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Antioxidative potential of melanoidins isolated from a roasted glucose–glycine model

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Abstract

The antioxidative activity of the total water soluble fraction (sol A), high molecular weight (HMW; MW > 12,400 Dalton), low molecular weight (LMW) and the insoluble fraction (IS) of a glucose–glycine model system roasted at 120°C was studied in hydrophilic solutions (PBS buffer, fruit juices; addition: 0.01–0.1%) at ambient temperature and lipophilic (coconut fat, triolein and corn oil; addition: 0.01–0.5%) matrices at 60°C and frying conditions at 200°C. The hydrophilic reducing power in the water-soluble fractions was evaluated with the ABTS [2,2′-azino-bis(3-ethylbenz-thiazoline-6-sulfonic acid)] method. Fat stability was determined by observing the development of the peroxide value and conjugated dienes. All fractions increased the hydrophilic reducing power in the water-soluble fractions as a matter of concentration with highest effects for the 0.1% enrichment. In PBS buffer the HMW was most effective, in fruit juices the IS. A slight antioxidative effect in fats was observed only for 0.5% IS and 0.5% sol A in corn oil. Neither in coconut fat at 200°C nor in triolein at 60°C Maillard reaction products (MRPs) were able to extend shelf life. The results performed describe MRPs as highly antioxidative in water-soluble but less effective in water-insoluble fractions.

Keywords: Maillard reaction products; Antioxidative activity; ABTS; Shelf life; Oxidation temperature

1. Introduction

The reduction of shelf life, nutritional quality and the generation of off-flavours in foods by rancidity is one of the main problems in food technology and food safety. Refined and processed foods are usually exposed to light, heat or metal ions which can cause structural degradation of their constituent lipids by triggering the process of lipid oxidation. Since the research activities were focused on identifying natural antioxidants a lot of papers have been published on this topic (Bedows, Jagait, & Kelly, 2001; Sanchez-Moreno, Satue-Gracia, & Frankel, 2000; Wagner, Wotruba, & Elmadfa, 2001; Yokozawa, Cho, Hara, & Kitani, 2000).

Maillard reaction products (MRPs) have been shown to efficiently suppress oxidation in different foods like cereals, milk, meat, juices or nuts (Anese, Manzocco, Nicoli, & Lenci, 1999; Bedinghaus & Ockerman, 1995; Hansen & Hemphill, 1984; Hwang, Shue, & Chang, 2001; Lenci & Nicoli, 1996; Wijewickreme & Kitts, 1998) and in model systems (Jing & Kitts, 2000; Wijewickreme & Kitts, 1997; Yoshimura, Iijima, Watanabe, & Nakazawa, 1997).

However, little information is available on the antioxidative potential of MRPs, formed under defined conditions, either on water soluble or fat soluble matrixes, as a result of concentration and considering the impact of the oxidation temperature. Although reports identified hydrophilic substances like gallic acid or ascorbic acid as antioxidants that are very active in preventing oils from lipid oxidation (Frankel, 1996; Huang, Frankel, Schwarz, Aeschbach, & German, 1996) MRPs have not been investigated in fats or oils with regard to different oxidation conditions.

This study was designed to investigate the antioxidative effects of different fractions of model MRPs generated from a glucose–glycine model. Antioxidative properties were evaluated in buffer, fruit juices, coconut fat, triolein and corn oil at different oxidation temperatures (Table 1).

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2. Material and methods

2.1. Test chemicals

All chemicals were purchased from Sigma unless otherwise stated.

2.2. Preparation of the melanoidin solutions in the reaction mixture of glucose–glycine

The instruction for the separation of the melanoidin solutions were prepared in the COST Action 919 "Melanoidins in Food and Health" and previously published (Hofmann, Ames, Krome, & Faist, 2001). Briefly, 0.05 mol of both glucose and glycine were dissolved in 20 ml distilled water, frozen, freeze dried over night and heated at 120°C for exactly 2 h. Thereafter the beaker was removed and cooled to room temperature. Two hundred milliliters of distilled water was added to 5 g of the beaker and stirred for 12 h at 4°C to dissolve as much material as possible. The mixture was filtered and the collected filtrate filled up to 250 ml. This mixture is called Solution A (Sol A). The sum of the UV-visible absorbance spectrum (200–600 nm) of Sol A measured at 280, 360, 420 and 520 nm was 0.967 nm (should be <1.0). One half of this solution was freeze dried, the other one dialysed at 4°C with double-distilled deionized water with a dialysis tubing to receive a low (LMW) and a high molecular weight fraction (HMW; molecular weight $>$ 12,400 Da). Both LMW and HMW as well as the water-insoluble fraction (IS), which remained in the filter, were freeze-dried and used as solid fraction.

