# Peroxyl Radical Scavenging Activity of Soluble High Molecular Weight Fraction from Coffee Brews

C. DELGADO-ANDRADE[1]* and F. J. MORALES[2]

[1]Consejo Superior de Investigaciones Científicas, Unidad de Nutrición Animal, Estación Experimental del Zaidín, Camino del Jueves, Granada, Spain, *E-mail: cdelgado@if.csic.es;
[2]Consejo Superior de Investigaciones Científicas, Instituto del Frío, Madrid, Spain

**Abstract**: Roasting process of coffee beans affects the final composition of coffee brew. While the presence of some of its antioxidant compounds (phenolic acids) decrease throughout the treatment, melanoidins are formed during the thermal process. Recent research into nutritional, physiological and functional properties of melanoidins has suggested that they have antioxidant activity. Instant coffees produced from roasted coffee beans were obtained from a company coffee in three different roasting degrees: light (CTn 110), medium (CTn 85) and dark (CTn 60). Melanoidins of high molecular weight (> 10 000 Da) were obtained from each coffee, as well as pure melanoidins by incubation in 2M ClNa and ultrafiltration. CGA contained in extractes of different melanoidins (1 mg/ml) was measured by HPLC. Peroxyl radical scavenging activity of melanoidins and pure melanoidins were tested using a procedure to inhibite the peroxidation of linoleic acid. Antioxidant efficiency ($AE_{50}$, ml/min/mg) was calculated as the slope of the time of inhibition at 50% (min) against concentration (mg/ml). No significant differences were found between melanoidins and pure melanoidins in the different roasting degrees. Peroxyl radical scavenging activity of melanoidins from coffee brew is mainly due to the chelated compounds by ionic binds.

**Keywords**: Maillard reaction; melanoidins; lipid oxidation; AAPH; coffee brew

## INTRODUCTION

Thermal processes utilised in the food industry often add value to raw materials and are applied to produce colour, texture, flavour and to sterilise the materials assessing longer shelf-life and enhancing product safety. One of the most common reactions related to heat treatment of foods is Maillard reaction (MR), which leads to melanoidins formation, among other products [1]. Melanoidins have been studied in recent years due to their nutritional, biological and health implication. Their antioxidant activity result especially interesting since these products are naturally formed during the food processing and storage [2]. A high proportion of daily melanoidins intake comes from coffee beverage, which antioxidant properties have frequently been attributed to certain phenolic compounds present in green coffee (chlorogenic acid, caffeic acid, ferulic acid or *p*-coumaric acid) [3]. Roasting process affects the final composition of coffee, thus, levels of phenolic acids vary depending on the treatment of beans, while the production of Maillard reaction products (MRP) maintain or even improve the antioxidant properties of coffee brew [4].

The aim of the present work is to evaluate the antiperoxyl radical scavenging properties of melanoidins in coffee brews from different roasting degrees, by applying an AAPH-induced oxidation of linoleic acid in aqueous dispersion. Contribution of this CGA content in coffees to the antioxidant activity was also studied. These findings will help to know the contribution of melanoidins to the overall antioxidant effect of coffee brew and their health implications.

## EXPERIMENTAL

***Preparation of coffee brews***. Instant coffees produced from roasted coffee beans were obtained from a coffee company in three different roasting

degrees: light (CTn 110), medium (CTn 85 and dark (CTn 60). 1 g of the different instant coffees was resuspended in 100 ml of hot water (50–60°C). The aqueous solutions obtained were then filtered (Whatman Filter Paper No. 40, ashless, Whatman, UK) and stored at 4°C until analysis were performed (CTn 110, CTn 85 and CTn 60 samples, respectively).

***Preparation of melanoidins extract and pure melanoidins extract from coffee brews***. An aliquot of each above described sample was subjected to ultrafiltration with a 10 000 Da nominal molecular mass cut-off membrane and the high molecular weight fraction corresponding to melanoidins was freeze-dried. Melanoidins (M) isolated from these systems were identified as M110, M85 and M60, respectively.

Pure melanoidins were obtained by ultrafiltration (10kDa) of a solution containing 5 mg of different melanoidins/ml in 2M NaCl. Retentates, containing pure melanoidins, were freeze-dried. Pure melanoidins (PM) obtained were named PM110, PM85 and PM60, respectively.

***Analytical techniques***. *High performance liquid chromatography (HPLC) analysis.* CGA extracted from the melanoidin was quantified by reversed phase HPLC. Degassed elution phases were prepared: (A) glacial acetic acid – milli-Q water (5%, v/v) and (B) acetonitrile. A Spherisorb ODS-2 analytical column (25 × 0.40 cm, 5 μm particle size, Analytical Tracer, Barcelona, Spain) was used at 32°C. The injection volume was 10 μl and detection at 320 nm (0.1 AUFS sensitivity and 0.5 s response time) was selected. An external standard method was used within the range 0.1–0.005 mg/ml ClNa 2M of CGA.

*Antioxidant assay.* Substrate – An aqueous solution of linoleic acid (LH, 16mM) was prepared according to Morales and Jiménez-Pérez [2]. The dispersion was checked for autooxidation before used. AAPH decomposes slightly and its products increase the absorbance at 234 nm. Therefore, this AAPH decomposition absorbance was subtracted from all sample absorbance data from this point on.

