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1 # GLYCATION IS REGULATED BY ISOFLAVONES

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16

17 **Abstract**

18 The effect of soy isoflavones on the Maillard reaction (MR) was investigated. Model
19 systems composed of the soy protein glycinin (10 mg mL^{-1}) and fructose (40 mg mL^{-1})
20 under basic pH conditions were employed for testing the anti-glycative effect of the
21 major antioxidant soy isoflavones (genistin and genistein) and a soy isoflavone-rich
22 extract. The progress of MR was estimated by analysis of free amino groups; sugar
23 covalently bound to protein, protein-bound N^{ε}-(carboxymethyl)lysine (CML) and
24 fluorescence spectra. Genistin (10 μg mL^{-1}, 23 μM) and its metabolite genistein (10 μg
25 mL^{-1}, 37 μM) did not prevent protein glycation. The soy isoflavone-rich extract (5 mg
26 mL^{-1}) efficiently decreased bound of sugar to the protein skeleton (20%) and formation
27 of advanced glycation end products (AGEs) (> 80%). The anti-glycative mechanism of
28 isoflavones may be related to its conjugation to glycation sites of the protein structure
29 (free amino groups), their antioxidant character and trapping of dicarbonyl
30 intermediates. Extracts based on mixtures of isoflavones may be useful for producing
31 glycated conjugates avoiding the substantial formation of AGEs bound to protein.

32

33 **Introduction**

34 Glycation, also known as Maillard reaction (MR), is a non-enzymatic reaction between
35 carbonyl groups of reducing sugar and free amino groups of proteins. Non-oxidative
36 and/or oxidative modifications of the early MR products (Amadori and Heyns products)
37 result in the formation of a variety of advanced glycation endproducts (AGEs). AGEs
38 can be fluorescent crosslinked structures such as pentosidine, or non-fluorescent
39 structures such as N^{ε}-(carboxymethyl)lysine (CML)[1]. AGEs are formed in foods and in
40 the human body and they are considered to be undesirable compounds[2]. The search for
41 natural inhibitors of AGEs formation is of great interest and a priority research line[3-4].
42 Compounds with antioxidant properties may be powerful inhibitors of the formation of
43 AGEs.

44 Soy isoflavones are phytochemicals that exist in two chemical forms, glucosidic
45 conjugates (daidzin, genistin, and glycitin), and the unconjugated forms, or aglycones
46 (daidzein, genistein, and glycitein)[5]. Isoflavones are powerful natural antioxidants[6]. In a
47 previous study, the relationship between the effect on AGEs formation and radical
48 scavenging activity of 62 flavonoids, including 7 isoflavones (diadzein, diadzin,
49 genistein, genistin, tectoridin, puerarin and biochanin), was examined[7]. AGEs formation
50 was assessed solely by measuring characteristic fluorescence at an excitation
51 wavelength of 370 nm and an emission wavelength of 440 nm. Soy isoflavones (at 200
52 µM) inhibited formation of fluorescent AGEs either completely (daidzin) or by 12-34%
53 (daidzein, genistein, genistin). To the best of our knowledge no previous studies
54 regarding the inhibitory effect of soy isoflavones on the formation of non-fluorescent
55 AGEs, such as CML, have been published.

56 Formation of MRPs derived from the interaction of soy proteins and fructose
57 may be inhibited by isoflavones. As a consequence, isoflavones may be used to form

