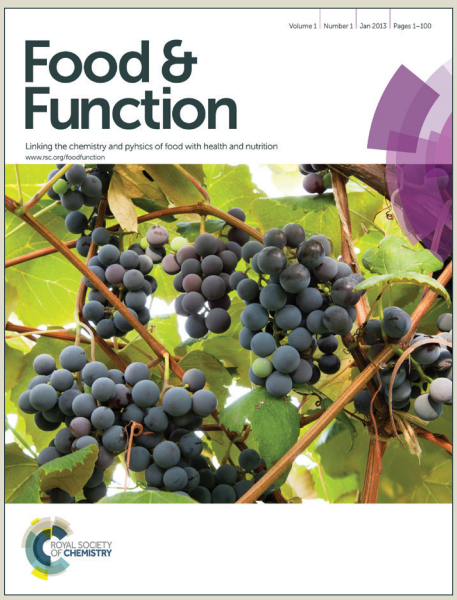
Food & Function

Accepted Manuscript



This is an *Accepted Manuscript*, which has been through the Royal Society of Chemistry peer review process and has been accepted for publication.

Accepted Manuscripts are published online shortly after acceptance, before technical editing, formatting and proof reading. Using this free service, authors can make their results available to the community, in citable form, before we publish the edited article. We will replace this *Accepted Manuscript* with the edited and formatted *Advance Article* as soon as it is available.

You can find more information about *Accepted Manuscripts* in the Information for Authors.

Please note that technical editing may introduce minor changes to the text and/or graphics, which may alter content. The journal's standard Terms & Conditions and the Ethical guidelines still apply. In no event shall the Royal Society of Chemistry be held responsible for any errors or omissions in this *Accepted Manuscript* or any consequences arising from the use of any information it contains.

ROYAL SOCIETY OF CHEMISTRY

www.rsc.org/foodfunction

38x38mm (96 x 96 DPI)

Treatment of proteins with dietary polyphenols lowers the formation of AGEs and AGEs-induced toxicity

Xinchen Zhang, [a] Shuting Hu, [a] Feng Chen, [b] and Mingfu Wang*[a]

[a] *School of Biological Sciences, The University of Hong Kong, Pokfulam, Hong Kong. Email: mfwang@hku.hk; Tel: +852-22990338; Fax: +852-22990348.*

[b] *Institute for Food & Bioresource Engineering, College of Engineering, Peking University, Beijing 100871, China. Email: sfchencoe@pku.edu.cn; Tel: +86-10-82529003.*

Abstract

The advanced glycation endproducts (AGEs) are a group of harmful compounds produced either endogenously or during thermal food processing. Once absorbed by humans via food intake, AGEs can cause oxidative cell damage and contribute to pathological development of various diseases. The AGEs-inhibitory activity of dietary polyphenols *in vitro* has been extensively reported before, but the current study is pioneering in examining the antiglycation activity of five selected dietary polyphenols (phloretin, naringenin, epicatechin, chlorogenic acid, and rosmarinic acid) during thermal protein glycation process. When added into the glucose-casein glycation model heated at 120 °C for 2 hrs, these polyphenols were capable of inhibiting the formation of both total fluorescent AGEs and nonfluorescent carboxymethyllysine (CML). The thermal stability and transformation of polyphenols is likely an important factor affecting their antioxidant activity and inhibitory efficacy of reactive carbonyl species formation. Treatment with epicatechin would not only lower AGEs formation but AGEs-induced cytotoxicity and oxidative stress to human retinal pigment epithelial (ARPE-19) cells.

1. Introduction

The advanced glycation endproducts (AGEs) are a group of compounds produced in the advanced stage of protein glycation reactions, including both fluorescent products such as pentosidine and nonfluorescent products like carboxymethyllysine (CML). Formation of AGEs have been reported both *in vivo* via metabolism and *in vitro* from heating sugar and protein, and the latter accounts for the extraneous source of dietary AGEs ingested by humans in a wide variety of foodstuffs, such as meat, milk products and bakery products. [1] Compared with endogenous formation occurring in physiological conditions at 37 °C, formation of AGEs during food processing is usually at a much higher temperature. As indicated by a recent survey, the amount of AGEs in processed foods depends on the parameters of thermal treatments, including temperature, time and moisture. Among different cooking methods, broiling (225 °C) and frying (177 °C) showed highest AGEs formation capacity, followed by roasting (177 °C) and boiling (100 °C). [1] It is also interesting to know that in saccharide-lysine model systems, microwave-heating method had higher CML formation capacity than water-heating and drying oven-heating methods. [2]

About 10% of consumed dietary AGEs are absorbed by humans and correlate with circulating and tissue levels of AGEs. [3] The ability of AGEs to form cross-links with intracellular proteins, receptors and extracellular matrix components will lead to structural and functional modifications of these molecules. [4,5] Meantime, AGEs promote the cellular oxidative stress and damage. [6] The associations between AGEs with pathological development of diabetic complications, renal failure, atherosclerosis, Alzheimer's disease,

arthritis, and chronic heart failure have been well documented. [7,8] Hence, the strategies to reduce the dietary AGEs formation during thermal food processing and alleviate their toxicity will be of clinical importance to human health.

