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EFFECT OF PRESSURE ON PROCESSES MODELLING THE MAILLARD REACTION

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The Maillard reaction between tryptophan and glucose or xylose was studied as a function of pressure. Using model reactions, volumes of activation for the formation of the intermediate imine and the Amadori rearrangement and for the decomposition of the aminoketose were measured as −14, 8 and 17 $cm^3 mol^{-1}$, respectively. Pressure therefore accelerates the initial reactions but retards the formation of the final heterocyclic products and melanoidins. Oxygen was found to accelerate the latter reaction.

INTRODUCTION

Food processing using high pressures (up to about 6 kbar) is becoming increasingly a commercial possibility[1] and it is therefore important to understand how aspects of food chemistry may change under these extreme conditions. The Maillard reaction is a complex sequence of events beginning with reactions between proteins and carbohydrates at temperatures of *ca* 100 °C and above, leading ultimately to a range of products, some volatile and others highly complex, which impart flavour and colour to cooked foodstuffs.[2]

Despite its complexity, the Maillard reaction has been studied in some detail in the form of model reactions between simple carbohydrates and amino acids and an overall scheme due to Hodge and co-workers[3] is still regarded as the basis of the process. The mechanisms of the initial stages are also understood as occurring according to Scheme 1. Thus, tryptophan and glucose, for example, in water at *ca* 100 °C condense initially to form an imine, **1**, which rapidly undergoes the Amadori rearrangement to the aminoketose **2**, existing in various tautomeric forms.[4] This in turn is degraded to a variety of small molecules which are important for flavour production. Among these, 5-hydroxymethylfurfural, norharman and maltol have been recognized as prominent while some tryptophan is recovered, although the formation of pyrroles, pyrazines and other nitrogen heterocyclics account for the loss of some of the amino acids. The appearance of a brown colour also signals the formation of more complex, possibly polymeric products ('melanoidins'). Other pathways involving intermediates reacting with further carbohydrates or proteins, although implicated in cooking of real food systems, are probably of only minor importance in such a model reaction.

It has been reported[5] that the browning reaction is suppressed by pressure but, since there are numerous stages involved in the reaction before coloured products are formed, few conclusions can be drawn as to the reasons for this and accordingly we have examined the effect of pressure on the rates of several of the early stages in addition to this overall sequence using the model reaction between tryptophan and glucose. The advantage of using this amino acid is that the indole nucleus has a characteristic UV absorption and therefore it is easy to identify indole fragments in any of the degradation products, many of which have already been structurally assigned.

EXPERIMENTAL

Preparation of tryptophan methyl ester. L-Tryptophan (2·5 g, 12·2 mmol) was added to a solution of thionyl chloride (1·1 ml, 15 mmol) in methanol (35 ml) and the resulting solution was heated under reflux for 18 h. After cooling and evaporation of the solvent, a white crystalline residue of the ester hydrochloride was obtained. This was neutralized in sodium hydroxide solution (200 ml, 10%) and the ester was extracted with chloroform (2 × 50 ml). After drying and rotary evaporation, a yellow oil remained which crystallised on cooling and was recrystallized from diethyl ether, m.p. 89 °C (lit. 89·5 °C).[6]