AAPH solution – 40 mM AAPH was prepared in 50mM phosphate buffer (pH 7.4). Portions were distributed in 1 ml test tubes and stored at –20°C until use.

Antioxidant activity – It was applied the procedure of Morales and Jimenez-Perez [2]. Sample (CTn, M, PM) was added, followed by 150 μl of the 40mM AAPH solution. The tube was quickly vortexed for a few seconds, avoiding the formation of foam, and put in a UV cuvette within 30 s. The reading chamber was thermostated at 38°C. The rate of oxidation of LH was monitored by recording the increase in absorption at 234 nm caused by conjugated diene hydroperoxides. A Shimadzu UV-visible 1601 spectrophotometer (Shimadzu) equipped with a thermostated automatic sample positioner was used. Measurements were recorded every 60 s. Antioxidant efficiency ($AE_{50}$, ml/min/mg) was calculated as the slope of the time of inhibition at 50% (min) against concentration (mg/ml).

***Statistical treatment***. All of the analyses were performed at least in triplicate. The Statgraphics v. 5.1 statistical procedures were performed at a significance level of 95%.

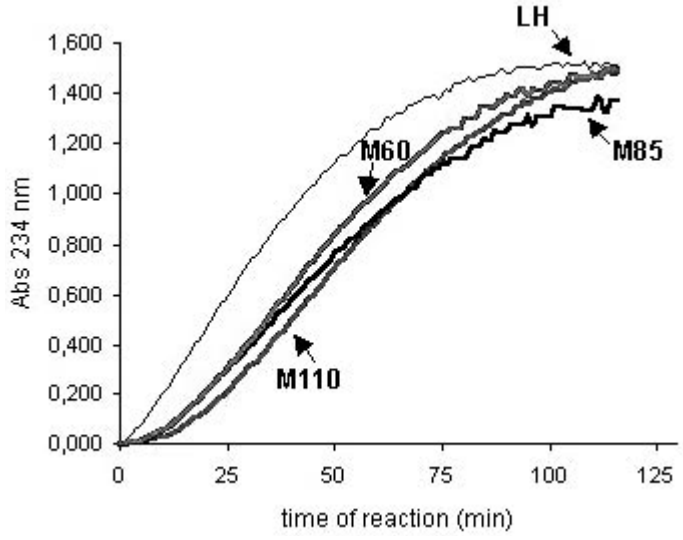


Figure 1. Classical time course of conjugated diene formation from control (LH) against conjugates diene formation from M60, M85 and M110 (0.1 mg/ml). Residual absorbance from metabolites of AAPH decomposition is already subtracted

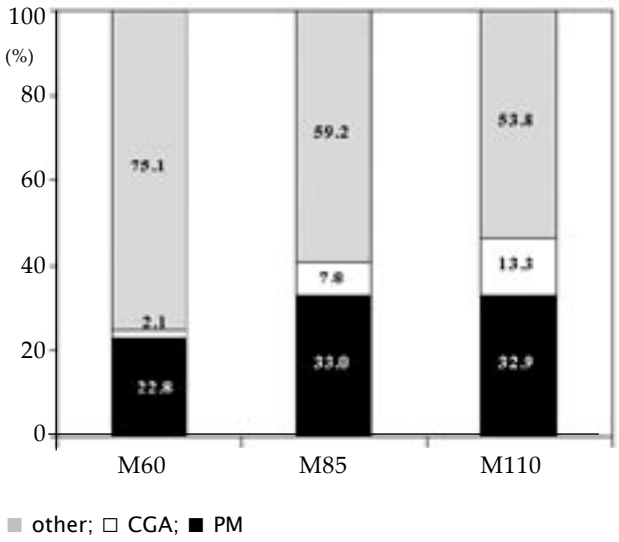


Figure 2. Contribution of CGA, pure melanoidins and other components to the total peroxyl radical scavenging activity of coffee melanoidins. No significant differences were found between PM60, PM85 and PM110 samples

## RESULTS AND DISCUSSION

$AE_{50}$ was calculated for each melanoidin in an appropriate range of concentration. The upper limit was related to the possible interference of colour with the analytical procedure. Figure 1 shows a classical representation of conjugated diene formation from AAPH-induced oxidation of LH alone and in the presence of 0.1 mg/ml different melanoidins. CGA was used as reference because it is one of the most active compounds involved in the overall antioxidant activity [4].

To make data comparatives, it was selected the concentration of 1 mg/ml for the different melanoidins. Values of CGA extracted from different melanoidins were as follow: 3.15 μg/ml for M60; 10.60 μg/ml for M85 and 20.20 μg/ml for M110. CGA was linked in a non-covalent way to the melanoidin core. These concentrations were replaced in the calibration curve of CGA peroxyl radical scavenging activity (standard) to obtain the theoretic values for time of inhibition. The percentages of contribution of each component (pure melanoidins, CGA and other compounds) are represented in Figure 2.

## CONCLUSIONS

No significant differences were found among the different roasting degrees for melanoidins and pure melanoidins concerning to the antiperoxyl radical activity. Peroxyl radical scavenging activity of melanoidins from coffee brew is mainly due to the chelated compounds to the high molecular weight fraction. Probably, this kind of compounds has been generated throughout the roasting process and they are related with the development and progress of Maillard reaction.

## References

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