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Formation of coloured Maillard reaction products in a gluten-glucose model system

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This paper is dedicated to the co-author Professor G. Randazzo who died on 1 July 1998.

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Abstract

A pasta model system consisting of *durum* wheat gluten proteins and glucose was heated at different times and temperatures under wet (6.6% water) and dry conditions in order to study conditions which promote the formation of Maillard compounds in pasta. Formation of coloured compounds was very slow up to 120°C and increased seven-fold at 150°C. Under wet conditions, the coloured material formed could be better extracted with polar solvents whereas, under dry conditions, more hydrophobic coloured compounds were extracted. Methanol extracts of the wet and dry gluten–glucose mixtures were separated by HPLC gel filtration. Two high molecular weight peaks were collected. They showed different UV-vis properties: the first peak was colourless and the second one was brown and was absent from extracts of gluten heated without glucose. Both peaks were dialysed through a 12-kDa membrane and analysed by C_{18} reverse phase HPLC with diode array detection before and after tryptic hydrolysis. Analysis of chromatograms revealed that coloured compounds were present only in peak 2 and were better detectable after proteolysis. It is concluded that, in the gluten-glucose system, coloured low molecular weight molecules became entrapped in the high molecular weight polymers formed by gluten proteins and that trypsin treatment of gluten favours the release of the coloured compounds.

Keywords: Maillard reaction; Gluten; Glucose; Model system; Melanoidins; Colour

1. Introduction

Non-enzymatic browning is of considerable importance to food companies. In particular, pasta industries need more knowledge to control browning during processing; in fact, pasta colour is generally considered as one of the major components of quality. Consumers like an amber-yellow colour while an intense brown tone causes a decrease of the commercial pasta value. Beside the colour due to the content of carotenoids and their oxidation (Feillet, Jeanjean, Kobrehel & Laignelet, 1975), the Maillard reaction (MR) is also responsible for browning in foods containing reducing sugars and free amino groups (Hodge, 1953). This reaction influences flavour, functional and nutritional properties of processed foods (Friedman, 1996). The MR produces a multitude of small molecular weight intermediates, collectively referred to as Maillard reaction products (MRPs), and high molecular weight polymeric compounds known as melanoidins. Melanoidins were isolated from different model systems consisting of a single amino acid and carbohydrate. A basic melanoidin structure with a backbone constituted of amino acid and sugar has been proposed by Kato and Tsuchida (1981), by Benzing-Purdie, Ripmeester and Ratcliffe (1985), and by Cämmerer and Kroh (1995) who demonstrate a ratio of about one to one between amino acid and sugar. However, it is generally accepted that variation of the reaction conditions strongly affects the structure of brown polymer (Wedzicha & Kaputo, 1992).

Several low molecular weight chromophores were isolated from model systems consisting of different sugars and amino acids (Ames, 1987; Ledl & Schleicher, 1990). The structure of a compound constituted of 4 rings with a high conjugation level has been elucidated by Hofmann (1998a) who hypothesised that low molecular weight chromophores rather than high molecular weight melanoidins are in the main responsible for colour formation in foods. The same author demonstrated

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that coloured low molecular weight compounds can cross-link proteins (Hofmann, 1998b).

In wheat-based food, such as pasta, the extent of MR depends on the intensity of thermal treatment. Yoong, Walters, Tester, Gomes and Ledward (1994), studying a gluten carbohydrate model system heated at 160°C for 30 min, reported that brown colour formation paralleled a loss of free lysine and glutamine.

The "high temperature process" of pasta drying is widely used by companies since it enhances the overall quality of the final product in terms of firmness and colour (Didonè & Pollini, 1990). However, an uncontrolled MR reduces the nutritional value of wheat protein by lowering the level of nutritionally-available lysine and causing excessive browning. Many efforts have been devoted to developing processing conditions to find a balance between these two opposite tendencies (Didonè & Pollini, 1990).

In this paper we devised a "pasta-like" model system consisting of *durum* wheat gluten proteins and glucose in order to develop a better understanding of the molecular nature of the coloured compounds formed and to evaluate colour-formation dependence on heating conditions.

2. Materials and methods

All solvents and reagents were of analytical grade and were supplied by Fluka. HPLC chromatography was performed on a Shimadzu HPLC apparatus (Japan) equipped with two LC10 pumps, Reodyne injector and diode-array detection. Data were processed using Class M-10 Shimadzu HPLC software.

Absorption spectra of crude MRPs and absorbance of MR solutions at 420 nm were recorded using a UV2100 (Shimadzu).

2.1. Model system preparation

A protocol described by Izzo and Ho (1993) was followed with minor modifications. Gluten was prepared by extensive washing under tap water of dough obtained from *durum* wheat flour variety "Ferro" containing 15% proteins. Gluten (10 g) and glucose (2 g) were added to 30 ml of deionized water. After vigorous mixing, the sample was freeze dried at 4°C for 48 h. The solid residue was finely ground in a Waring blender, passed through a sieve and stored desiccated at 4°C. A control system was prepared by freeze-drying gluten only.