Polyphenols are reputable antioxidants and given their natural origins, they are regarded as functional food ingredients safer than synthetic compounds. As the advanced stage of protein glycation reactions is facilitated by free radicals, polyphenols have been investigated on their inhibitory effects on the AGEs formation. Compared with experiments carried out in simulated physiological conditions, there are scarce investigations on the antiglycation effects of polyphenols in thermal protein glycation models. As examples, in protein glycation model composed of fructose and soy glycinin or bovine serum albumin (BSA) incubated at 60 °C for 60 mins, ferulic acid reduced fluorescent AGEs and CML formation by nearly 90% and 85%, respectively. [9] Addition of ferulic acid to sponge cake baked at 190 °C for 30 mins also significantly lowered the level of CML. [10] Grape seed extract rich in phenolic compounds could reduce CML in baked bread dose-dependently. [11] The ability of polyphenols to inhibit the AGEs formation would possibly be affected by factors such as heating time and temperature. For example, rutin is a potent inhibitor toward AGEs formation from glucose and BSA incubated at 37 °C, but no inhibition of CML was observed in the baked sponge cake model possibly due to thermal instability of rutin. [10] Therefore, it's essential to explore the antiglycation activity of polyphenols in thermal models so as to find out ways to minimize the levels of AGEs in heat-treated foods.

The current study established a thermal protein glycation model composed of glucose and casein heated at 120 °C for 2 hrs to mimic the real thermal food processing conditions. Five selected types of dietary polyphenols were added to the model system and their impacts on the formation of reactive carbonyls, fluorescent AGEs and nonfluorescent CML were examined. Finally, the effects of polyphenol-fortified protein glycation products on cell proliferation and oxidative status were explored in human retinal pigment epithelial (ARPE-19) cells.

2. Materials and methods

2.1 Reagents and chemicals

Epicatechin was purchased from Biopurify Phytochemicals Ltd. (Chengdu, China). Phloretin, naringenin, chlorogenic acid, rosmarinic acid, glucose, casein, disodium hydrogen phosphate, sodium dihydrogen phosphate, sodium sulfite, sodium hydroxide, sodium carbonate, sodium bicarbonate, TNBS (2,4,6-trinitrobenzenesulfonic acid), NBT (nitro blue tetrozolium), OPD (*o*-phenylenediamine), quinoxaline, 2-methylquinoxaline, tween-20, p-nitrophenyl phosphate substrate, HEPES (4-(2-hydroxyethyl) piperazine-1-ethanesulfonic acid), and DCFH-DA (dichloro-dihydro-fluorescein diacetate) were all purchased from Sigma-Aldrich (St. Louis, MO, USA). Rabbit polyclonal antibody to carboxymethyllysine and alkaline phosphatase conjugated goat polyclonal antibody to rabbit IgG were purchased from Abcam (Cambridge, UK). The ARPE-19 cell line was kindly provided by Dr. Amy Lo (Eye Institute, The University of Hong Kong). Low glucose DMEM (Dulbecco's Modified Eagle medium) and Ham's F-12 nutrient mix, FBS fetal bovine serum, penicillin-streptomycin solution, trypsin, PBS (phosphate buffered saline) were obtained from Invitrogen (Grand island, NY, USA).

CCK-8 (cell counting kit-8) was purchased from Dojindo China Co., Ltd. (Shanghai, China). All solvents and acids used were of analytical or HPLC grade and were obtained from BDH Laboratory Supplies (Poole, UK).

2.2 Thermal protein glycation model

2 mL of solution containing 3.2% casein and 400 mM D-glucose in 0.1 M sodium phosphate buffer (pH=7 or 10) was aliquoted to each 14-mL screw cap septum vials (Thermo scientific, IL, USA). Dietary polyphenols (phloretin, naringenin, epicatechin, chlorogenic acid, rosmarinic acid) were added to the reaction mixture at a concentration of 10 mM. The solutions were then subject to heating at 120 °C in silicone oil bath for 2 hrs. After heating, the samples were immediately removed from oil bath and cooled in ice-water before further analysis.

2.3 Measurement of free amino group content and fructosamine

The free amino group content was measured by TNBS method. [12] 125 μL properly diluted sample solution was thoroughly mixed with 2 mL of 0.2125 M phosphate buffer (pH=8.2) and 1 mL of 0.01% TNBS solution. The solutions were then incubated in 50 °C water bath for 30 mins in dark. 2 mL of 0.1 M sodium sulfite was used as stopping solution. The solutions were cooled at room temperature for 15 mins before taken absorbance at 420 nm. The blank was prepared in the same manner as the samples except that Milli-Q water was used in place of 0.01% TNBS. Free amino group content was expressed in terms of L-leucine.