58 particular glycoconjugates with specific and improved functional properties. Other
59 phytochemicals, such as ferulic acid, have been successfully used to achieve this aim[8].
60 The present work aimed to gain new knowledge on the anti-glycation capacity of
61 soy isoflavones (pure and soy isoflavone-rich extract) and their potential for the
62 formation of glycoconjugates avoiding tedious and high cost purification processes for
63 removing contaminants (AGEs and unreactive fructose and proteins). Fructose is
64 commonly used as a sweetener in processed foods and beverages, such as soy milk[9-10].
65 Nowadays, soy based foods are very popular because their health promoting properties,
66 which are mainly associated with the proteins[11] and isoflavones[12]. Therefore, their
67 presence as contaminants in glycoconjugates preparations is not considered a
68 disadvantage. On the under hand, glycation reduces soy protein immnureactivity[13]. The
69 effect of soy isoflavones on the formation of early MRPs and AGEs, both fluorescent
70 and non-fluorescent (CML), in reaction mixtures composed by soy proteins and fructose
71 under basic conditions was investigated. A high pH of reaction was used to enhance the
72 solubility of soy proteins and to catalyse the advanced stages of the MR.
73
74 **Materials and methods**
75 **Materials**
76 All chemicals and solvents were of analytical grade. Fructose, genistin, genistein,
77 lysine, N^{α}-acetyl-L-lysine, nonafluoropentanoic acid (NFPA), phenol, sodium bisulfite,
78 sodium borohydride, sodium dodecyl sulfate (SDS), and trifluoroacetic acid (TFA) were
79 purchased from Sigma-Aldrich (St. Louis, MO, USA). Biuret reagent, Folin-Ciocalteu's
80 phenol reagent, hydrochloric acid 37% (HCl), methanol, sodium hydroxide (NaOH),
81 sulfuric acid 96%, potassium hydroxide (KOH), and trichloroacetic acid (TCA) were
82 purchased from Panreac Química S.A. (Barcelona, Spain). Other chemicals and their

83 suppliers were as follows: β-mercaptoethanol (Merck, Hohenbrunn, Germany),
84 acetonitrile HPLC grade (ACN) (Chromanorm, Leuven, Belgium), CML (NeoMPS,
85 Strasbourg, France), d_4-lysine (Cambridge Isotopes, Andover, MA, USA), *o*-
86 phthaldialdehyde (OPA) (Fluka, Buchs, Switzerland), and sodium tetraborate (Acros-
87 Organics, Geel, Belgium). d_4-CML was kindly provided by Professor S.R. Thorpe
88 (Department of Chemistry and Biochemistry, University of South Carolina, SC, USA).
89 Microtest 96-well plates were purchased from Sarstedt AG & Co. (Nümbrecht,
90 Germany). The Amicon® Ultra-4 centrifugal filter unit fitted with an Ultracel-50
91 cellulose membrane (50 kDa cut-off) were from Millipore Co. (Billerica, MA, USA).
92 Soybean flour (from organically grown; 35% protein, 3.1% carbohydrates, and 18% fat)
93 and commercial isoflavone preparation (capsules active of ethanolic extract 117.85 mg
94 60% v/v dry hypocotyls *Glycine max* (L) Merr. (Soybean) containing 30% of total
95 isoflavones) were purchased from a local supermarket in Spain.
96

97 **Isolation of soy glycinin protein**

98 Soy glycinin was extracted from defatted soy flour as described by Wu *et al*[14]. Briefly,
99 defatted soybean flour (150 mg) was resuspended in deionized water, 1:15 (w/v), the pH
100 was adjusted to 7.5 with 2 M NaOH, and the resulting slurry was mechanically stirred
101 for 1 hour at room temperature. After centrifugation at 14,250*g* for 30 min at 20ºC, the
102 insoluble residue was discarded. Sodium bisulfite was added to the supernatant to
103 achieve a final concentration of 10 mM of SO_2 (0.98 g L^{-1}). Glycinin was precipitated
104 by decreasing the pH to 6.4 with 2 M HCl and incubating at 4ºC overnight. Then the
105 protein solution was centrifuged at 7,500*g* for 20 min at 4ºC. The precipitated glycinin
106 was resuspended in deionized water, freeze-dried and stored at -20ºC until use.
107

108 **Preparation of soy isoflavone-rich extract**

109 The commercial preparation containing a mixture of daidzein, glycitein and genistein
110 (600 mg) was dissolved in 10 mL of reaction medium (0.2% KOH solution) and stirred
111 for 1 hour at room temperature. The preparation was centrifuged at 4,000*g* for 15 min
112 and the supernatant (soluble fraction) was collected. Finally, the concentration of
113 soluble isoflavones was estimated by the Folin-Ciocalteu micromethod assay[15] using
114 genistein (0.5-5 mg mL^{-1}) for obtaining the calibration curve. All measurements were
115 performed in triplicate.