2.3. Assay to determine the hydrophilic reducing power (HRP)

The generation and detection of ABTS was a modification of the spectrophotometric method with a fixed moment in time and previously described (Wagner, Haberzettl, & Elmadfa, 2000).

Trolox (Hoffman-La Roche, Vienna, Austria) (6-hydroxy-2,5,7,8-tetramethychroman-2-carboxylic acid) was used as antioxidant standard. Trolox (2.5 mM) was prepared in mM phosphate buffered saline, pH 7.4 (PBS), for use as a stock standard. Fresh working standards (0.5, 1, 1.5, 2 mM) were prepared daily on dilution with PBS. The reaction mixture consisted of: 400 μl of PBS, 10 μl standard (20 μl sample), 20 μl metmyoglobin (135 μM) and 400 μl ABTS (150 μM), with vortexing. The reaction was started with the addition of 170 μl of 75 μM H_2O_2, the clock set, and the tube mixed. A quantitative relationship exists between the absorbance at 734 nm at 6 min and the antioxidant activity of the sample or standard.

PBS-buffer and fruit juices (apple, orange and grape juice) with and without melanoidin enrichment were diluted in buffer prior to the test. The percentage inhibition of absorbance at 734 nm was calculated and plotted as a function of concentration of Trolox for the standard reference data.

Table 1
Concentrations tested in each approach

	Water soluble matrices		Fats and oils	
	PBS	Juices[a]	60 °C	200 °C
Sol A (%)	0.01, 0.05, 0.1	0.01, 0.05, 0.1	0.01, 0.1, 0.5	0.01, 0.1, 0.5
HMW (%)	0.01, 0.05, 0.1	0.01, 0.05, 0.1	0.01, 0.1, 0.5	0.01, 0.1, 0.5
LMW(%)	0.01, 0.05, 0.1	0.01, 0.05, 0.1	–	
IS (%)	0.01, 0.05, 0.1	0.01, 0.05, 0.1	–	–
αToc[b]	–	–	0.01, 0.1	0.01, 0.1
γToc[b]	–	–	0.01, 0.1	0.01, 0.1

Sol A: total water soluble fraction; HMW: high molecular weight fraction (MW > 12,400 Da); LMW: low molecular weight fraction; IS: water insoluble fraction.

[a] Apple, orange and grape juice.

[b] α-Tocopherol, γ-tocopherol: used as reference substances for comparisons with well established antioxidants.

2.4. Fat enrichment with melanoidins/tocopherols

Coconut fat was liquefied at 40°C and the solid melanoidin fractions were added to obtain final concentrations of 0.01–0.5%. The same procedure was applied to corn oil and triolein. α- and γ-tocopherol as reference antioxidants were dissolved in hexane, which was thereafter evaporated at 35°C. An appropriate amount of the fats was added to the evaporated tocopherols to obtain final concentrations of 0.01–0.1%. The final tocopherol concentrations were monitored with HPLC.

All enriched fats were stirred in an ultrasonic bath for 30 min.

2.5. Purification of the corn oil

Corn oil and activated carbon (1:1; v/v) were mixed and incubated in an ultrasonic bath for 30 min. This prepared oil was separated by centrifugation at 2500 U/mm for 1 h. The tocopherol content was determined photometrically at 294 nm, the reduction after the separation was 55%.

2.6. Fatty acid composition

The fatty acids of the coconut fat, corn oil and triolein were converted into methyl esters and analyzed with gas chromatography (GC) by using an Auto-System Gas Chromatograph, Perkin Elmer, equipped with a splitsplitless capillar injector as described previously (Wagner, Auer, & Elmadfa, 2000). FAME were separated by a 30 m × 0.25 mm ID fused silica column (RTx-2330) and detected with a flame ionisation detector (FID). The FID temperature was set at 250°C. The fatty acid pattern was analyzed in duplicate.

2.7. Low temperature/flying experiments

The finally enriched fat (0.01–0.5%) was filled into screw-capped flasks and oxidized in the dark at 60 or at 200°C. Oxidative changes were observed by analyzing the peroxide value (POV) and the formation of conjugated dienes (CD).

