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Reactivity of acrylamide with coffee melanoidins in model systems

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ABSTRACT

Coffee and its substitutes have been described as complex matrices for acrylamide (ACR) analysis due to both analytical interferences and ACR instability in the matrix. Melanoidins are multifunctional and biochemically active polymers which are formed in large extent during coffee roasting. Model systems composed of ACR (elimination studies) or glucose-asparagine (ACR formation/elimination studies) with/without melanoidins was heated at 180 °C. Washed sea sand and cellulose microcrystalline were used as matrix. Coffee melanoidins had a direct influence on the fate of ACR under heating, while the effect was not observed at room temperature. In addition, ACR decrease was also related to the reaction time and the initial amount of melanoidins in the media, where clearly a dose-response was observed. In contrast, pH (from 3.5 to 7.0) had no significant effect on ACR reactivity towards melanoidins. It is hypothesized that nucleophilic amino groups of amino acids from the proteinaceous backbone of melanoidins react via the Michael addition reaction with ACR, although the exact mechanism is unknown. Then, melanoidins could modulate the reaction pathways of ACR formation and elimination during coffee roasting and serve as acrylamide-mitigation substance.

1. Introduction

Melanoidins are brown anionic nitrogenous polymers formed during the final stage of the Maillard reaction (Borrelli, Visconti, Mennella, Anese, & Fogliano, 2002). Melanoidins formation is a direct consequence of the thermal process applied to food, such as roasting, baking or toasting. Although their chemical structure remains largely unknown, there is increasing evidence that arabinogalactan-like carbohydrates, proteins, phenols and Maillard reaction compounds are constituents of coffee melanoidins (Bekedam, Schols, van Boekel, & Smit, 2007).

There is a growing interest in coffee melanoidins, since they are not biologically inert food constituent and may exert various technological, nutritional, biological and health effects (Rufián-Henares & Morales, 2007; Somoza, 2005). In addition to their contribution to colour, texture (foam stability) and antioxidant capacity (Delgado-Andrade & Morales, 2005), melanoidins play an important role in the binding of nutritionally important metals (Morales, Fernandez-Fraguas, & Jiménez-Pérez, 2005), undesirable dietary compounds (Solyakov, Skog, & Jägerstad, 2002) and odourants (Hofmann & Schieberle, 2002). Moreover, their chelating properties towards metal ions further contribute to the antioxidant and antimicrobial properties of melanoidins in food (Rufián-Henares & De la Cueva, 2009). In summary, melanoidins reactivity is a relevant issue in food science from a technological (Petracco et al., 1999), safety (Jägerstad, Skog, & Solyakov, 2002), nutritional (O'Brien & Morrissey, 1997) and physiological (Somoza, 2005) points of view.

Acrylamide (ACR) is a processing contaminant with potential harmful consequences to humans (i.e. EFSA, 2010). ACR is naturally formed during roasting of green coffee beans, and it is found at levels of 200 μg/kg (maximum 958 μg/kg) and 188 μg/kg (maximum 1047 μg/kg) in roasted and instant coffee, respectively (EFSA, 2010). The contribution of coffee to the dietary daily intake of ACR is significant in countries with high coffee consumption, thus, level of 30% is reached in Scandinavian countries (Dybing et al., 2005). Since the presence of naturally formed ACR in foods was detected, different mitigation strategies have been attempted (CIAA, 2009). Nowadays, there is not an effective strategy of ACR mitigation or alternative process applicable for coffee and its derivatives (EFSA, 2010). In addition, there is some controversy regarding the measurement of ACR in coffee since values decrease with storage time and temperature (Delatour, Périsset, Goldmann, Riediker, & Stadler, 2004; Hoenicke & Gatermann, 2005; Wenzl, Klaffke, Mothar, Palavinkas, & Anklam, 2005). In this context, Baum et al. (2008) confirmed that close to 90% of ACR remained

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firmly bound to the coffee matrix and it was hypothesized that ACR might be integrated with material eluting into the brew together with colouring material.

In view of their multifunctional properties, anionic behaviour and high abundance in coffee, melanoidins could have a direct implication in the ACR formation and elimination during coffee roasting. This investigation examines the role of coffee melanoidins in the formation and elimination of ACR.

2. Materials and methods

2.1. Chemicals

D-glucose, L-asparagine, acrylamide, and cellulose microcrystalline were purchased from Sigma–Aldrich (Madrid, Spain). All other chemicals were purchased from Aldrich (Milwaukee, WI), or Merck (Darmstadt, Germany) and were of analytical grade. The water used was double-distilled (18.2 meqX/cm) using a Milli-Q System (Millipore Ibérica, Madrid, Spain).

2.2. Isolation of coffee melanoidins

Coffee melanoidins were prepared following the recommendation of the COST-919 group for coffee melanoidin analysis (COST-919, 1998) and as described elsewhere (Delgado-Andrade & Morales, 2005). Roasted coffee beans (Coffea arabica) were provided by a local factory; a moderate degree of roasting was applied, producing a weight loss of 16.2% (w/w) dry matter, in relation to green coffee weight. Ground coffee (100 g) was stirred in 300 mL of distilled water at 75 °C for 5 min. The solution was filtered and an aliquot of filtrate was de-fatted with dichloromethane (2 x 200 mL). The coffee brew was then subjected to ultrafiltration (Amicon ultrafiltration cell model 8400, Amicon, Beverly, MA, USA) with a 10 kDa nominal molecular mass cut-off membrane. The retentate corresponding to melanoidins was completed to 200 mL with water and washed again at least three times, after which the high molecular weight fraction was freeze dried and stored.