116

117 **Model system preparation**

118 To prepare the glycated model systems, soy glycinin (10 mg mL^{-1}) was mixed with
119 genistin (10 µg mL^{-1}), genistein (10 µg mL^{-1}) or soy isoflavone-rich extract (5 mg mL^{-1})
120 in 0.2% KOH solution (pH 12) and pre-incubated for 1 h or 16 h at 60°C. Fructose (40
121 mg mL^{-1}) was then added and incubation continued for a further hour. Glycation was
122 stopped by cooling the samples on ice. The pH of the model glycation systems at the
123 beginning and end of the reaction was 12. Some samples were also prepared in the
124 absence of fructose. All samples were prepared in triplicate.

125

126 **Sample fractionation**

127 Incubated samples were fractionated by ultrafiltration in order to recover unmodified
128 and glycated proteins for further analysis. Briefly, sample (2 mL) was placed in the
129 sample reservoir of an Amicon® Ultra-4 centrifugal filter unit fitted with an Ultracel-50
130 cellulose membrane (50 kDa cut-off) and centrifuged at 7,500*g* for 20 min at room
131 temperature. The filters were washed with distilled water (4 mL). The concentrated
132 samples were recovered, dissolved in water (2 mL) and stored at -20ºC until analysis.

133 Recovery of protein (RMM > 50 kDa) was determined by the Biuret method in
134 microplate format. Free amino groups, incorporation of sugar into the protein backbone,
135 CML, and fluorescecent AGEs were determined as described below.
136

137 **Determination of free amino groups: available lysine**

138 Free amino groups of samples were determined by the *o*-phthalaldehyde (OPA)
139 method[16]. OPA reagent was prepared fresh before use by mixing 0.1 M sodium
140 tetraborate (pH 9.5, 50 mL), 20% (w/v) SDS (5 mL), β-mercaptoethanol (0.2 mL) and
141 OPA (80 mg dissolved in 2 mL of methanol), and adjusting the final volume to 100 mL
142 with distilled water. An aliquot of protein solution containing 25 µg protein was mixed
143 with OPA reagent (3 mL). After incubation for 5 min at room temperature, the
144 fluorescence was read against a blank containing the OPA reagent using a Shimadzu
145 spectrofluorometer RF-1501 (Shimadzu Co., Kyoto, Japan). The wavelength of
146 maximum excitation was 340 nm and the wavelength of maximum emission was 455
147 nm. Calibration curves were constructed using standard solutions of N^{α}-acetyl-L-lysine
148 (10-1000 µM). All measurements were performed in triplicate and data were expressed
149 as a % of free amino groups. Untreated glycinin (control) was assumed to have 100%
150 amino groups available.
151

152 **Estimation of carbohydrate covalently bound to the protein backbone**

153 Carbohydrate bound to the protein backbone was analysed by the phenol-sulfuric acid
154 method in microplate format[17]. An appropriate dilution of sample (100 µL) was pipetted
155 into a glass vial, to which was added concentrated sulfuric acid (300 µL) and 5% phenol
156 in water (60 µL). After incubating at 90ºC for 5 min, samples were cooled to room
157 temperature for 5 min. Finally, 200 µL aliquots were placed in the wells of a 96-well

158 microplate and the absorbance was measured at 490 nm by employing a microplate
159 reader BioTek PowerWave™ XS (BioTek Instruments, Inc., Winooski, VT, USA). A
160 calibration curve of fructose (5-40 µg per well) was constructed and employed for
161 quantification. Data were expressed as µg of fructose/100 mg of protein. All
162 measurements were performed in triplicate.

163

164 **CML analysis**

165 Protein-bound CML was determined by ultra-performance liquid chromatography
166 (UPLC)-MS/MS according to the procedure recently described by Assar *et al*[18]. Prior to
167 analysis, samples were reduced with sodium borohydride, protein was isolated by TCA
168 precipitation and hydrolysed with 6M HCl. The protein hydrolyzate was purified by
169 solid phase extraction prior to CML analysis by UPLC-MS/MS. Samples were analysed
170 in triplicate.