POV was determined by the AOCS Cd 8–53 acetic acid–chloroform procedure [American Oil Chemists' Society (AOCS), 1998a]. CD were observed according to the official method Ti 1a-64 of the AOCS (1998b).

2.8. Statistics

Obtained data were analyzed by one way ANOVA, statistical analyses were conducted using SPSS for Windows 10.0. Differences were considered significant at $P<0.05$.

3. Results and discussion

The non-enzymatic browning reaction between sugars and amino acids which occurs during food processing or cooking, affects the development of flavoring, colour and organoleptic properties of foods and therefore arises the special interest of the food industry. The antioxidative potential of MRPs started with a report in 1954 (Franzke & Iwainsky, 1954), followed by publications on this topic with different kind of food or model systems. However, less information is available on the antioxidative capacity of MRPs in fat soluble matrixes subjected to the oxidation temperature. Since reports have shown that hydrophilic substances like ascorbic acid and citric acid are more effective as antioxidants in food systems of low surface-to-volume ratio like bulk oils based on their interfacial phenomenia (Frankel, 1996) corn oil, triolein and coconut fat were chosen as test systems in these experiments. To compare their effects with hydrophilic test systems, buffer and fruit juices, were tested. The MRPs used were formed after roasting glucose–glycine for 2 h at 120°C, which was selected because it also represents a temperature which is comparable with the sterilization temperature of canned food, which is 121°C (Powrie, Wu, Rosin, & Stich, 1981). Special focus was given to the antioxidative activity of the formed MRPs with respect to their molecular weight and solubility.

3.1. HRP in buffer and fruit juices

With the HRP assay, the ability of suppressing the ABTS radical formation was tested. Reports of tests with juices or vegetable extracts using this assay were recently performed (Scalfi, et al., 2000; Van den Berg, Haenen, Van den Berg, Van der Vijgh, & Bast, 2000; Wagner, Auer, et al., 2000; Wagner, Haberzettl, et al., 2000). To prevent interactions between matrixes and solvents, the freeze dried melanoidin fractions were solved in PBS buffer, which was also used for the test assay. As the control sample either the basic buffer or the fruit juices without melanoidin additions were used. The hydrophilic reducing power of the four fractions tested in PBS-buffer is shown in Fig. 1. Especially the HMW fraction (MW >12,400 Da) was significantly more effective than all other fractions, followed by the IS and the Sol A. Only the LMW fraction was weaker in suppressing the ABTS radical formation. However, for all fractions the increase in activity was significant, the capacity after 0.1% HMW was about four times higher compared to the lowest enrichment of 0.01% HMW. (0.3 mM vs. 1.1 mM; $P<0.001$). These results are in agreement with the report of Yoshimura et al. (1997), who found that the HMW fraction had the highest potential to inhibit the hydroxyl radical formation, due not only to a direct scavenging ability of radicals but also to its stronger metal-chelating capability.

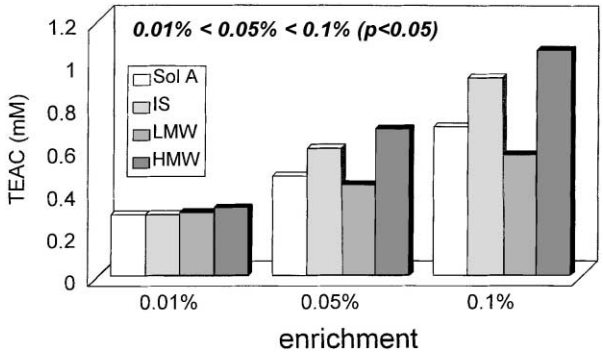

Fig. 1. Hydrophilic reducing power of melanoidins with different molecular weight in PBS-buffer presented as Trolox equivalents (TEAC in mM). Sol A: total water soluble fraction; HMW: high molecular weight fraction (MW > 12,400 Da); LMW: low molecular weight fraction; IS: water insoluble fraction.

The applied ABTS-test mainly depends on the ability of iron to induce the ABTS-radical formation, therefore a chelating ability of melanoidins is also one explanation for their effects.