2.2. Thermal treatments

For the dry system, aliquots (3 g) of gluten–glucose freeze dried mixture were placed in a glass beaker and treated in air at 16 different time-temperature conditions as follows: temperatures 80, 100, 120 and 150°C; times 15, 30, 45 and 60 min. The wet system was added to 0.2 ml of deionized water, mixed to give homogeneous dough, and treated as described above.

2.3. Extraction of coloured material

After heating, 0.5 g of each sample was finely ground and placed in glass tubes. Five different solvents (4 ml) were used for colour extraction: water, methanol, ethanol, acetone and 1 M NaOH. Tubes were hermetically closed and placed in an orbital shaker. After 1 h, tubes were centrifuged at 5000*g* for 10 min. Pellets were discarded and UV-visible spectra of the supernatants were recorded.

2.4. HPLC separations and trypsin digestion

Methanol extracts were dried under vacuum and resuspended in 1 ml of water. Aliquots of 0.1 ml were loaded at a flow rate of 1 ml/min on an TSK-gel 2000 SW gel filtration column (60-cm length, i.d. 4.6 mm; TosoHaas, Japan) using deionized water as mobile phase. Raw data were collected from 200 to 600 nm. Peaks related to high molecular weight materials, designated as peak 1 and peak 2, were manually collected and concentrated to 400 ml using a microfiltration centrifuge filter having a cut-off of 12 kDa (Amicon).

Tryptic digestion was performed by adding to 0.1 ml of samples 50 ml of a 0.1 M carbonate buffer, pH 8, containing 1 mg/ml of trypsin (Sigma). The reaction was maintained for 12 h at 37°C and then stopped by adding 5 μl of trifluoroacetic acid (TFA).

Reverse-phase HPLC was performed on both digested and undigested peaks 1 and 2, collected by gel filtration chromatography using a 5μ 250×4.6 mm C_{18} column Primesphere 110Å (Phenomenex). The column was equilibrated in 0.1% TFA in water at a flow rate of 1 ml/ min. Elution was achieved by linear gradient from 0 to 98% CH_3CN, containing 0.08% TFA in 40 min and monitored by diode array detection from 200 to 600 nm.

3. Results and discussion

The experimental procedure used allowed the coating of reducing carbohydrates on the gluten proteins, thus enhancing strong colour formation upon heating both in the wet and dry states. This coloured material was partially extracted with methanol and colour formation was monitored by UV-vis spectra. A time-course of heat treatment at 150°C is shown in Fig. 1 where the UV-vis spectra of methanol extracts from the wet system are recorded. The first event in colour formation is an increase in the absorbance at 320 nm (up to 30 min).