171

172 **Fluorescence measurement**

173 Fluorescent protein-bound AGEs were measured as previously reported by Wang *et*
174 *al*.[19] by measuring the fluorescence intensity of samples using an excitation wavelength
175 of 337 nm and emission wavelengths ranging from 350 nm to 550 nm with a Shimadzu
176 spectrofluorometer RF-1501 (Shimadzu Co., Kyoto, Japan). Glycinin samples at a
177 concentration of 10 mg mL^{-1} in distilled water were positioned in a cuvette of 1 cm path
178 length. All emission spectra were recorded at 0.5 nm wavelengths intervals.

179

180 **Statistical analysis**

181 Data are expressed as the mean ± standard deviation (SD) of triplicates from three
182 independent experiments and analysed by IBM SPSS version 21.0 (SPSS Inc., Chicago,

183 IL, USA). Analysis of variance was performed using ANOVA procedures. Significant
184 differences between means were determined using Duncan's multiple-range test ($p <$
185 0.05).
186
187 **Results**
188 The loss of available primary amino groups is an indicator used to estimate the extent of
189 the MR[20]. Fig. 1 shows amino groups availability obtained by OPA assay. The
190 percentage of available amino groups in all samples was determined as the relative
191 difference between the percentage of reactive amino groups in the unheated soy glycinin
192 protein sample and that in the glycated models. Heating of soy glycinin protein (heated
193 control) did not significantly ($p > 0.05$) affect the availability of free amino groups. A
194 significant decrease ($p < 0.05$) in free amino groups availability of soy glycinin protein
195 was observed by heating in presence of fructose (glycation model system) suggesting
196 the occurrence of the MR. Values of availability of free amino groups in the samples
197 containing the soy isoflavone-rich extract and glycation model system did not
198 significantly differ ($p > 0.05$). Interestingly, the addition of pure genistin and genistein
199 together with fructose caused a significant decrease in free amino groups' availability (p
200 < 0.05).
201 Monitoring sugar conjugation to protein allows evaluation of the degree of
202 formation of the earliest MRPs[21]. Sugar conjugation was detected by the phenol-sulfuric
203 acid method. As shown in Fig. 2, fructose was successfully bound to soy glycinin
204 protein (glycation model system) indicating the formation of early MRPs (Heyns
205 compounds) ($p < 0.05$). As expected, controls (unheated soy glycinin and protein heated
206 in absence of fructose) proved that reactivity could not be attributed to possible release
207 of carbonyl functions from the protein. Similar levels of early MRPs were formed in the

208 inhibition model systems containing genistin and genistein and the glycation model
209 system (absence of isoflavones). The content of protein-bound early MRPs was
210 significantly ($p < 0.05$) lower (20%) in samples treated with isoflavone extract
211 compared to that found in the sample corresponding to positive control of the Maillard
212 reaction (glycation model).

213 CML, a non-fluorescent AGE, is an important specific biomarker that increases
214 during the MR and correlates with the severity of the reaction. We evaluated whether
215 isoflavones could inhibit CML formation during soy glycinin glycation by fructose. Fig.
216 3 shows the effect of genistin, genistein and soy isoflavone-rich extract on protein-
217 bound CML formation. CML was readily formed under the experimental control
218 conditions (soy glycinin/fructose) and in the presence of genistin and genistein, but its
219 generation was significantly inhibited ($p < 0.05$) by 87% and 92% in glycation models
220 treated with the soy isoflavone-rich extract for 1 and 16 hours, respectively. The level of
221 CML inhibition was greater than that observed for the formation of early MRPs (20%).
222 This is a novel result.

223 Fig. 4 illustrates the effect of genistin, genistein and soy isoflavone-rich extract
224 on the formation of fluorescent AGEs. The fluorescence spectrum of glycinin treated
225 with carbohydrate and isoflavones showed modifications with respect to the control and
226 heated glycinin spectrum. Glycinin reaction with fructose caused formation of
227 fluorescent compounds with emission maxima between 420-425 nm and intensity of
228 70.1 arbitrary units of fluorescence. The addition of genistin and genistein in the
229 reaction mixtures did not inhibit the formation of fluorescence compounds, identical
230 spectra to that obtained for the glycation control being observed. These results support
231 the lack of inhibitory activity in the formation of AGEs at naturally occurring
232 concentrations of these compounds in soy proteins. The addition of the soy isoflavone-