The fructosamine was measured by spectrophotometric method. [13] The sample solutions were properly diluted and 0.2 mL of diluted solution was reacted with 0.8 mL of 300 μM NBT

reagent in 100 mM sodium carbonate buffer (pH=10.3) at room temperature for 15 mins. The amount of fructosamine was indicated by reading absorbance at 530 nm using a spectrophotometer (UV-1206, Shimadzu, Japan).

2.4 Determination of glyoxal (GO) and methylglyoxal (MGO)

The two typical representatives of reactive carbonyls, GO and MGO, were quantified by the derivatization HPLC/DAD method. [14] Derivatization was conducted by mixing 300 μL of sample with 300 μL of methanol and 200 μL of 10 mM OPD and afterwards incubating the solutions for 3 hrs at 70 ℃ in an oven. After cooling to room temperature, 200 μL of acetic acid was mixed with the derivatized mixture. The samples were then filtered by 0.45 μm membrane before subject to HPLC/DAD analysis. After derivatization with OPD, GO and MGO could be measured as quinoxaline (Q) and 2-methylquinoxaline (2-MQ) detectable by UV absorbance at 315 nm correspondingly. The analysis was performed on a Shimadzu HPLC system which is composed of a separation module (LC-20AT), an autosampler (SIL-20A), a degasser (DGU-20A3), and a photodiode array detector (SPD-M20A). Separation was conducted on an ACE C18 column (5 μm, 250×4.6 mm, Advanced Chromatography Technologies, Aberdeen, U.K.). Elution was performed with a mixture of A: 0.5% (*v/v*) acetic acid in water and B: methanol and gradient flow was as followed: 0 min, 16% B; 2 min, 16% B; 22 min, 32% B; 37 min, 80% B; 42 min, 80% B; 43 min, 16% B; 53 min, 16% B. Flow rate was 0.8 mL/min and injection volume equaled to 10 μL. Quantitative interpolation was facilitated by calibration curves constructed by Q and 2-MQ standards.

2.5 Measurement of total fluorescent AGEs and nonfluorescent CML

100 μL of sample solution was pippeted to each well of 96-well plate and fluorescent AGEs

were indicated by fluorescence reading with excitation wavelength of 355/40 nm and emission wavelength of 405/10 nm (Victor X4 Multilabel Plate Reader, PerkinElmer, USA). [15]

CML was analyzed by enzyme-linked immunosorbent assay (ELISA). [15] The sample solution was properly diluted in 50 mM of sodium carbonate buffer (pH=9.5-9.7) and 100 μL of diluted solution was loaded to each well of 96-well polystyrene plate. Antigen coating lasted overnight at 4 °C. After coating, wells were washed by 200 μL of washing solution (1×PBS with 0.05% tween-20) for 3 times. Blocking was achieved by adding 300 μL of 0.5% gelatin solution and incubating for 2 hrs at 37 °C. The wells were washed 3 times by 200 μL of washing solution before adding 100 μL of 500 times diluted rabbit polyclonal antibody to CML and incubating for 1 hr at 37 °C. After washing 3 times with 200 μL of washing solution, 100 μL of 1000 times diluted alkaline phosphatase conjugated goat polyclonal antibody to rabbit IgG was loaded to each well and the plate was incubated for 1 hr at 37 °C. Subsequent to a final washing step, 100 μL p-nitrophenyl phosphate substrate solution was added to each well. Incubation lasted 15 mins at room temperature in dark and absorbance was read at 405 nm by a Multilabel Plate Reader (Victor X4, PerkinElmer, USA).

2.6 Cell culture

The ARPE-19 cells were cultured in 15 mL (75 cm^2 flask) low glucose DMEM/F12 medium containing 10% FBS and 1% penicillin/streptomycin. The cell culture was maintained in a humidified incubator at 37 °C, 5% CO_2. Cells were subcultured when they reached over 80% confluence. Cell density was estimated by a haemocytometer.

133 **2.7 Assessment of cell proliferation**

134 The cell proliferation was assessed by CCK-8. Cells were seeded in wells of 96-well plates.
135 After cell attachment, the medium was removed and cells were exposed to different
136 concentrations of normal or epicatechin-fortified protein glycation solution prepared at pH=7
137 for 24, 48 and 72 hrs respectively. After treatment, the medium was disposed and cells were
138 washed for 3 times by 1× PBS. 100 μL 10% CCK-8 was aliquoted to each well and
139 incubation lasted for 1-4 hrs at 37 ℃. Finally, the absorbance at 450 nm was determined by a
140 micro-plate reader (Bio-Rad, CA, USA).