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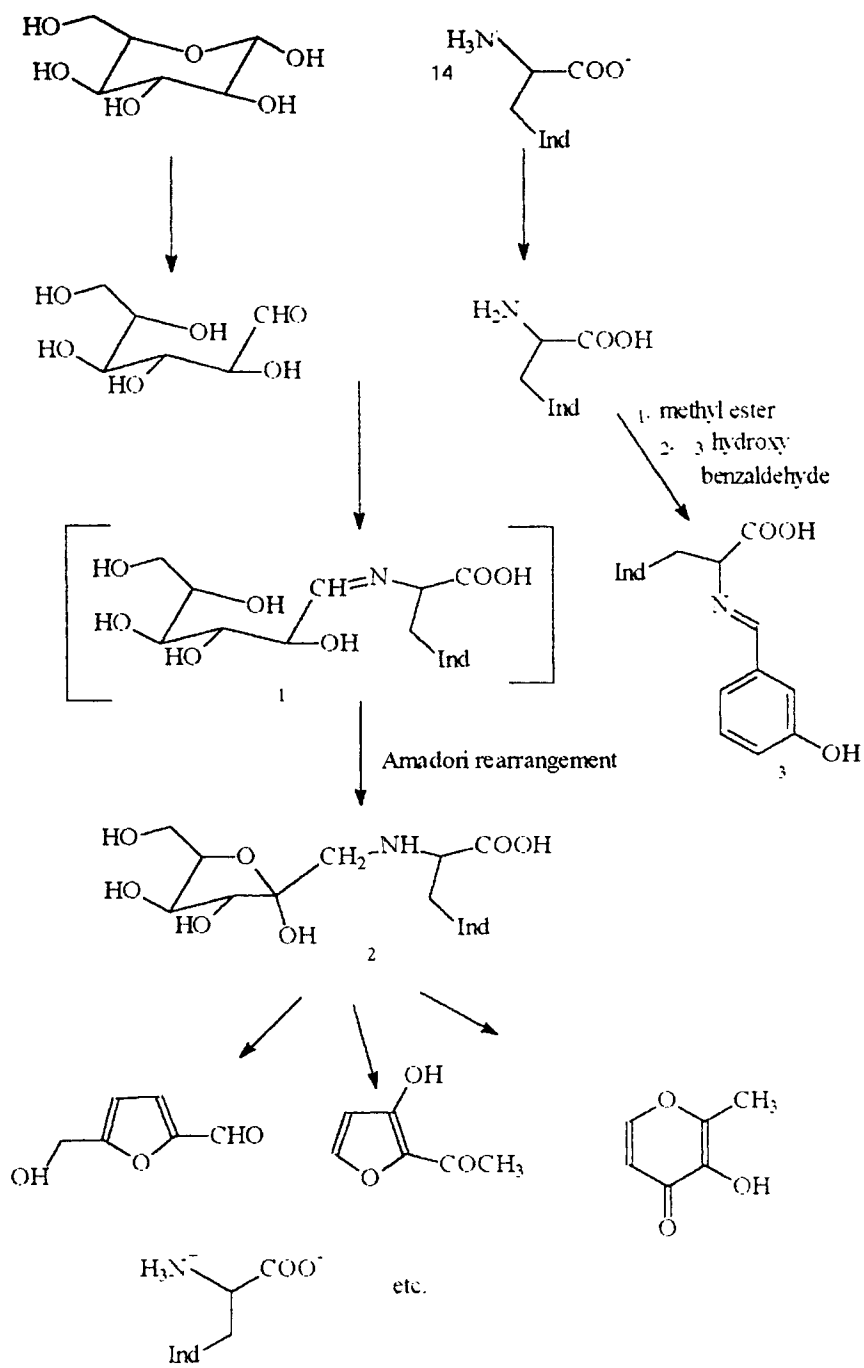
HO
HO
HO
O
OH
OH
H3N
14
COO-
Ind
CHO
OH
COOH
1. methyl ester
2. 3 hydroxy
benzaldehyde
CH=N
1
COOH
3
Amadori rearrangement
CH2-NH
2
CHO
COCH3
CH3
etc.

Scheme 1

Reaction of tryptophan methyl ester with 3-hydroxybenzaldehyde Tryptophan methyl ester (1·0 g, 5 mmol) was dissolved in THF (5 ml) and 3-hydroxybenzaldehyde (0·61 g, 5 mmol) was added. The solution was allowed to stand overnight and the solvent was evaporated under vacuum to leave a yellowish oil, which resisted attempts at crystallization. The formation and identity of the anil, however, were revealed by the mass spectrum (chemical ionization), which had a prominent peak at m/z 323·1395 which corresponds to MH^+ (theoretical for $C_{19}H_{18}N_2O_3 = 323·1395$).