233 rich extract inhibited fluorescence formation. The resulting emission spectrum showed a
234 maximum at 470 nm with an intensity of 15.8 arbitrary units of fluorescence. This
235 intensity value was similar to that detected for the unheated and heated protein;
236 however, the shape of the spectra of these samples differed.
237

238 **Discussion**

239 Results on availability of free amino groups (Fig. 1) and level of sugar bound to the
240 proteins (Fig. 2) suggest that the MR is the major chemical reaction involving free
241 amino groups of the protein polypeptide chain in the model systems**.** Although other
242 reactions, e.g., cross-linking of proteins, may also decrease the level of free amino
243 groups, no evidence was obtained for such chemical events being important under our
244 experimental conditions. Pure isoflavones (genistin and genistein) at the concentrations
245 tested in the present research did not significantly inhibit the progress of the Maillard
246 reaction (Fig. 2-4). However, the soy isoflavone-rich extract (composed of a mixture of
247 soy isoflavones) significantly decreased the formation of early MR products (Fig. 2) and
248 also the progress of the reaction to the advanced stage. Thus, the formation of both non-
249 fluorescent (CML) (Fig. 3) and fluorescent AGEs (Fig. 4) was significantly inhibited.

250 In the present study, the availability of free amino groups on soy glycinin protein
251 was reduced in all glycation models at 60°C (Fig. 1). This suggests that amino groups
252 on soy glycinin were progressively bound to the carbonyl moiety of fructose and/or
253 isoflavones. In addition, lower levels (20%) of sugar bound to the protein were found in
254 samples treated with the isoflavone enriched extract compared to the glycation control
255 or samples containing the pure isoflavones. These data suggest that isoflavones are able
256 to decrease the reactivity of amino groups on the soy glycinin with fructose molecules
257 in the reaction mixture during the early steps of the MR. In addition, our findings seem

258 to indicate the possible reaction of phenolic compounds with soy glycinin and
259 consequent formation of glycinin-isoflavone complexes. Although the mechanisms of
260 inhibition by polyphenolic compounds of glycation are not completely understood, it is
261 possible that some of these natural compounds bind to protein inhibiting Amadori
262 product generation and subsequent AGEs formation[22]. Further analysis employing
263 advanced analytical tools for the identification of the novel structures is needed to
264 confirm this hypothesis.

265 Several *in vivo* and *in vitro* studies have indicated that dietary phenolic
266 compounds could inhibit the formation of AGEs[7,8,23]. The inhibitory effects of flavonoid
267 compounds on AGEs formation are mainly thought to involve their potent antioxidant
268 activity, leading to scavenging of free radicals formed during glycation, and thus
269 inhibiting the subsequent formation of AGEs. However, limited effort has been devoted
270 to understanding the underlying mechanisms of action of effective natural AGE
271 inhibitors. To inhibit AGEs formation, anti-AGE agents, such as flavonoids, may act
272 through different mechanisms.

273 Reactive dicarbonyl intermediates, such as MGO, play an important role in the
274 chemistry of AGEs formation. Wu *et al.*[24] found that protein co-incubated with
275 genistein and MGO could inhibit MGO-induced reactive oxygen species. Lv *et al.*[25]
276 have hypothesized that dietary flavonoids such as genistein can inhibit the formation of
277 AGEs by trapping reactive dicarbonyl intermediates under neutral and alkaline
278 conditions *in vitro*. Dicarbonyl intermediates may be produced by degradation of
279 fructose. Our data for sugar bound to protein also suggest a major formation of early
280 Maillard reaction products in all samples containing fructose (Fig. 2). In agreement, the
281 trapping of dicarbonyl intermediate seems to be not the only anti-AGEs pathway by
282 which the isoflavones enriched extract operates.

283 It has been proposed that no oxidation reaction is involved in the formation of
284 Amadori or Heyns rearrangement products, whereas oxidation plays a role in the
285 formation of AGEs. Flavonoids with antioxidant properties, such isoflavones, may
286 protect against glycation-derived free-radical-mediated oxidation by acting as transition
287 metal ion chelators, and preventing the self-oxidation of reducing sugars, Amadori
288 products and reactive carbonyl species[26]. It has been reported by Jang *et al.*[27] that
289 daidzein and genistein isoflavones, obtained from *P. lobata* root extracts, possess
290 significant inhibitory activity against fluorescent AGE formation with IC_{50} values of
291 12.0 μg mL^{-1} and 70.1 μg mL^{-1}, respectively. We found no anti-AGEs activity with
292 genistin and genistein at 10 μg mL^{-1} concentration; however, the soy isoflavone-rich
293 extract containing a mixture of different isoflavones at higher concentration (5 mg mL^{-1})
294 was an effective anti-glycation agent.