141 **2.8 Measurement of intracellular reactive oxygen species (ROS)**

142 The amount of intracellular ROS was determined by the fluorescent products of DCFH-DA.
143 [16] Cells were seeded in wells of black 96-well plates. After cell attachment, the medium was
144 removed and cells were incubated in different concentrations of normal or
145 epicatechin-fortified protein glycation solution prepared at pH=7 for 24 hrs. After treatment,
146 the medium was disposed and cells were washed for 3 times by 1× PBS. 100 μL of 25 μM
147 DCFH-DA was aliquoted to each well and incubation lasted for 30 mins at 37 ℃. The
148 formation of fluorescent products induced by ROS was indicated by fluorescence reading
149 with excitation wavelength of 485 nm and emission wavelength of 535 nm (Victor X4
150 Multilabel Plate Reader, PerkinElmer, USA).

151 **2.9 Statistical analysis**

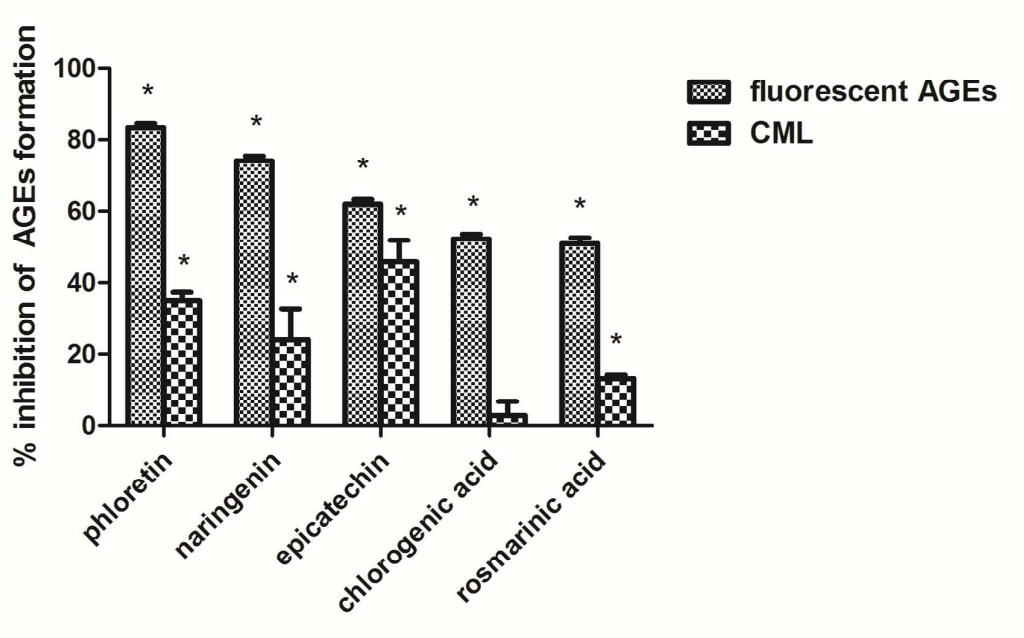
152 Statistical analyses were carried out by Graphpad Prism 5 software package (GraphPad
153 Software, Inc., La Jolla, CA, USA). Data are expressed as the mean ± standard error of
154 triplicate determinations and differences with $P<0.05$ were considered to be statistically

155 significant.

156 **3. Results and discussion**

157 **3.1 Impacts of polyphenols on the formation of AGEs**

158 Previous studies have indicated that a variety of plant extracts were capable of inhibiting the
159 formation of AGEs from sugar and protein co-incubated in the simulated physiological
160 conditions and the antiglycation activities were found to be positively correlated with the
161 total phenolic content, suggesting that the dietary polyphenols were likely the primary
162 antiglycation agents in the extract. [13, 17- 22] However, the results obtained in these *in vitro*
163 studies could not be relied on to deduce the antiglycation potential of dietary polyphenols in
164 thermal conditions, and there were examples that polyphenols' antilgycation activity was lost
165 possibly attributable to thermally-induced structural decomposition. [10] Current study
166 attempted to fill the gap of polyphenols' thermal antiglycation properties and results
167 demonstrated that in thermal glucose-casein glycation model, all tested polyphenols exhibited
168 over 50% inhibition on the production of fluorescent AGEs at neutral pH and phloretin and
169 naringenin were the most potent inhibitors among tested categories with over 70% inhibition
170 (Fig. 1). The inhibitory activities on CML formation were generally weaker that four out of
171 five polyphenols reduced CML level by 13% to 45% at neutral pH (Fig. 1). The order of
172 phenolics' antiglycation activity followed the same pattern at alkalinic pH but the inhibition
173 rate was generally lower (data not shown).



174

175 **Fig. 1** Inhibitory effects of polyphenols on the formation of total fluorescent AGEs and CML
176 in the thermal casein glycation model at pH=7. Bars with an asterisk indicate significant
177 difference from control ($P<0.05$).

