

Bleaching Of Components Responsible For Tooth Discoloration by Therapeutic Ozonated Water

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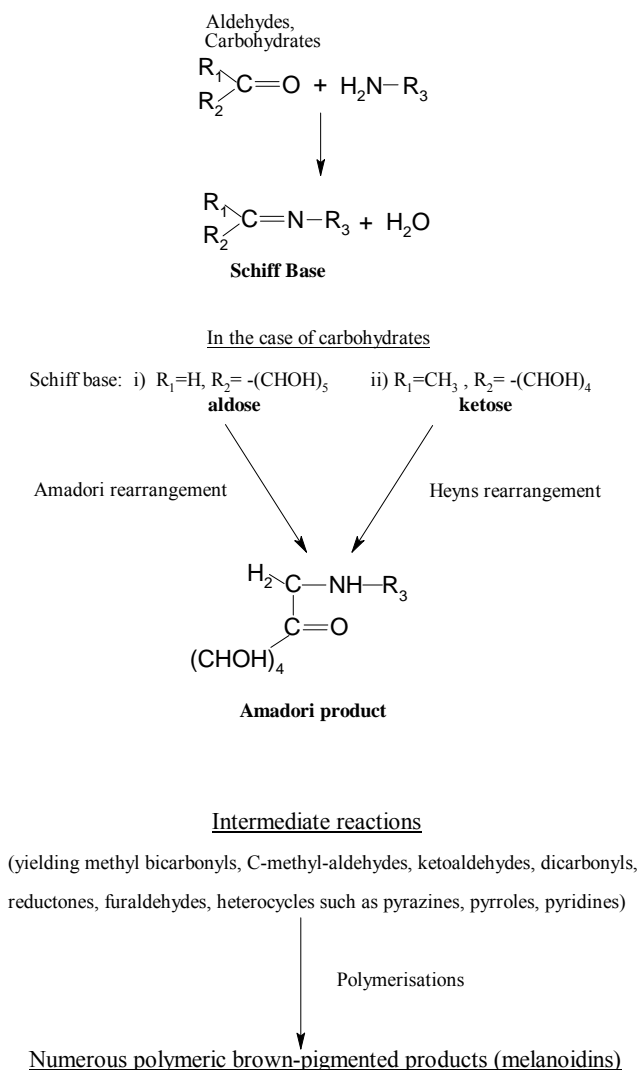
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Introduction

Extrinsic tooth discoloration (ETD) can arise from brown-coloured melanoidins, products derived from the condensation reaction of reducing sugars or carbonyl compounds with amino functional groups contained in, for example, amino acids, peptides, polypeptides and proteins. These are termed Maillard reactions, and glycoproteins of the acquired pellicle serve as possible substrates for these processes.

The Maillard reaction is a general term used to describe a whole network of processes involving amino acids, reducing sugars, nucleic acid bases and sugar moieties, unsaturated fatty acids, nitrogen-containing vitamins, together with their derivatives including fragmentation products, either as single molecules or as polymers. It provides the underlying mechanisms for some causes of, e.g., mutagenesis, carcinogenesis, protein molecule unfolding, cross-linking and denaturation, modified and impaired antibody-antigen reactions, specific enzyme inhibition, the promotion of growth of microbial pathogens such as bacteria, viruses, yeasts, fungi and protozoa, the suppression of growth of microbial non-pathogens, etc. The Maillard reaction is of much relevance to dental aesthetics, cariology and periodontology since many independent investigations have suggested that non-enzymatic browning has an aetiological importance in the development of extrinsic tooth discoloration.

Such processes arise from the condensation reaction of carbonyl compounds or reducing sugars with free amino groups. These can be typical α -terminal amino acid groups or the ϵ -NH₂ side-chain group of a lysine residue. Such reactions, in the first instance, yield relatively simple pre-melanoidins. Subsequent reactions are more complex and can yield a diverse range of soluble and volatile products ranging from straight-chain enol derivatives, carbocycles and heterocycles resulting from further reactions at the carbonyl and amino acid units, the separate fusion of carbonyl units, and subsequently simple addition polymers of such cyclic units. These products can then undergo further reactions and more complex polymerizations, yielding numerous brown-coloured, high-molecular-mass species (melanoidins or "browning products").