295 The isoflavone enriched extract greatly inhibited the formation of non-
296 fluorescent (Fig. 3) and fluorescent (Fig. 4) AGEs. The characteristic fluorescence
297 spectrum of AGEs changed in samples containing the isoflavone extract. This is
298 attributed to the absorption of the AGE glycophore, formed by the linking of protein
299 and glucose molecules, in accordance with data obtained by Rondeau *et al.*[28] These
300 results agree with those obtained by Wang *et al.*[19] using ferulic acid and feruloyl-
301 oligosaccharides as glycation inhibitors. They obtained a progressive decrease in
302 fluorescence with increasing concentration of inhibitor.

303 The formation of CML and fluorescent AGEs like pentosidine is catalysed under
304 oxidative conditions. In agreement with this, our data seem to indicate that although part
305 of the AGEs formed in our particular system can come from oxidative sugar
306 degradation (CML), they are also being generated from early MR products (Heyns
307 rearrangement products).

308 The primary structure of isoflavones is three benzene rings with one or more
309 hydroxyl groups; this structure is the key factor that determines their anti-oxidant
310 activity. Matsuda *et al.*[7] examined several flavonoids for inhibitory activity towards
311 AGE formation. Compared to the well-known AGE inhibitor, aminoguanidine,
312 flavonoids showed stronger inhibitory effects. Nevertheless, isoflavones only weakly
313 inhibited AGEs formation (by 25-46% at 200 μM). In the current study, the isoflavones
314 enriched extract showed strong inhibitory activity, therefore, the antioxidative effects of
315 isoflavones are apparently, at least in part, involved in AGEs inhibition mechanisms.

316 In the present *in vitro* study, we demonstrate that a mixture of isoflavones (soy
317 isoflavone-rich extract) is an effective inhibitor of the formation of early MR products
318 and AGEs. Our data suggest that the formation of early MR products may be inhibited
319 by conjugation of isoflavones to the active site of glycation, while AGEs formation may
320 be modulated by trapping of dicarbonyl intermediates and oxygen radical species. In
321 addition, our results suggest that a soy isoflavone-rich extract might be useful for the
322 generation of particular glycoconjugates with improved functional properties. Further
323 research should be performed to confirm this hypothesis. Phytochemomics[29] may be an
324 appropriate tool for the generation of this necessary knowledge.

325

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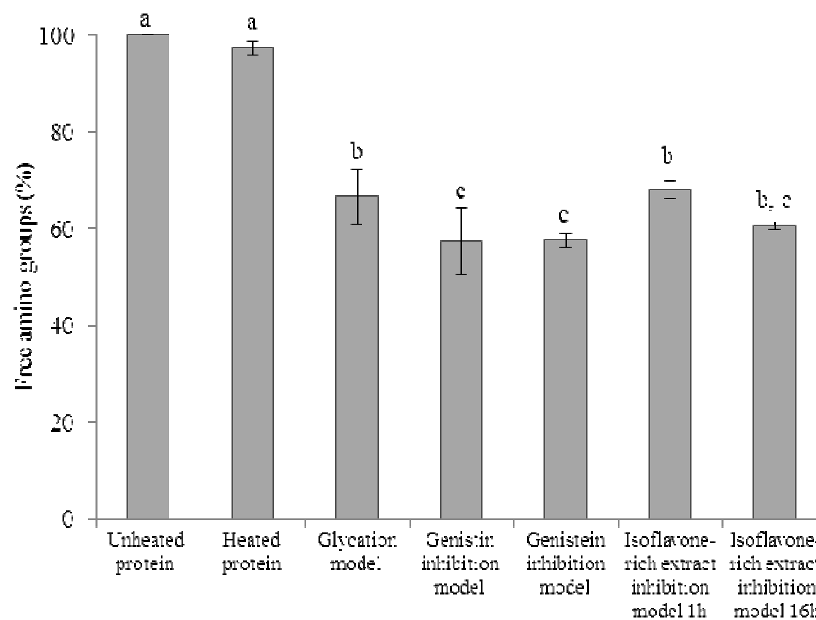
410