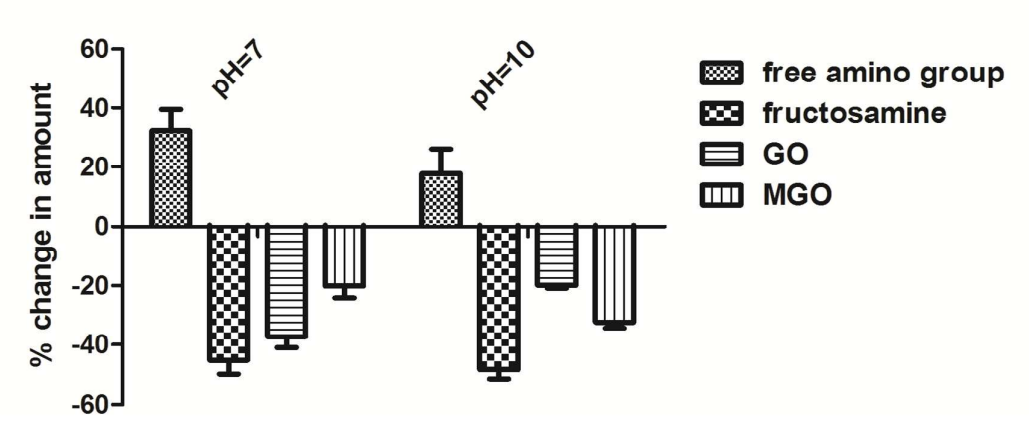
178

179 Free radical scavenging is one of the most recognizable antiglycation mechanisms. In our
180 study, only weak to moderate correlation could be established between the free radical
181 scavenging capacity of polyphenols and their inhibitory activity of AGEs formation,
182 suggesting that the antiglycation activity of tested polyphenols under thermal treatment may
183 depend on factors other than antioxidative capacity, such as the solubility and thermal
184 stability of phenolics in the model system. [10] Compared with physiological conditions,
185 polyphenols were subject to much more severe structural degradation under thermal
186 conditions and alkalinic pH may be associated with further decrease in thermal stability.
187 Taking epicatechin as an example, we have previously reported that heating the polyphenol
188 alone at 120 °C for 2 hrs led to a great reduction in its antioxidant capacity, probably due to

loss of the particular structure carrying antioxidant capacity. [23] For phloretin and naringenin, decrease in the antioxidant capacity could also be induced by thermal reaction between polyphenols and Maillard reaction intermediates (e.g. sugar fragments). [23] Moreover, the temperature rise may introduce alterations in the reactivity of polyphenols towards protein glycation intermediates. [24]

3.2 Impacts of polyphenols on the formation of glycation intermediates

The α-dicarbonyl compounds are important protein glycation intermediates. They are generated via sugar autoxidation or thermal degradation of Schiff base or Amadori rearrangement products. GO and MGO are two representative reactive carbonyls leading to formation of AGEs. [9, 14] The levels of GO and MGO were quantified as 11.7 and 9.3 μg/mL at pH=7 while the values were 19.2 and 23.5 μg/mL at pH=10. The production of α-dicarbonyls was promoted at alkaline pH, which condition may favor the oxidation of Amadori products. [25] Previously published reports suggested that phloretin and epicatechin were capable of trapping these two reactive carbonyls under simulated physiological conditions. [26, 27] However, the results collected in this study suggest that phloretin and epicatechin could not lower the content of MGO or GO in this thermal model, possibly because their active binding sites for the carbonyls were lost by thermal degradation or interaction with other reaction fragments. [23, 24] Chlorogenic acid was the only phenolic compound that can reduce both GO and MGO levels in our reaction model system (Fig. 2).



208

209 **Fig. 2** The impacts of chlorogenic acid on the amounts of free amino group, fructosamine,

210 GO and MGO in the thermal casein glycation model.

211

212 In order to gain more information on the mechanism of chlorogenic acid's inhibitory activity

213 against α-dicarbonyls formation, the amount of free amino group and early-stage

214 fructosamine were quantified. Gradual decrease in the free amino group content is a

215 noticeable phenomenon as Maillard reaction proceeds, [12, 28] which reflects covalent

216 attachment of sugar and the so-called "glycation" process. Phloretin and epicatechin did not

217 significantly influence the free amino group content of the protein glycation solution whilst

218 chlorogenic acid-fortified solution contained a significantly higher quantity of free amino

219 group (Fig. 2). Chlorogenic acid addition was further found to reduce the amount of

220 early-stage fructosamine (Fig. 2). Reduction in the degree of blocking of free amino groups

221 suggests a lower level of Schiff base formation from glucose incorporation onto casein, and

222 therefore decrease in the quantity of the rearrangement product of Schiff base – fructoamine.