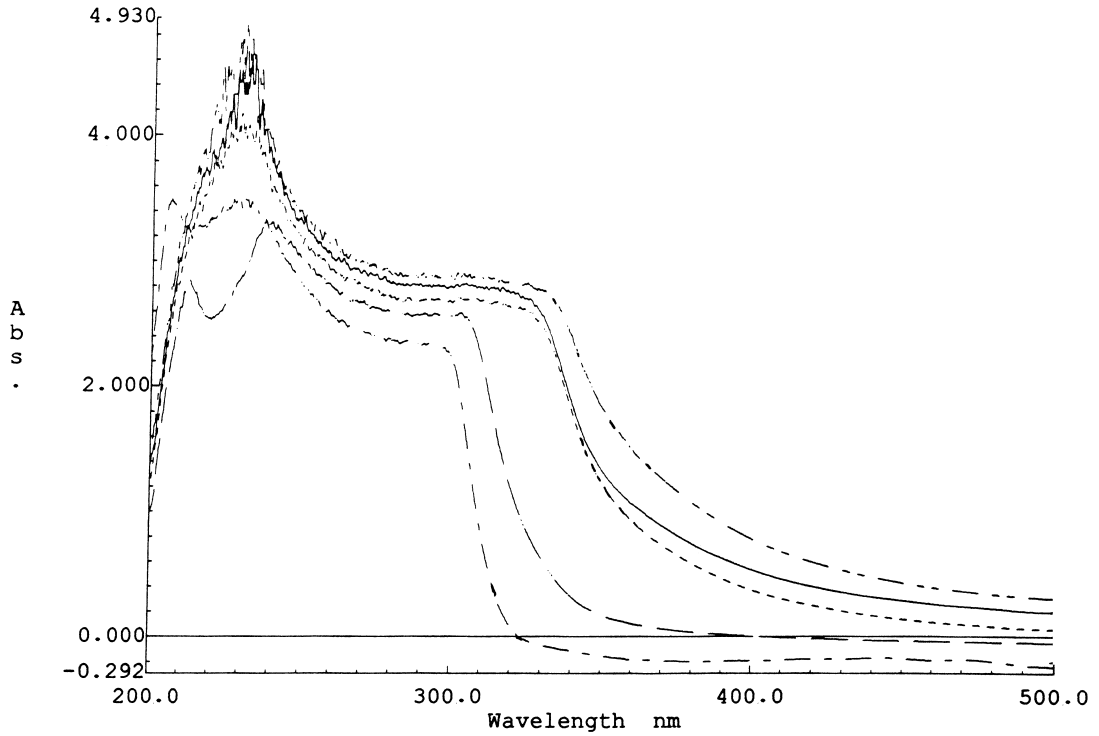

Fig. 1. UV-Visible spectra of the methanol extract obtained from a wet gluten–glucose model system after different times of heating at 150°C: ---, time zero; ——, 15 min, – – –, 30 min, ——,45 min, ———— 60 min.

The increase of 420 nm absorbance begins after 30 min and continues up to 1 h. This behaviour is very similar to that observed with model systems comprised of one sugar and one amino acid (Wijewickreme, Kitts & Durance, 1997). The appearance of the absorbance at 320 nm is due to the formation of soluble pre-melanoidins, while the absorbance at 420 nm has been used as the end-point measurement for quantifying the yield of the high molecular weight melanoidins (Wijewickreme et al., 1997).

The 420 nm absorbance, depending on the heating temperature for the wet gluten–glucose mixture, is reported in Fig. 2. Colour formation is poor and not many differences were detectable for temperatures up to 120°C. A dramatic increase of browning was evident at 150°C. After 45 min, the absorbance at 420 nm is seven times higher than at 120°C.

The time-course of colour formation in this system had a similarity to those observed in model systems made from glucose and free amino acids (Bailey, Ames & Monti, 1996; Monti, Bailey & Ames, 1998). This evidence was not surprising: in fact, although gluten proteins are rather hydrophobic and do not possess free

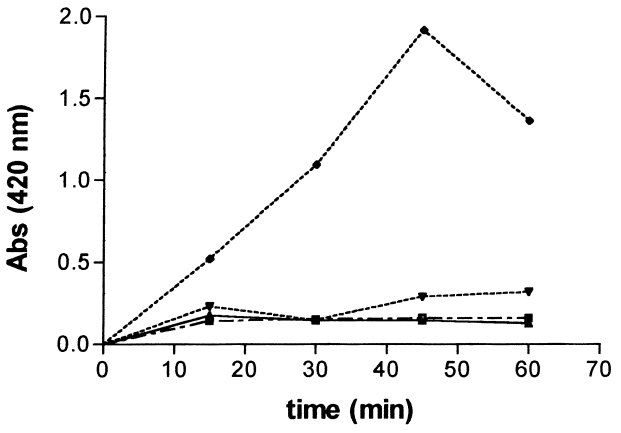

Fig. 2. Time course of colour formation in the wet gluten–glucose system at different temperatures. ■80°C; ▲100°C; ▼120°C; ◆150°C. Data are representative of five independent sets of experiments. Inter-experiment variation was below 5%.

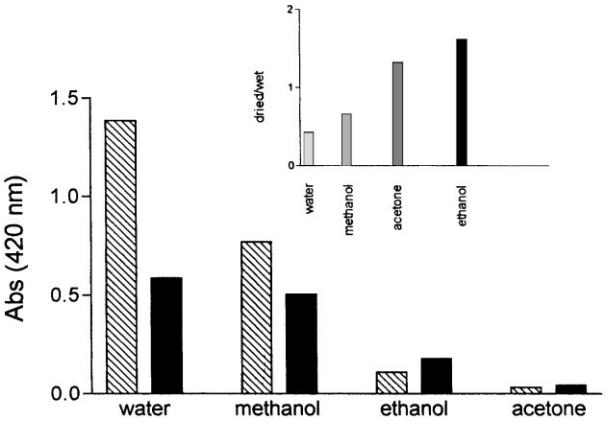

Fig. 3. Absorbance at 420 nm of coloured material extracted after 1 h using different solvents. Shaded bars: wet system; grey bars: dry system. Inset: ratio between absorbance of extracts from wet vs dried material. Data are representative of three independent sets of experiments. Inter-experiment variation was below 10%.

amino acids, at 150°C glutamine residues can undergo deamidation (Wright, 1991). In fact, it has been reported that free NH_3 may be responsible for MRPs formation (Izzo & Ho, 1992; Riha, Izzo & Ho, 1996). The main differences between the gluten-glucose and the single amino acids–glucose model systems is the higher temperature required by the former to observe the browning formation (150°C vs 100°C).

Results obtained by Yoong et al. (1994) strengthen the role played by MR in browning development. They reported that the heat treatment of dry gluten results in a loss of 25% of available lysine and of 10% of glutamine residues. The addition of carbohydrates further increased these values.