2.3. Preparation of model systems

2.3.1. Study of acrylamide elimination (static system)

Double-washed sea sand (30 mg ± 0.1 mg) was carefully placed at the bottom of a Pyrex test tube (100 × 15 mm). Then, 50 μL of melanoidin solution (20 mg/mL) and 50 μL of ACR solution (corresponding to 10000, 5000, 2000, 500 and 100 μg/L) were added. The melanoidin solution was replaced by buffer (0.2 M in phosphate buffer 0.1 M, pH 6.8) for control samples. In parallel, a model system with cellulose microcrystalline was designed as a polymer control instead of melanoidins.

2.3.2. Study of formation/elimination of acrylamide (dynamic system)

Double-washed sea sand (30 mg, ±0.1 mg) was carefully placed at the bottom of a Pyrex test tube (100 × 15 mm). Then, 20 μL of asparagine solution (0.2 M in phosphate buffer 0.1 M, pH 6.8), 20 μL of glucose solution (0.2 M in phosphate buffer 0.1 M, pH 6.8) and 50 μL of coffee melanoidin solution (20 mg/mL) were added. The melanoidin solution was replaced by buffer for control samples.

2.3.3. Effect of pH on acrylamide formation

Double-washed sea sand (30 mg, ± 0.1 mg) was carefully placed at the bottom of a Pyrex test tube (100 × 15 mm). Then, 20 μL of asparagine solution (0.2 M), 20 μL of glucose solution (0.2 M) and 50 μL of coffee melanoidin solution (20 mg/mL) were added. Both asparagine and glucose were dissolved in phosphate buffer 0.1 M pH 7.0, distilled water, sodium citrate 0.1 M pH 5.5 or sodium citrate 0.1 M pH 3.5 as necessary. Each melanoidin solution was replaced by the corresponding buffer for control samples.

2.3.4. Effect of staling on acrylamide elimination

Double-washed sea sand (30 mg, ± 0.1 mg) was carefully placed at the bottom of a Pyrex test tube (100 × 15 mm). Then, 50 μL of melanoidin solution (20 mg/mL) and 50 μL of ACR solution (500 μg/L) were added. The melanoidin solution was replaced by buffer for controls. After thermal treatment, the samples were rapidly reconstituted with 2 mL water, mixed and kept at room temperature. An aliquot (50 μL) was taken every 5 min for 60 min.

2.4. Heat treatment

The samples were heated in Pyrex test tubes (100 × 15 mm). Heat treatments were performed in a thermostated polyethylene glycol bath (GFL 1086, Großburgwede, Germany) equipped with an Omron E5J temperature controller (Omron Electronics, CA, USA) at 180 °C for 2, 4, 6 and 12 min. After thermal treatment, the samples were immediately cooled in ice water. Thermal treatments were carried out in parallel in open and hermetically closed screw-capped tubes to minimize the effect of evaporation.

2.5. Analysis of acrylamide

The samples were reconstituted with 2 mL water and 200 μL were then ultrafiltrated using Vivaspin 500 disposable units (Sartorius Stedim Biotech, Göttingen, Germany) in order to remove the melanoidins. Finally, 50 μL of the ultrafiltrated ACR solution was injected into an Accela 600 HPLC system (Thermo-Fisher Scientific, Palo Alto, USA) and analyzed in accordance with Knol et al. (2005) and Barber, Hunt, LoPachin, and Ehrich (2001) with minor modifications. Such method consists in the use of a reversed-phase column specially designed for separation of small molecules in highly aqueous solutions and a mobile phase composed of water:methanol (99:1) with a counter ion (heptane sulphonic acid) in order to elute compounds like acrylamide or its metabolite glycidamide. In our study, the reversed-phase column was replaced by a gel-permeation column (Discovery Bio GPC 150, 30 cm × 4.6 mm, 150Å, Supelco, Madrid, Spain) thermostated at 25 °C to avoid interference with the retained melanoidins. In addition, the mobile phase was water 100% delivered at 1 mL/min. ACR was detected at 210 nm in a PDA detector equipped with a 5 cm flow cell, which also improve the detection limit compared with the former method. The method was linear between 50 and 5000 μg/L, presenting 3.1% precision and a detection limit of 20 μg/L.

2.6. Statistical analysis

Results are expressed as mean values ± standard deviation. All experiments were carried out in triplicate. Means were compared by one way analysis of variance (ANOVA) and Student's *t*-test at a significance level of P-values < 0.05. All analyses were carried out with Microsoft Excel statistical software (Microsoft Office Excel 2003, Microsoft Corp., Redmond, WA).

3. Results and discussion

ACR content in roasted coffee decreases during storage following a temperature-dependent model (Hoenicke & Gatermann, 2005; Lantz et al., 2006), and its variability during storage is probably due to ACR instability in the matrix (Wenzl et al., 2005). Recently, Baum et al. (2008) stated that ACR may