223 On the basis of the above findings, the capability of reducing the amount of Schiff base and

224 fructosamine, which are precursors of α-dicarbonyls, may partially explain chlorogenic acid's

225 inhibition of α-dicarbonyls formation. The polyphenols' role in the early stage of protein

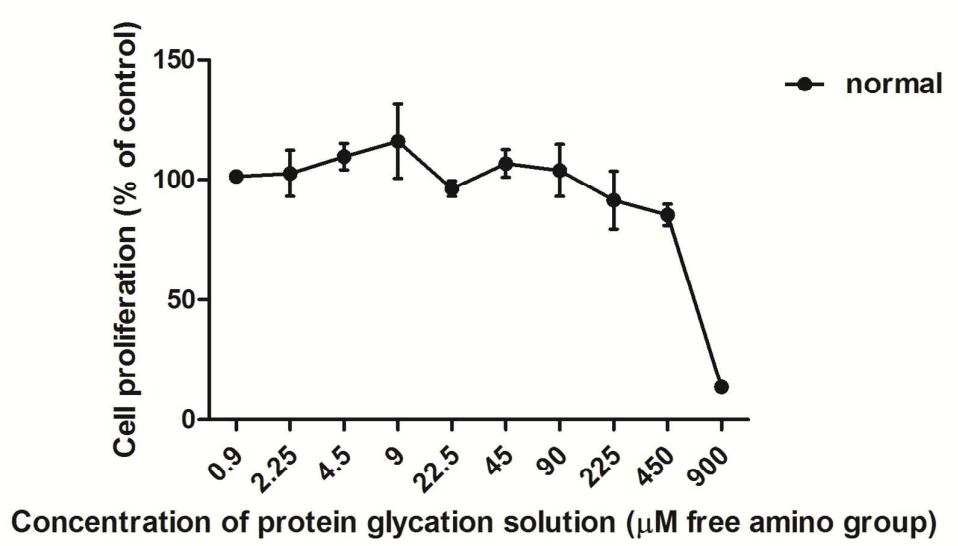
glycation may be further investigated in the context of phospholipids glycation, which is also an important process in foods and *in vivo*. [29, 30]

3.3 The protection of epicatechin fortification against cell damage induced by glycation products

Based on previous discussions, epicatechin fortification during thermal casein glycation process led to significantly reduced amount of both fluorescent AGEs and CML in the final protein glycation solution. It remained to be seen whether epicatechin fortification could alleviate the oxidative damage of the fortified glycation products in cells accordingly. ARPE-19 cells were selected for investigation because early studies have demonstrated that exogenous AGEs were correlated with pathological development of diabetic retinopathy. [16] The content of free amino group expressed in term of μM of L-leucine was used in Fig. 3 and the following discussions to indicate the concentration of protein glycation solution that cells were exposed to. The free amino group content in original normal protein glycation solution prepared at pH=7 was around 9 mM and as mentioned above, epicatechin fortification had no effect on the free amino group content. In agreement to early observation, [31] exposure to normal protein glycation solution for 24 hrs showed no impact on the proliferation of ARPE-19 cells when the content of free amino group was not higher than 225 μM (Fig. 3A). There's no significant difference on the proliferative rates of cells treated by normal or epicatechin-fortified protein glycation solution. The cytotoxicity of protein glycation solution at higher concentrations (free amino group content equaled 450 or 900 μM), however, was significantly lowered by epicatechin fortification, as justified by higher cell viability (Fig.3B). The protective effect of epicatechin fortifcation against the cytotoxicity induced by high

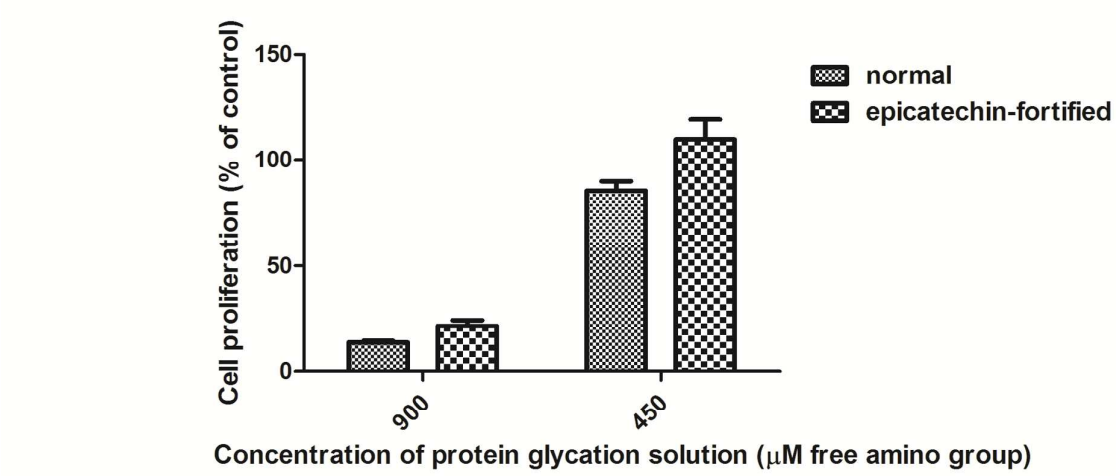
248 concentrations of protein glycation solution was consistently observed when the exposure
249 time was elongated to 48 and 72 hrs (data not shown).