In contrast, Clawson and Taylor (1993), based on chemical changes occurring during cooking of wheat, concluded that colour formation was only partially due to the MR while phenolic and lipid oxidation have a greater influence on colour.

The significant role played by MR in colour formation observed in the gluten–glucose system was confirmed by the observed dependence of browning on water activity (Eichner, 1975). Samples heated under wet conditions produced more colour at all temperatures compared to those heated under dry conditions. Fig. 3 shows the 420 nm absorbance of materials extracted using different solvents after 1 h of treatment at 150°C under wet and dry conditions. It is notable that the absorbance rate was higher for the wet system when extracted with water and methanol and for the dry system when extracted with ethanol and acetone. The inset of Fig. 3 reports the ratio between the amount of colour extracted from the dry and wet systems: the ratio is less than one using water and methanol, and more than one using ethanol and acetone. Data suggest that less hydrophilic coloured material is formed under dry conditions.

Extraction with 1 M NaOH (data not shown) resulted in a complete solubilization of the sample leading to the formation of brown syrup. This behaviour was probably due to the well-known alkali-solubility of glutenins (Khan & Bushuk, 1979).

The following step was to investigate the chemical nature of the extracted coloured material. Different authors (Bailey et al., 1996; Cämmerer & Kroh, 1995; Hayase, Kim & Kato, 1986) have related the development of brown colour to the formation of high molecular weight polymers, called melanoidins. Hofmann (1998a,b) and Arnoldi, Corain, Scaglioni and Ames (1997) have elucidated the chemical structures of several

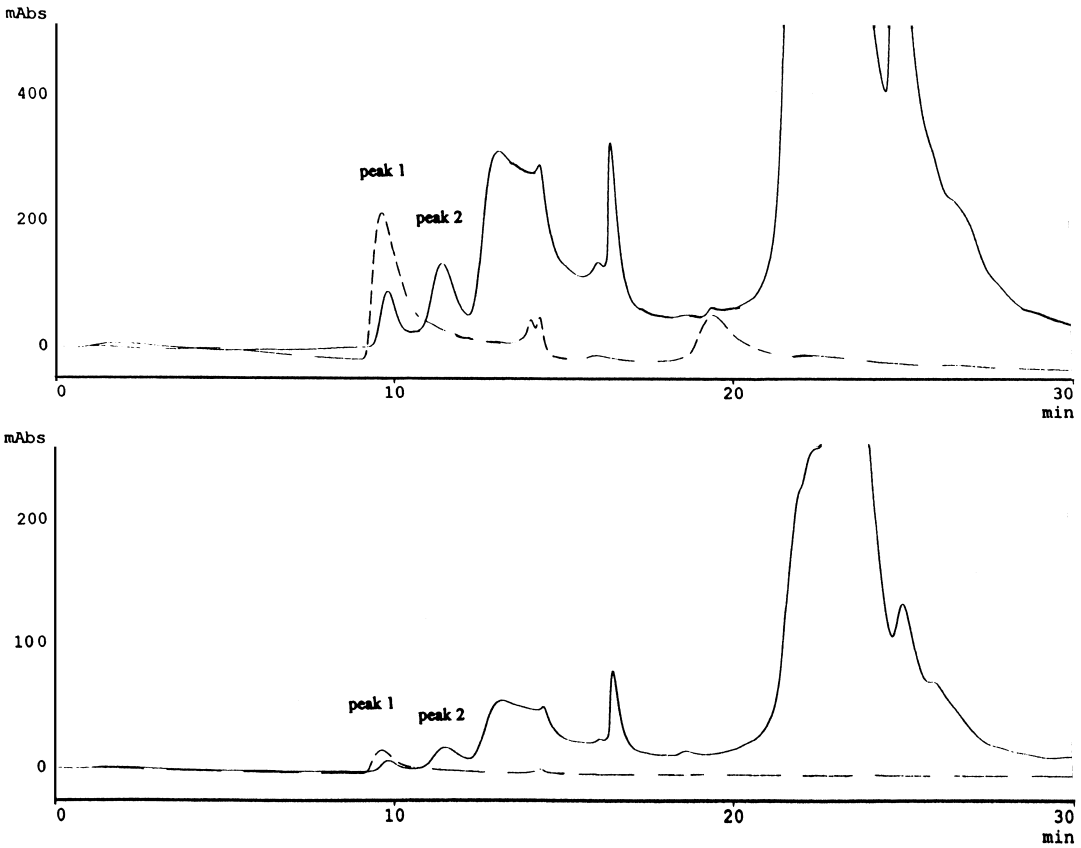

Fig. 4. HPLC gel filtration of methanol extract from gluten (control) and gluten–glucose system heated at 150°C. Gluten (- - -); gluten-glucose (—) 150. Up: detection at 220 nm; down: detection at 360 nm.

small chromophores formed in different amino acid sugar model systems. Hofmann (1998c), studying a casein–glucose model system, hypothesised that the main part of the colour is given by small compounds which cross-link the reactive side-chain of proteins forming oligomers. Ames, Arnoldi, Bates and Negroni (1997), working on a model extrusion-cooked cereal product (constituted of starch glucose and lysine), isolated 4-hydroxy-2-(hydroxymethyl)-5-methyl-3(2H) furanone and other compounds having a molecular mass ranging from 250 to 350 Da. These authors reported that most of the coloured material was not extracted in methanol but was retained by the starch matrix.