The activities of the glucose–glycine model might also be explained by the formation of hydroxyl containing products within the Maillard reaction, which are already known in the literature:

Glucose + glycine→	4-hydroxy-3(2H)-furanones (Kikugawa, Hiramoto, Kato, & Yanagawa, 2000)
	2,3-dihydro-3,5-dihydroxy-6-methyl-4H-pyran-4-one (Hiramoto, Nasuhara, Michikoshi, Kato, & Kikugawa, 1997)
	5-(hydroxymethyl)-fuirfural, 2-acetyl-6-hydroxy-7-(hydroxymethyl)- 1,5,6,7-tetrahydro-4H-azepin-4-one (Ames, Bailey, & Mann, 1999)

All these reaction products contain at least one hydroxyl group, which might act as hydrogen donator and therefore have an ability to scavenge free radicals.

To also consider possible synergistic or antagonistic effects with kinds of food which are rich in initial antioxidants, fruit juices were tested. Orange juice is known to be rich in vitamin C and terpenoids, grape juice rich in polyphenols, whereas apple juice contains only low amounts of secondary plant substances.

In orange juice, a concentration dependent increase of the HRP was observed, with the highest potential for the IS fraction, followed by the LMW (Fig. 2). All fractions added in 0.1% increased the HRP of orange juice significantly ($P<0.05$) compared to the control and the lowest addition of 0.0 1%. Similar results, even more pronounced with regard to the relative activity compared to the control, were observed for the apple juice test. Again, the IS and LMW were more active than Sol A and HMW. In grape juice, the initial HRP was comparable to that of orange juice, only the HMW was found to show a corresponding increase. However, the trend of an increased HRP as a result of increased melanoidin addition was observed for both test matrices, buffer and the fruit juices.

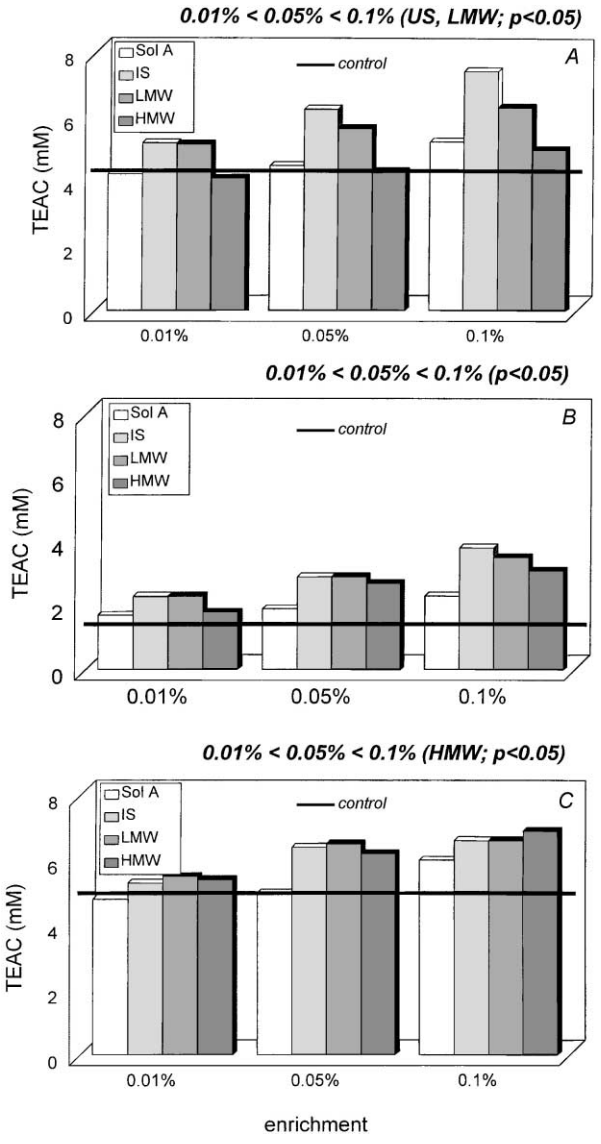

Fig. 2. Hydrophilic reducing power of different molecular weight melanoidin fractions in orange juice (A), apple juice (B) and grape juice (C); TEAC. Trolox equivalents in mM.