250 (A)



251

252 (B)



253

254 (C)

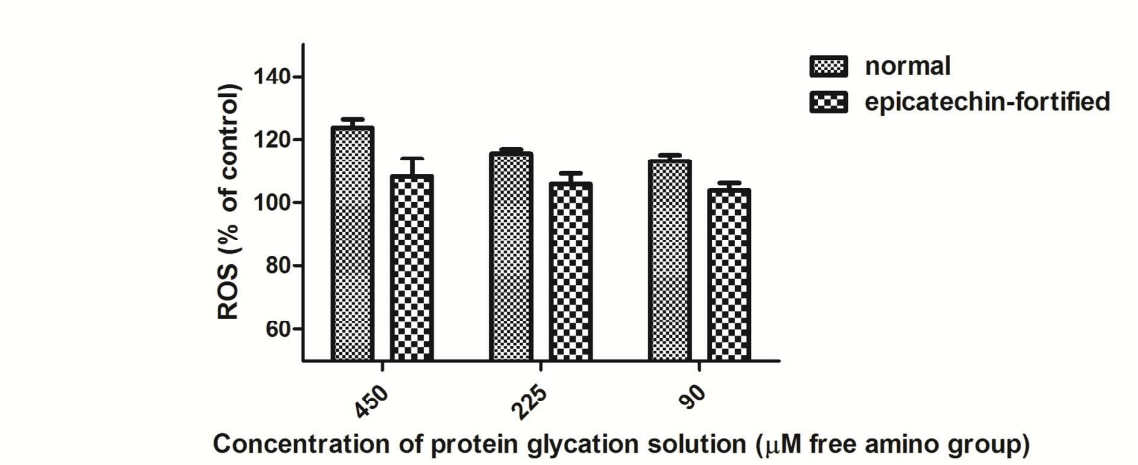


Fig. 3 (A) Proliferation of ARPE-19 cells after 24 hrs exposure to normal protein glycation solution; (B) Cell viability comparison after 24 hrs exposure to either normal or epicatechin-fortified protein glycation solution; (C) Intracellular ROS comparison after 24 hrs exposure to either normal or epicatechin-fortified protein glycation solution. The concentration of solution was expressed in term of free amino group content.

Cultured in the protein glycation solution diluted in the medium, ARPE-19 cells were exposed to AGEs, which are among recognizable factors promoting cell apoptosis via inducing oxidative stress and inflammation. [32] As shown in Fig. 3C, there were about 13-20% increases in the intracellular ROS after cell exposure to normal protein glycation solution (free amino group content equaled 90 to 450 μM) for 24 hrs whereas exposure to epicatechin-fortified protein glycation solution of same concentrations resulted in around 10% less in oxidative stress. Hence, it's suggested that epicatechin fortification during thermal casein glycation process reduced the amount of AGEs in the fortified solution and therefore alleviated the AGEs-mediated oxidative damage to cells. Remaining epicatechin and its thermal reaction products possessing antioxidative capacity in the fortified protein glycation

solution may also clear the free radicals, increase cell's antioxidant ability and therefore promote the cell viability.

4. Conclusions

In this paper, the antiglycation activity of five selected dietary polyphenols in thermal protein glycation process was discussed. Further to AGEs-inhibitory potential in simulated physiological conditions, polyphenols were found to be capable of reducing AGEs formation under thermal conditions. The thermal stability and transformation of polyphenols is likely an important factor affecting their antioxidant activity and inhibitory efficacy of reactive carbonyl species formation. Therefore, the antiglycation potential of polyphenols during thermal processing may quite differ from that shown in physiological conditions. Finally, polyphenol fortification during thermal protein glycation process could be a promising way to alleviate the toxicity and oxidative stress brought by AGEs to ARPE-19 cells.

References

1 T. Goldberg, W. Cai, M. Peppa, V. Dardaine, B. S. Baliga, J. Uribarri, and H. Vlassara, *J. Amer. Diet. Assn.,* 2004, **104**, 1287-1291.

2 Q. Fu, L. Li, and B. Li, *Int. J. Food Eng.,* 2012, **8**, Article 1.

3 T. Koschinsky, C. J. He, T. Mitsuhashi, R. Bucala, C. Liu, C. Buenting, K. Heitmann, H. Vlassara, *Proc. Natl. Acad. Sci. USA*, 1997, **94**, 6474-6479.

4 A. M. Schmidt, O. Hori, J. Brett, S. D. Yan, J. L. Wautier, D. Stern, *Arterioscler. Thromb.*, 1994, **14**, 1521-1528.

5 H. Vlassara. *Diabetes/Metab. Rev.*, 2001, **17**, 436-443.

6 F. Imani, Y. Horii, M. Suthanthiran, E. Y. Skolnik, Z. Makita, V. Sharma, P. Sehajpal, H.

294 Vlassara. *J. Exp. Med.*, 1993, **178**, 2165-2172.

295 7 A. J. Smit, J. W. Hartog, A. A. Voors, D. J. van Veldhuisen, *Ann. N. Y. Acad. Sci.,* 2008,

296 **1126**, 225-230.