In this framework, it is of interest to investigate what happens in a pasta-like model system where the MR occurs in the presence of a protein polymer. Further experiments work on the methanol extracts, that are easy to handle and contain large amounts of coloured compounds, were carried out using samples treated under wet conditions.

HPLC gel filtration was performed on the methanol extracts obtained from samples treated under wet conditions at different time-temperatures. Fig. 4 compares chromatograms obtained after 1 h at 150°C to those of a control sample (gluten without glucose) at two different wavelengths (220 and 360 nm). As expected, low molecular weight compounds eluted after 20 min constituted most of the extracted material (Hofmann, 1998c). The first peak of the chromatogram (eluted at 10 min) is present also in the control system. It was rather small in the chromatogram obtained at 360 nm. Therefore, peak 1 did not contribute to the overall colour, and was likely due to gluten proteins. It is notable that the high molecular weight material eluted between 11 and 16 min (the first part of which was collected and indicated as peak 2) parallels the thermal treatments and shows absorption in the yellow–brown region. The comparison between chromatograms obtained at intermediate temperature (not shown) demonstrates that the formation of peak 2 is temperature-dependent.

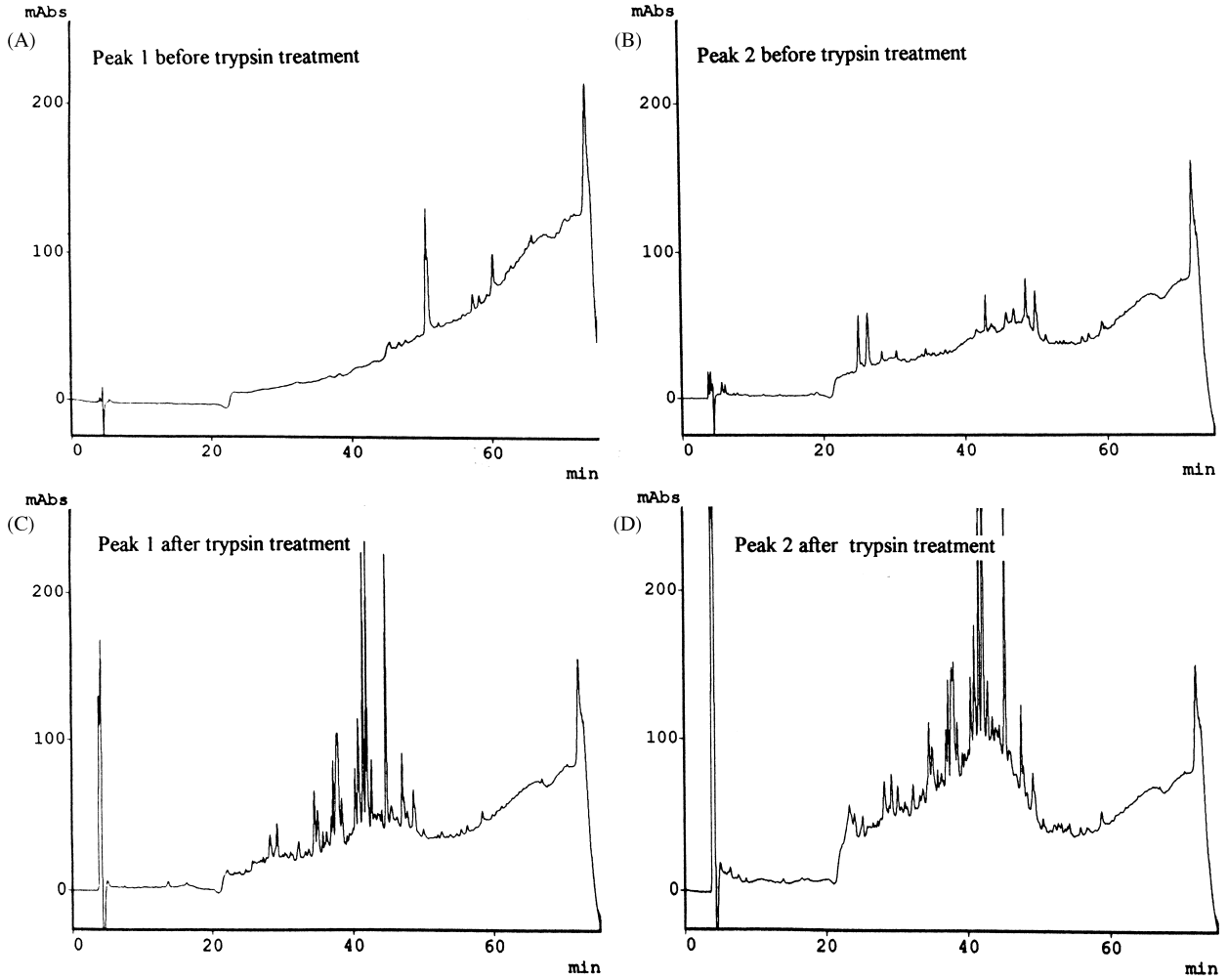

Fig. 5. HPLC reverse phase analysis with detection at 220 nm of peak 1 and 2 collected from gel filtration column as shown in Fig. 4. Panel A: peak 1 without trypsin treatment; Panel B: peak 2 without trypsin treatment; Panel C: Peak 1 after trypsin treatment; Panel D, Peak 2 after trypsin treatment.