411 **Figure 1.** Free amino group content of unheated and heated protein (control), glycation
412 model (soy glycinin protein-fructose) and isoflavones inhibition model in 0.2% KOH
413 solution in presence of genistin, genistein and soy isoflavone-rich extract (60ºC for 60
414 min). Data are expressed as a percentage of the control (unheated protein) value. Data
415 are means of triplicate analyses. Error bars denote the relative standard deviation.
416 Values with different letters are significantly different ($p<0.05$).
417



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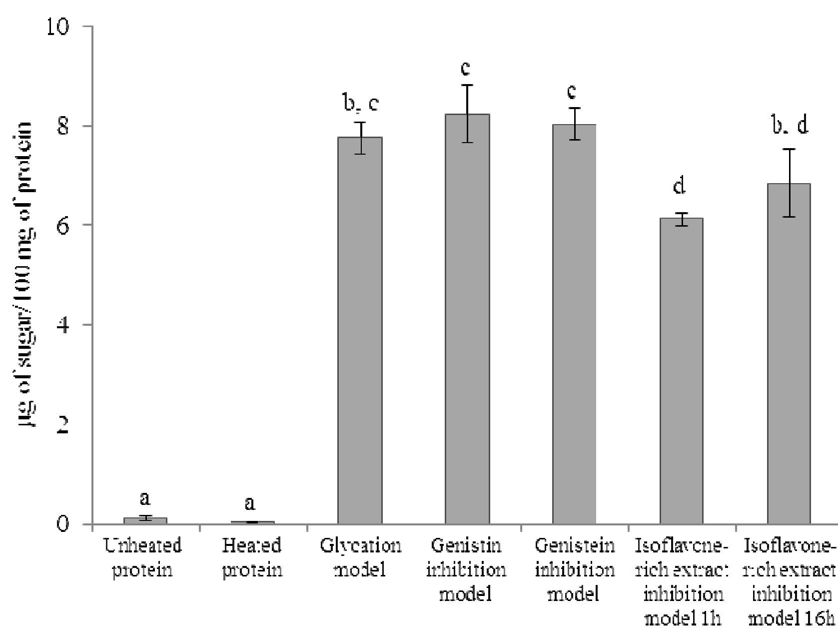
420 **Figure 2.** Sugar content of unheated and heated protein (control), glycation model (soy
421 glycinin protein-fructose) and isoflavones inhibition model in 0.2% KOH solution in
422 presence of genistin, genistein and soy isoflavone-rich extract (60ºC for 60 min). Data
423 are expressed as µg sugar/100 mg protein. Data are means of triplicate analyses. Error
424 bars denote the relative standard deviation. Values with different letters are significantly
425 different (p<0.05).
426