Melanoidins can also arise from the interactions of carbonyl compounds regularly consumed in the diet. For example, reactive aldehydes generated from culinary oils that have been subjected to episodes of thermal stressing have been detected by high resolution proton (^1H) NMR spectroscopy, and these may also play an important role in the discoloration of human teeth. Many commonly-administered antibiotics, anti-fungal and other anti-microbial agents have amino groups and/or carbonyl groups and consequently Maillard reactions may be partially responsible for their therapeutic activities, especially since many of them are specific enzyme inhibitors. Further sources of melanoidins include chlorhexidine, quinone species (derived via autoxidation of plant polyphenols occurring in beverages such as tea and red wine), together with acetaldehyde present in cigarette smoke.

In this study we have conducted a spectrophotometric evaluation of the ability of ozone (O_3) present in a novel ozonated water product [1] to bleach brown-coloured melanoidins.

Materials and Methods

Melanoidins were generated from the reaction of L-lysine (1.25-250.00 mM) with an equivalent concentration of α -D-glucose in 40.0 mM phosphate buffer (pH 7.00) at 80°C for a period of 240 hr. After cooling to ambient temperature, each brown-coloured reaction mixtures were subjected to a series of selected dilutions with further 40.0 mM phosphate diluted to a final volume of 1.00 ml with additional 40.0 mM phosphate buffer. Aliquots (18.00 ml) of each of the above solutions were then divided into six equivalent portions (3.00 ml). These portions were then treated with 0.00, 3.00, 6.00, 9.00, 12.00 and 15.00 ml volumes of ozonated water [product [1], generated by the TherOzone unit (CA, USA)] and 15.00, 12.00, 9.00, 6.00, 3.00 and 0.00 ml of untreated, doubly-distilled water respectively (yielding a final volume of 18.00 ml), the first sample serving as an untreated control. Subsequently, samples were equilibrated at ambient temperature for a period of 15 min. The extent of bleaching of these brown-coloured melanoidin solutions was then monitored spectrophotometrically at a wavelength of 350 nm (Unicam UV-2 spectrophotometer). Moreover, complete electronic absorption spectra of these reaction mixtures were also recorded (260-700 nm wavelength range). Each sample treatment was conducted in triplicate.

Results

Results acquired revealed an O_3 concentration ($[\text{O}_3]$)-dependent decolourisation (bleaching) of brown-coloured melanoidins by the product tested, i.e. decreases in absorbance in the visible region of the above wavelength range (λ_{max} . 350 nm) for each solution investigated (Figure 1 and Table 1). Indeed, as expected, the extent of this process increased with increasing added O_3 concentration (i.e., added volume of ozonated water).

Conclusion

Data acquired here clearly demonstrate that O_3 present in the product investigated effectively bleaches models of agents responsible for extrinsic tooth discoloration; indeed, our results demonstrate a concentration-dependent level of 'browning-product' discoloration. The methodology developed here can also be employed to investigate the kinetics, mechanisms and extent of bleaching by alternative tooth-whitening products. The mechanism of this process clearly involves the oxidative consumption of brown-coloured melanoidins.