Despite the dark brown colour of the generated melanoidins, no interference with the analytical procedure at the experimental conditions described took place, due to diluting them in PBS buffer prior to determination. Surprisingly, the IS fraction was able to suppress the ABTS radical formation very efficiently in both systems. Since reports with the IS fraction are missing, we can only hypothesize that the stirring of the IS in buffer in the ultrasonic bath 30 min prior to determination may unhinge some water soluble parts. This might also explain the brown colour of the samples enriched with the IS fraction after ultrasonic bath treatment. In addition to the above mentioned radical scavenging and metal-chelating abilities, an interaction with initial antioxidants in the juice may have taken place and therefore the tested MRPs might act as synergists. This would also explain the higher reducing power of orange and grape juice tests, which are both rich in initial antioxidants. A similar reducing power of MRPs after roasting of peanut kernels was recently shown by Hwang et al. (2001), they concluded that the roasting process and the MRPs that were thereby generated, were highly effective in enhancing the antioxidative activity.

3.2. Shelf life tests with fats

The development of rancidity in edible oils is a serious problem in some sectors of the food industry, especially due to the increasing emphasis on the use of polyunsaturated oils. Additionally, the use of synthetic antioxidants is beeing abandoned in favor of an increased search for potent natural antioxidants like spices, tea or plant extracts (Lindberg Madsen, Sørensen, Skibsted, & Bertelsen, 1998; Mansour & Khalil, 2000). Since MRPs are natural products and formed at higher temperatures, their activity in bulk oils was tested with regard to their molecular weight, solubility and the oxidation temperature. To discuss the results obtained and compare them with well established antioxidants, α-tocopherol and γ-tocopherol were used as reference substances.

3.3. Low temperature experiments (60°C)

Melanoidins were tested in fats and oils with different fatty acid compositions. Tests were performed with corn oil (p/s ratio: 4.2), which was stripped of 55% of the

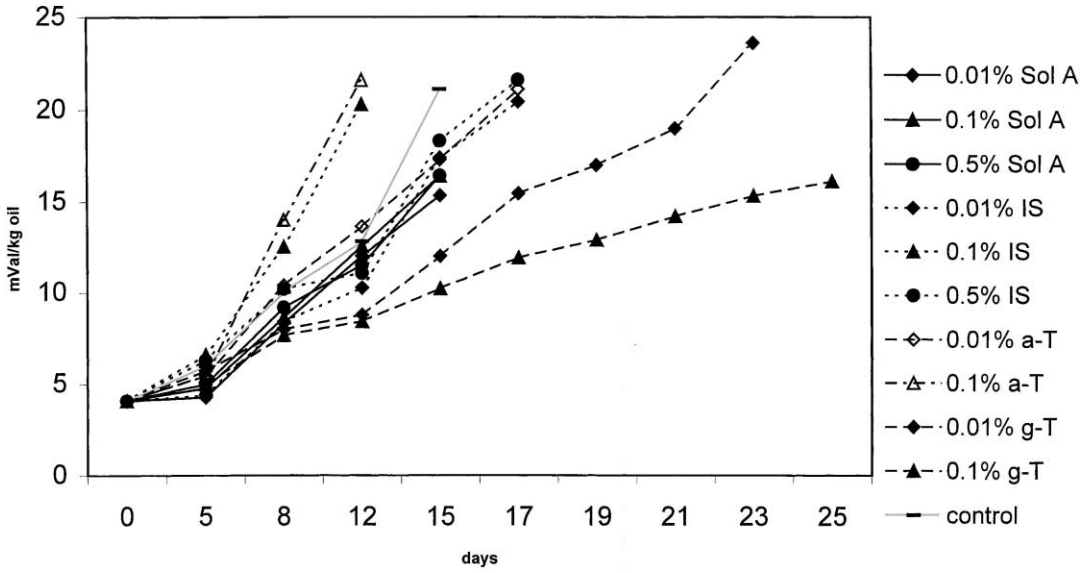

Fig. 3. Changes in peroxide values of triolein following melanoidin additions in comparison to tocopherol additions (a-T = alpha tocopherol, g-T = gamma tocopherol) at 60 °C.