The presence of the coloured material in peak 2 could be explained in three different ways: (i) colour is due to high molecular weight compounds resulting from polymerisation of low molecular weight MRPs formed by glucose and free ammonia generated by protein deamidation (Rihja et al., 1996); (ii) reaction of glucose with the side chain of protein residues leads to the formation of chromophores covalently linked to gluten proteins as shown by Hofmann (1998b); (iii) the low molecular weight MRPs formed by reaction of glucose and free ammonia are incorporated into the gluten polymer and behave as macromolecules.

To elucidate this point, the two high molecular weight peaks (1 and 2) obtained from gel filtration chromatography were collected, dialysed and concentrated using a microfiltration centrifuge filter having a cut off of 12 kDa. No coloured material was recovered in the filtrate of either peak, confirming that non-dialyzable coloured compounds behave as macromolecules.

After loading the two concentrated peaks on a C_{18} reverse phase column under denaturing conditions (i.e. TFA 0.1% and CH_3CN), most of the material was not separated and was eluted as an unresolved broad band (Panel A and B of Figs. 5 and 6). This chromatographic behaviour is characteristic of polymeric material (Bailey et al., 1996). The chromatograms at 360 nm, obtained for peak 2 (Panel B of Fig. 6), allows observation of some resolved peaks. This observation suggests that the chromatographic conditions caused the desorption of some small compounds from the polymer.

A tryptic hydrolysis was performed on both peaks. The digested material was again separated by C_{18} reverse phase under the same conditions as described above. Results shown in Panels C and D of Figs. 5 and 6 confirmed that peaks 1 and 2 are mainly proteins. In fact, chromatograms recorded at 220 nm after trypsin digestion revealed several peaks probably due to peptides formed by tryptic hydrolysis. This indicates that methanol extracted coloured material together with some of the gluten proteins. Among the peaks detectable after proteolysis, only a few, all from peak 2, are

