic acid); GAsn (glucose/asparagine); Gasp(glucose/asparagine) with 1.2 $g{\cdot}kg^{-1}$ concentration*

CONCLUSIONS

In this research, the optimum processing parameters to yield melanoidins of a single combination of sugar (fructose or glucose) and amino acid (Asn) model systems are determined. This process was designed and analyzed using the composite design and RSM. The RSM were applied to optimize the operating variables of the initial temperature, time and amino acid concentration for all combinations of sugar/amino acid. This study showed that RSM was a suitable method for optimizing the operating conditions and maximizing the yield of the melanoidin production with different optimum conditions: temperature 81.5 ºC, heating time 109.7 hours and amino acid concentration 0.77 $g{\cdot}kg^{-1}$ for glucose/Asn. For fructose / Asn the optimal conditions values were: 89.6 ºC, 105 h and 0.76 $g{\cdot}kg^{-1}$ respectively.

The results indicate that colour can be considered an index of the overall antioxidant properties of melanoidins whenever the mechanisms responsible for forming antioxidants and colour follow the optimal conditions obtained. The antioxidant activity of melanoidins increased by the optimum conditions; time, temperature and amino acid concentration. It can be also noted that the fructose/Asn model system showed the highest antioxidant activity values. The correlation between colour and antioxidant properties can be achieved in melanoidins where the formation of antioxidant MRPs is the prevalent event during processing. The colour of melanoidins formed in the model systems decreased progressively with time. This indicates that there is an aggregation of particles of melanoidin with time at high temperature.

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