297 8 J. Smit, H. L. Lutgers, *Curr. Med. Chem.,* 2004, **11**, 2767-2784.

298 9 J. M. Silvan, S. H. Assar, C. Srey, M. D. del Castillo, and J. M. Ames, *Food Chem.,* 2011,

299 **128**, 208-213.

300 10 C. Srey, G. L. J. Hull, L. Connolly, C. T. Elliott, M. D. D. Castillo, and J. M. Ames, *J.*

301 *Agr. Food Chem.,* 2010, **58**, 12036-12041.

302 11 X. Peng, J. Ma, K. W. Cheng, Y. Jiang, F. Chen, and M. Wang, *Food Chem.,* 2010, **119**,

303 49-53.

304 12 W. Lertittikul, S. Benjakul, and M. Tanaka, M. *Food Chem.,* 2007, **100**, 669-677.

305 13 J. W. Wu, C. L. Hsieh, H. Y. Wang, and H. Y. Chen, *Food Chem.,* 2009, **113**, 78-84.

306 14 G. Arribas-Lorenzo, and F. J. Morales, *J. Agr. Food Chem.,* 2010, **58**, 2966-2972.

307 15 Z. Sun, X. F. Peng, J. Liu, K. W. Fan, M. F. Wang, and F. Chen, *Food Chem.,* 2010, **120**,

308 261-267.

309 16 Z. Sun, J. Liu, X. Zeng, J. Huangfu, Y. Jiang, M. Wang, and F. Chen, *Food Funct.,* 2011,

310 **2**, 251-258.

311 17 C. S. Harris, L. P. Beaulieu, M. H. Fraser, K. L. McIntyre, P. L. Owen, L. C.

312 Martineau, . . . J. T. Arnason. *Planta Med.,* 2011, **77**, 196-204.

313 18 W. Kusirisin, S. Srichairatanakool, P. Lerttrakarnnon, N. Lailerd, M. Suttajit, C. Jaikang,

314 and C. Chaiyasut. *Med. Chem.,* 2009, **5**, 139-147.

315 19 X. Peng, Z. Zheng, K. Cheng, F. Shan, G. Ren, F. Chen, and M. Wang. *Food Chem.,* 2008,

316 **106**, 475-481.

317 20 J. S. Ramkissoon, M. F. Mahomoodally, N. Ahmed, and A. H. Subratty. *Asian Pac. J.*

318 *Trop. Med.,* 2013, **6**, 561-569.

319 21 M. Rudnicki, M. R. de Oliveira, T. D. Veiga Pereira, F. H. Reginatto, F. Dal-Pizzol, and J.

320 C. Fonseca Moreira. *Food Chem.,* 2007, **100**, 719-724.

321 22 E. Verzelloni, D. Tagliazucchi, D. D. Rio, L. Calani, and A. Conte. *Food Chem.,* 2011,

322 **124**, 1430-1435.

323 23 X. C. Zhang, F. Chen, and M. F. Wang, *Food Chem.,* 2013, **141**, 3451-3458.

324 24 J. Ma, X. Peng, K. M. Ng, C. M. Che, and M. Wang, *Food Funct.,* 2012, **3**(2), 178-186.

325 25 R. Nagai, K. Ikeda, Y. Kawasaki, *et al.*, *FEBS Lett.,* 1998, **425**, 355-360.

326 26 C.Y. Lo, S. M. Li, D. Tan, M. H. Pan, S. M. Sang, and C. T. Ho, *Mol. Nutr. Food. Res.,*

327 2006, **50**, 1118-1128.

328 27 X. Shao, N. S. Bai, K. He, C. T. Ho, C. S. Yang, and S. M. Sang, *Chem. Res. Toxico.,*

329 2008, **21**, 2042-2050.

330 28 S. Dong, B. Wei, B. Chen, D. J. Mcclements, and E. A. Decker, *J. Agr. Food Chem.,*

331 2011, **59**, 13311-13317.

332 29 R. Bucala, Z. Makita, T. Koschinsky, A. Cerami, and H. Vlassara. *Proc. Natl. Acad. Sci.*,

333 1993, **90**, 6434-6438.

334 30 V. Levi, G. A. Villamil, P. Castello, J. P. F. C. Rossi, and F. F. Gonzalez. *Biochem. J.*,

335 2008, **416**, 145-152.

336 31 S. Hoffmann, U. Friedrichs, W. Eichler, A. Rosenthal, and P. Wiedemann, *Graefes Arch.*

337 *Clin. Exp. Ophthalmol.,* 2002, **240**, 996-1002.

32 Y. Xu, L. Feng, S. Wang, *et al.*, *J. Cell. Biochem.,* 2011, **112**, 2953-2965.