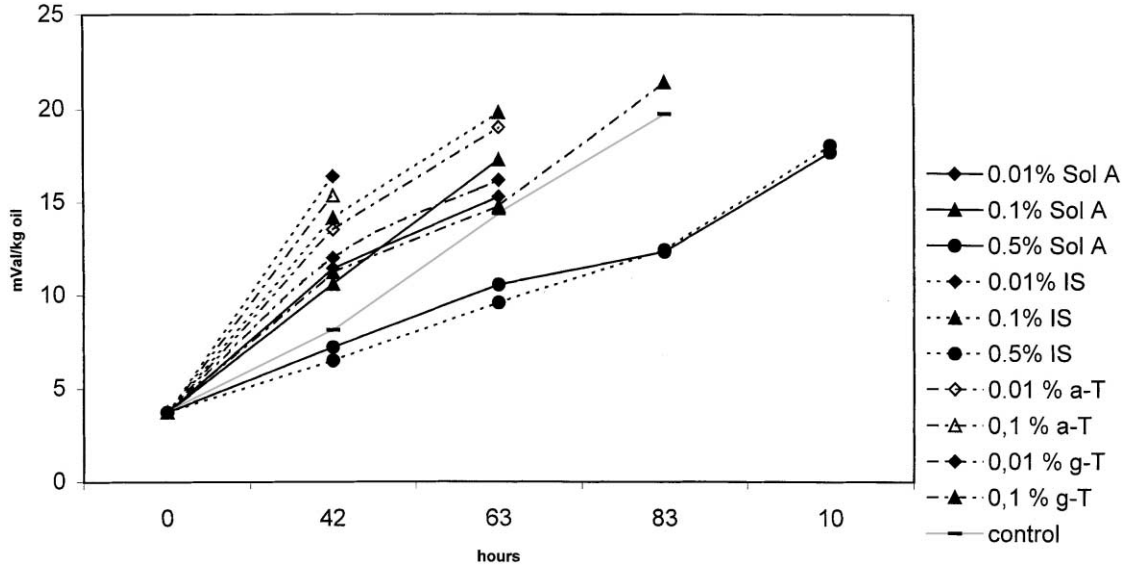

Fig. 4. Changes in peroxide values of corn oil following melanoidin additions in comparison to tocopherol additions (a-T = alpha tocopherol, g-T = gamma tocopherol) at 60 °C.

initial tocopherol concentration prior to the test. Additionally, triolein as a model substance was used containing *65%* oleic acid and no initial antioxidants.

Both were oxidized at 60°C and the POV and CD were determined periodically (Figs. 3–5).

In triolein, no significant prolongation of shelf life was obtained, the process was comparable with the progression of the control oil. However, in contrast to α-tocopherol, which was less effective, no pro-oxidative behavior was shown for the melanoidins. The most efficient substance was γ-tocopherol with a 40 and 66% increase of life time for the 0.01 and 0.1% addition, respectively (Fig. 3). In the corn oil regimen, the highest levels of 0.5% Sol A and the IS were both very active in prolonging shelf life by around 23% until a POV level of 15 mVal/kg sample was achieved (Fig. 4). However, in this high concentrations of Sol A and IS are not practicable for the food industry due to colour changes. Lower enrichments were less active than the control sample. Of the tocopherols only 0.1% of the γ-form was