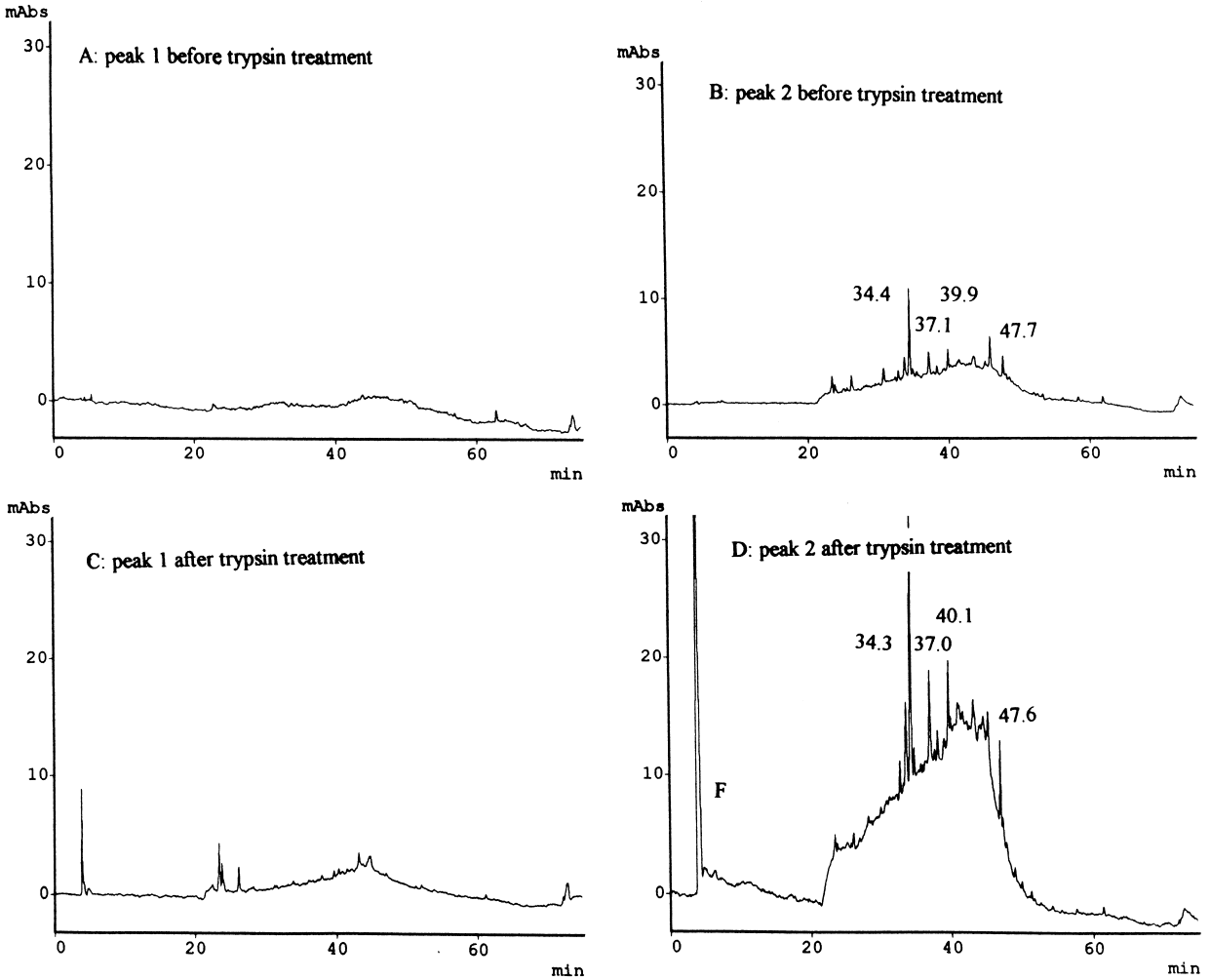

Fig. 6. HPLC reverse phase analysis with detection at 360 nm of peak 1 and 2 collected from gel filtration column as shown in Fig 4. Panel A: peak 1 without trypsin treatment; Panel B: peak 2 without trypsin treatment; Panel C: Peak 1 after trypsin treatment; Panel D, Peak 2 after trypsin treatment. A retention time of the major peaks was reported; F indicates the peak on the front of the chromatogram.

coloured, and detectable at 360 nm (Panels C and D of Fig. 6). By comparison of the profiles obtained before and after proteolysis of peak 2 (cf. Panel B and D of Fig. 6) it was clear that trypsin treatment did not induce formation of new compounds. The disruption of gluten polymers promoted the release of the same compounds present before proteolysis. In fact, retention times and UV spectra of the peaks are identical while their amount is about three times higher.

Trypsin digestion of peak 2 also produced a coloured peak eluting in front of the chromatographic column (indicated by "F" in Fig. 6, Panel D) which was absent before proteolysis. The spectrum of peak F is shown in Fig. 7. This is the only evidence for a coloured compound being directly generated by enzymatic digestion. It may be due to a peptide-bound chromophore as envisaged by Clark and Tannenbaum (1970). Further studies are necessary to ascertain the molecular nature of this compound.

In conclusion, our results suggest that, in a pasta-like model system, the presence of high molecular weight coloured material is mainly due to small MRPs which are physically entrapped into the gluten proteins polymer.

Acknowledgements

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References

Ames, J. M. (1987). A comparison of the colour properties of six heated sugar/amino acid model systems. *Chem. Ind. (London)*, 659–661.

Ames, J. M., Arnoldi, A., Bates, L., & Negroni, M. (1997). Analysis of the methanol-extractable non volatile Maillard reaction products of a model extrusion-cooked cereal product. *Journal of Agricultural and Food Chemistry, 45*, 1256–1263.

Arnoldi, A., Corain, E. A., Scaglioni, L., & Ames, J. M. (1997). New coloured compounds from the Maillard reaction between xylose and lysine. *Journal of Agricultural and Food Chemistry, 45*, 650–655.

Bailey, R. G., Ames, J. M., & Monti, S. M. (1996). An analysis of the non-volatile reaction products of aqueous Maillard model system at pH 5, using reversed phase HPLC with diode-array detection. *Journal of the Science of Food and Agriculture, 72*, 97–103.

Benzing-Purdie, L. M., Ripmeester, J. A., & Ratcliffe, C. I. (1985). Effects of temperature on Maillard reaction products. *Journal of Agricultural and Food Chemistry, 33*, 31–33.

Cämmerer, B., & Kroh, L. W. (1995). Investigation of the influence of reaction conditions on the elementary composition of melanoidins. *Food Chemistry, 53*, 55–59.