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429 **Figure 3.** Content of protein-bound CML in unheated and heated proteins (control),
430 glycation model (soy glycinin protein-fructose) and isoflavones inhibition model in
431 0.2% KOH solution in presence of genistin, genistein and soy isoflavone-rich extract
432 (60ºC for 60 min). Data are expressed in millimol of CML/mol of lysine. Values are
433 means of triplicate analyses. Data are means of triplicate analyses. Error bars denote the
434 relative standard deviation. Values with different letters are significantly different
435 (p<0.05).
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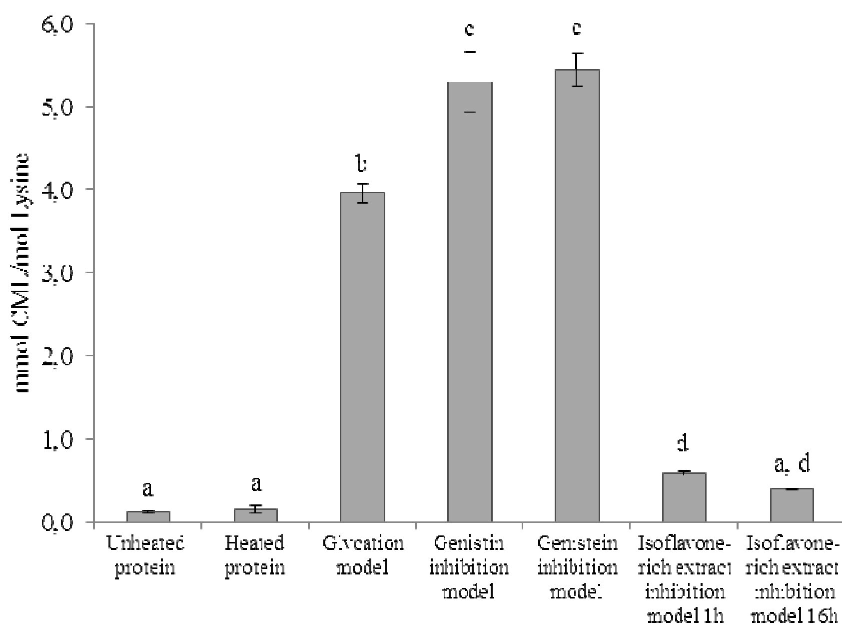

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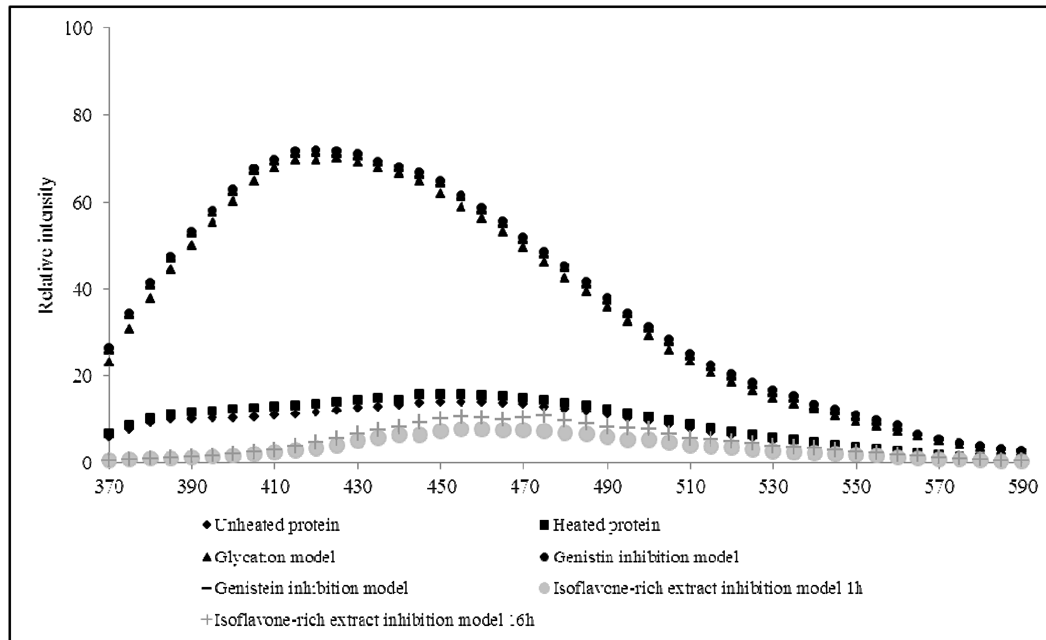
Figure 4. Fluorescence spectra of glycation model systems in 0.2% KOH solution in presence of genistin, genistein and soy isoflavone-rich extract.



Executive Editor,

Food & Function

23/12/2013

Dear Sir / Madam,

Re Silvan et al. **GLYCATION IS REGULATED BY ISOFLAVONES**

Please find attached an electronic version of the above manuscript, including figures and related material for consideration for publication as an original article.

The manuscript, as prepared by **Silván et al.** provides new knowledge on the anti-glycation capacity of soy isoflavones and their potential for the formation of glycoconjugates avoiding tedious and high cost purification processes for removing contaminants (AGEs and unreactive fructose and proteins).

We believe that the content of this paper corresponds well to the aims and scope of the journal.

Yours sincerely,

Dr. Dolores del Castillo (corresponding author)

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