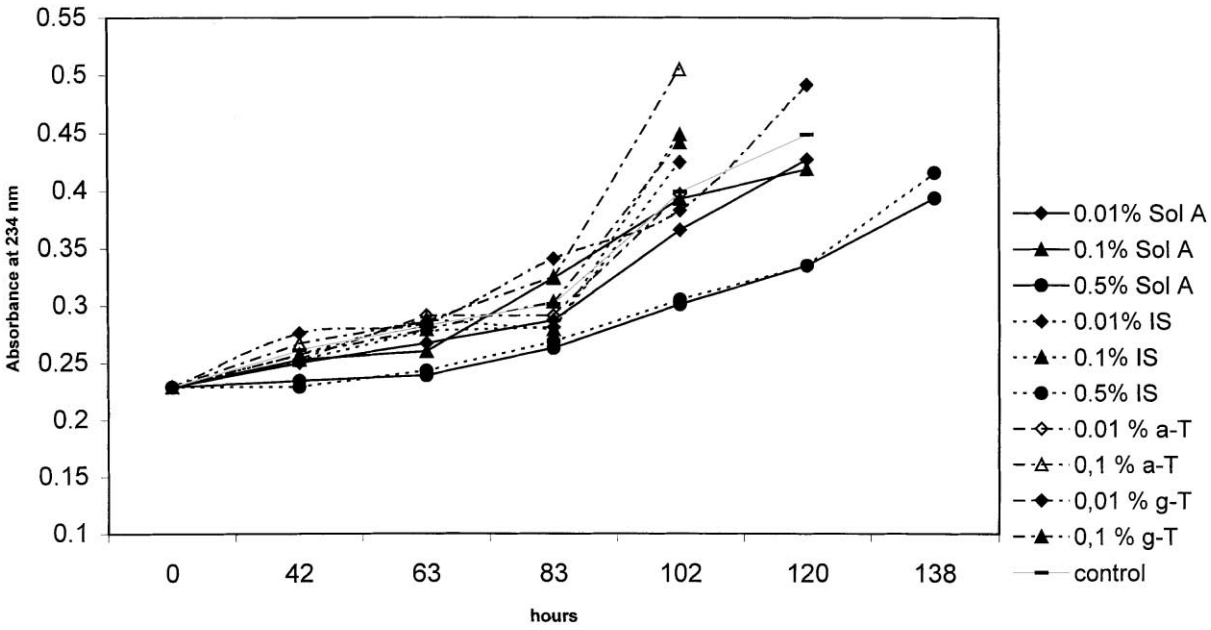

Fig. 5. Changes in conjugated dienes of corn oil following melanoidin additions in comparison to tocopherol additions (a-T = alpha tocopherol, g-T = gamma tocopherol) at 60 °C.

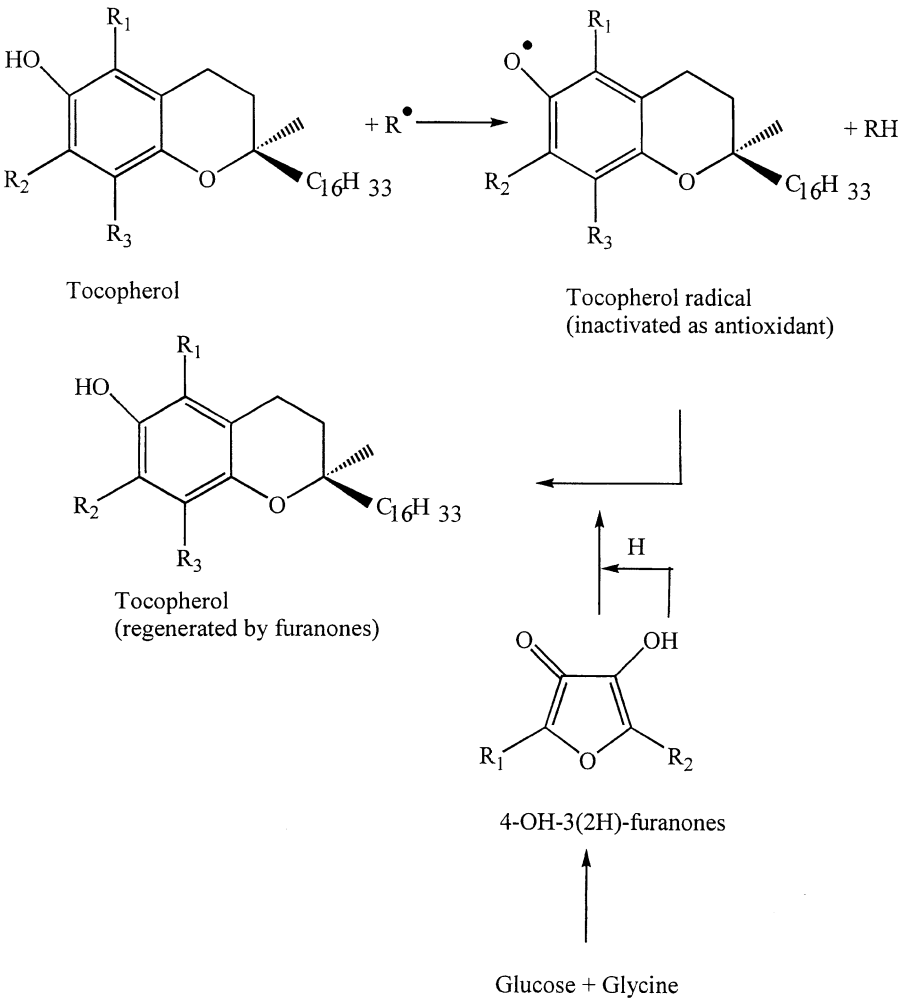

Fig. 6. Possible pathway for the regeneration of inactivated tocopheryl radicals by generated 4-OH–3(2H)-furanones in the reaction of glucose–glycine.