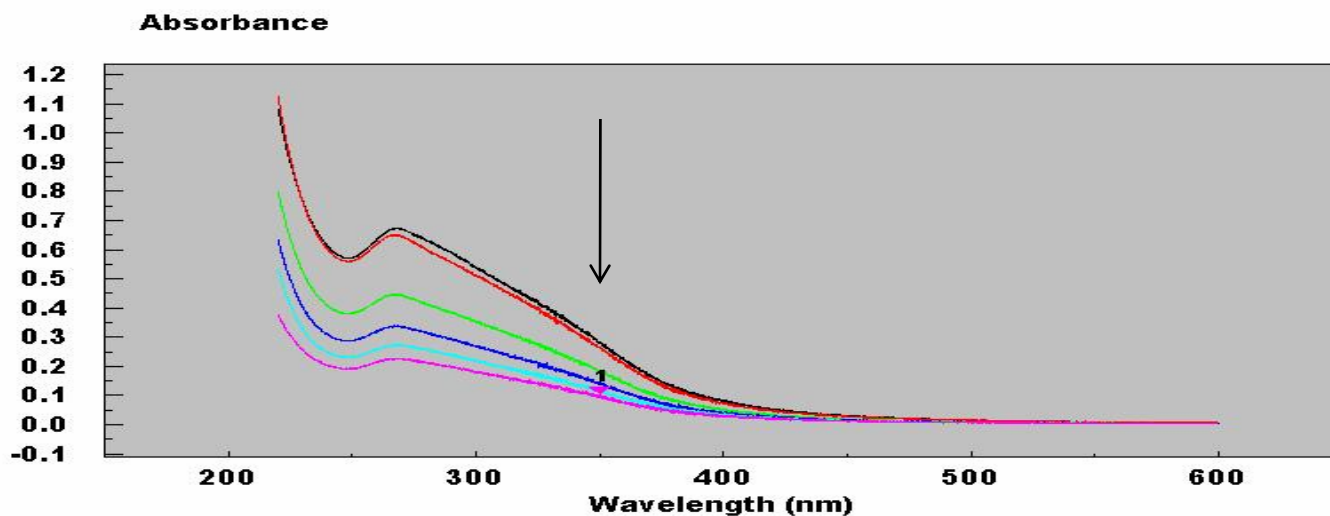


Figure 1. Full and expanded regions of the electronic absorption spectra of a model melanoidin-containing extrinsic tooth stain solution (generated from the reaction of 50 mM L-lysine with 50 mM a-D-glucose; 1/10 dilution in 40 mM phosphate buffer) treated with increasing levels of ozonated water.

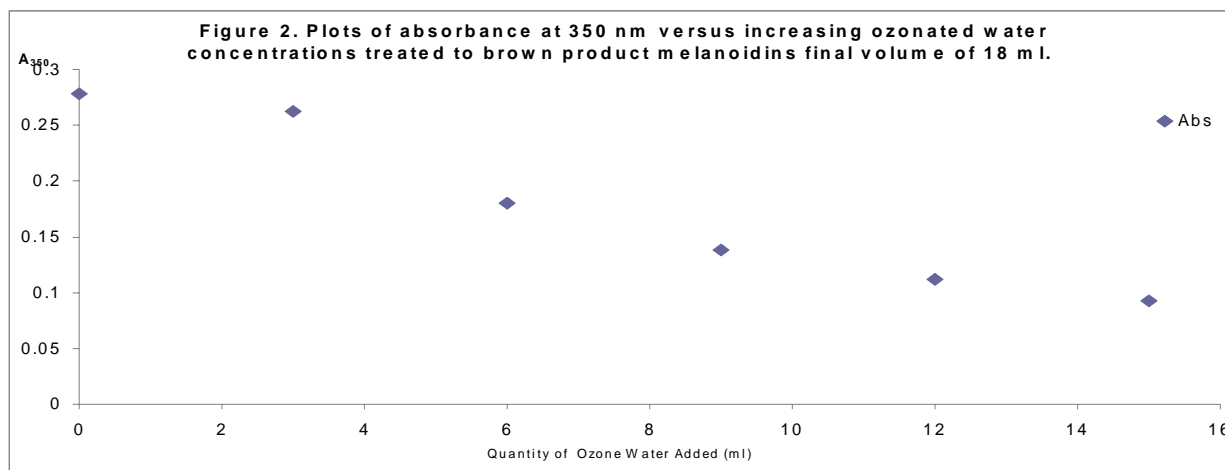


Figure 2. Plots of absorbance at 350 nm versus time for solutions containing melanoidins treated with increasing volumes of ozonated water