Clark, A. V., & Tannenbaum, S. R. (1970). Isolation and characterization of pigments from protein-carbonyl browning system. *Journal of Agricultural and Food Chemistry, 18*, 891–894.

Clawson, A. R., & Taylor, A. J. (1993). Chemical changes during cooking of wheat. *Food Chemistry, 47*, 337–343.

Didonè, G., & Pollini, C. M. (1990). The role of Maillard reaction in the THT technologies for pasta drying. *Tecnica molitoria*, 565–572.

Eichner, K. (1975). The influence of water content on non-enzymic browning reactions in dehydrated foods and model systems and the inhibition of fat oxidation by browning intermediates. In: R. B. Duckworth, (Ed.), *Proc Int Symp on Water relations of Foods* (pp. 417–434). New York: Academic Press.

Feillet, P., Jean, M. F., Kobrehel, K., & Laignelet, B. (1975). Macaroni brownness. *Tecnica molitoria*, 81–86.

Friedmann, M. (1996). Food browning and its prevention: an overview. *Journal of Agricultural and Food Chemistry, 44*, 631–653.

Hayase, F., Kim, S. B., & Kato, H. (1986). Analysis of the chemical structure of melanoidins by ^{13}C NMR, ^{13}C and ^{15}N CP-MAS NMR. *Agricultural and Biological Chemistry, 50*, 1951–1957.

Hodge, J. E. (1953). Chemistry of browning reactions in model systems. *Journal of Agricultural and Food Chemistry, 46*, 2599–2600.

Hofmann, T. (1998a). Characterization of the chemical structure of novel colored Maillard reaction products from furan-2-carboxaldehyde and amino acids. *Journal of Agricultural and Food Chemistry*, *46*, 932–940.

Hofmann, T. (1998b). 4-Alkylidene-2-imino-5-[4-alkylidene-5-oxo-1,3-imidazol-2-inyl]aza-methylidene-1,3-imidazoline. A novel colored substructure in melanoidins formed by Maillard reaction of bound arginine with glyoxal and furan-2-carboxaldehyde. *Journal of Agricultural and Food Chemistry*, *46*, 3896–3901.

Hofmann, T. (1998c). Studies on the relationship between molecular weight and the color potency of fractions obtained by thermal treatment of glucose/amino acid and glucose/protein solution by using ultracentrifugation and color dilution techniques. *Journal of Agricultural and Food Chemistry*, *46*, 3891–3895.

Kato, H., & Tsuchida, H. (1981). Estimation of melanoidin structure by pyrolysis and oxidation. *Progress in Food and Nutritional Science, 5*, 147.

Khan, K., & Bushuk, W. (1979). Studies of glutenin. XII. Gel filtration, isoelectrofocusing and amino acid composition studies. *Cereal Chemistry, 56*, 505–512.

Izzo, H. V., & Ho, C.-T. (1992). Ammonia effects Maillard chemistry of an extruded autolyzed yeast extract: pyrazine aroma generation and brown colour formation. *Journal of Food Science, 57*, 657–659.

Izzo, H. V., & Ho, C.-T. (1993). Effect of residual amide content on aroma generation and browning in heated gluten-glucose model systems. *Journal of Agricultural and Food Chemistry, 41*, 2364–2367.

Ledl, F., & Schleicher, E. (1990). New aspects of the Maillard reaction in foods and in the human body. *Angewante Chemie, 29*, 565–594.

Monti, S. M., Bailey, R. G., & Ames, J. M. (1998). A chromatographic investigation of the influence of pH on the non-volatile reaction products of aqueous Maillard model system by reverse phase HPLC with diode-array detection. *Food Chemistry, 62*, 369–375.

Riha, W. E., Izzo, H. V., & Ho, C.-T. (1996). Non-enzymatic deamidation of food proteins. *Critical Reviews in Food Science and Nutrition, 36*, 225–255.

Yoong, F. S., Walters, R. J., Tester, R. F., Gomes, M. R. A., & Ledward, D. A. (1994). The role of glutamic acid/glutamine and lysine during non-enzymic browning in heated gluten. *Food Chemistry, 51*, 271–274.

Wedzicha, B. L., & Kaputo, M. T. (1992). Melanoidins from glucose and glycine: composition, characteristics and reactivity towards sulphite ion. *Food Chemistry, 43*, 359–367.

Wijewickreme, A. N., Kitts, D. D., & Durance, T. D. (1997). Reaction conditions influence the elementary composition and metal chelating affinity of nondialyzable model Maillard reaction products. *Journal of Agricultural and Food Chemistry, 45*, 4577–4583.

Wright, H. T. (1991). Non-enzymatic deamidation of asparaginyl and glutaminyl residues in proteins. *Crit Rev Biochem Mol Biol, 26*, 1–51.