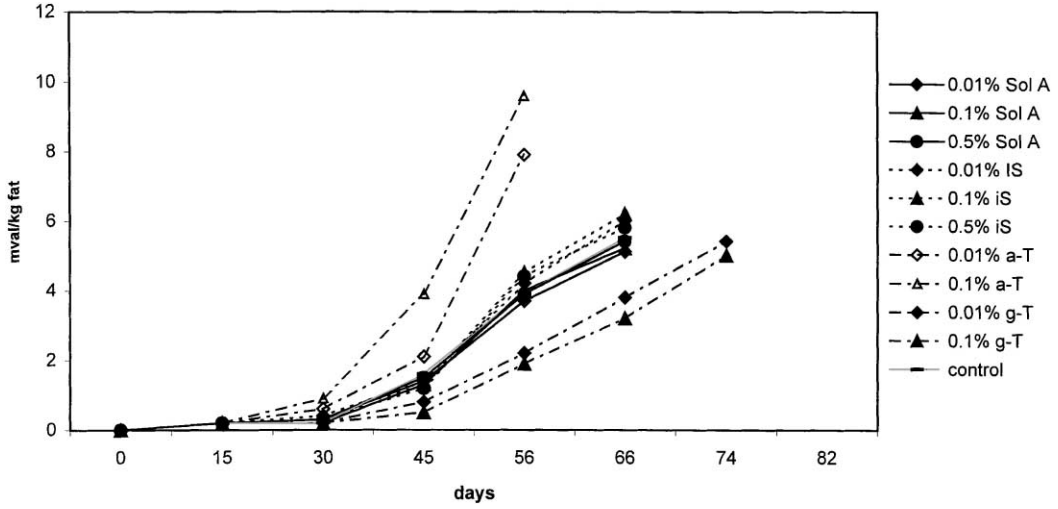

Fig. 7. Changes in peroxide values of coconut oil following melanoidin additions in comparison to tocopherol additions (a-T = alpha tocopherol, g-T = gamma tocopherol) at 200 °C.

comparable with the control sample, the lower enrichment and both α-tocopherol enriched samples were less effective. Similar results were obtained by assessing CD (Fig. 5).

The potential of the highest melanoidin fractions may also be discussed with regard to two main mechanisms proposed before. They probably were able to suppress the peroxide formation as scavenger of free radicals. Secondly, they might interact with the remaining tocopherols in the corn oil, and act synergistically by regenerating the oxidized tocopherols. A conceivable mechanism for this hypothesis is shown in Fig. 6. The second hypothesis might be of higher relevance due to the low activity in the tocopherol free triolein approach. Since the so-called melanoidins are a chemically undefined class (Hofmann, Czemy, Calligaris, & Schieberle, 2001) their mode of action is unknown. Tests in a powered model of linoleic acid with a phosvitingalactomanan conjugate, formed through a controlled Maillard reaction at 60°C, showed an increased antioxidative effect of the Maillard type product when compared to the initial phosvitin. Apart from this effect it also showed good emulsifying properties and heat stability (Nakamura, Ogawa, Nakai, Kato, & Kitts, 1998).

3.4. Frying experiments (200°C)

The development of the POV in coconut fat after melanoidin enrichment is presented in Fig. 7. No antioxidative behaviour was assessed, either as a matter of concentration or depending on the fraction added. The similar progression of the curves shows that the tested fractions were not active as antioxidants under frying conditions. The less effective behaviour of α-tocopherol and the 12% increase in shelf life by γ-tocopherol was in accordance with previous results (Wagner & Elmadfa, 2000) and similar studies (Gordon & Kourimska, 1995).

Since no studies under flying conditions were reported, it is not possible to compare and discuss the results. Nakamura et al. found non affected antioxidative effects of Maillard type phosvitin-galactomanan conjugates by autoclaving (121°C, 1.2 atm for 15 mm), therefore conditions are not comparable.

4. Conclusion

The data obtained clearly show that MRPs, formed by a glucose–glycine model at 120°C, were highly effective with regard to their antioxidative quality, in hydrophilic matrices. There were obvious differences in the radical suppressing ability between HMW and LMW, but also the IS fraction was highly efficient. In corn oil only the highest addition of 0.5% IS and Sol A were able to improve life time at 60°C, possibly by acting synergistically with the remaining tocopherols in the corn oil. Neither the shelf life of triolein at 60°C nor of coconut fat at 200°C were prolonged with the MRPs added.

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