Preparation of 1-(N-L-tryptophanyl)-1-deoxy-D-fructose[7] D-Glucose (10·0 g, 55·6 mmol) and L-tryptophan (1·2 g, 5·9 mmol) were dissolved in methanol (250 ml) and the solution was heated under reflux with stirring for 4·5 h. The resulting brown solution was concentrated by rotary evaporation to 50 ml and diluted with water (8 ml). This solution was applied to a preparative column (340 × 44 mm i.d.) packed with Merck Avicel microcrystalline cellulose and the product was eluted with water saturated with *n*-butanol. Aliquots (25 ml) were collected and cooled to 0 °C, from which solid product was filtered and recrystallized from methanol, m.p. >140 °C (decomp.). This probably contained several tautomers, as shown by Yaylayan and Forage.[4] The mass spectrum (chemical ionization) showed a molecular ion at m/z 367·1509 (MH^+) (theoretical for $C_{17}H_{23}N_2O_7 = 367·1505$).

ANALYTICAL METHODS

High-performance liquid chromatography (HPLC) was used to follow reactions and to estimate quantities of products, as follows: Varian Star system fitted with a multi-diode array detector (200–400 nm), monitoring at 278 nm; automatic sampling; and column, 25 cm Nucleosil 120 GA 7 μm NH_2 at 35 °C. In all cases, response factors were determined using solutions of known concentration of pure substances. Solvent system 1 was acetonitrile–1·36 g dm^{-3} aqueous KH_2PO_4 (80:20) at a flow rate of 0·7 ml min^{-1} and solvent system 2 was methanol–water–1·36 g dm^{-3} aqueous KH_2PO_4 (78:28:1) at a flow rate of 1·5 ml min^{-1}. The retention times with solvent 1 were tryptophan 12·5 and phenylalanine 14·1 min and with solvent 2 tryptophan 4·5 and Amadori compound 3·7 min.

High-pressure sampling apparatus

This consisted of a stainless-steel vessel (300 mm long × 60 mm o.d. × 25 mm i.d.) fitted with a flanged top sealed by two O-rings, to which was attached a high-pressure valve (Nova Swiss miniature 4 kbar valve) on the outside and connecting with a KelF cylinder fitted with a piston sealed by two O-rings on the inside. The whole was thermostated by a heating coil controlled by a proportional controller. At the bottom of the cylinder was connected a high-pressure hand pump containing a 1:1 mixture of paraffin oil and light petroleum (b.p. 120 °C) as pressure-transmitting fluid. The reaction mixture was placed in the inner KelF cylinder and pressure was applied. Samples were then withdrawn through the valve at appropriate time intervals and analysed by HPLC, pressure being restored immediately to the cylinder by means of the hand pump. Specific rate constants were calculated from concentration vs time data using the program KINFIT (supplied by Olis Kinfit, On Line Instrument Systems, Olis, GA, USA) and were averaged over several runs. Volumes of activation were adjusted to a standard median temperature (50 °C) for comparison using a value of $-d\Delta V^*/dT = 0·16$.[8]

Reaction between tryptophan and 3-hydroxybenzaldehyde

A methanolic solution of tryptophan (0·01 M) and 3-hydroxybenzaldehyde (0·1 M) was prepared and reaction proceeded at 70 °C and at pressures up to 800 bar. Samples were taken periodically and analysed by HPLC (solvent system 1), measuring the disappearance of the tryptophan and the appearance of the imine, **3**, to obtain pseudo-first-order rate constants and a volume of activation according to the relationship[7] $\Delta V^* = RTd(\ln k)/dp$.

Reaction between tryptophan methyl ester and 3-hydroxybenzaldehyde

In an analogous manner, rates of imine formation between the methyl ester of tryptophan and 3-hydroxybenzaldehyde were measured at pressures up to 600 bar by sampling and HPLC analysis (solvent system 1). The ester proved more reactive than tryptophan itself since no zwitterion formation is possible and rates were measured at 20 °C.

Reaction between L-tryptophan and D-glucose

L-Tryptophan (0·0128 g, 0·063 mmol) and D-glucose (0·1126 g, 0·63 mmol) were dissolved in methanol (40 ml) and maintained at 70°C and pressures up to 600 bar. Samples were removed periodically for HPLC analysis (solvent system 2), by which both tryptophan and the Amadori rearrangement product could be estimated. Pseudo-first-order rate constants were estimated as usual and the volume of activation evaluated.

Thermal degradation of 1-{N-L-tryptophanyl)-1-deoxy-D-fructose

Solutions of 1-(*N*-L-tryptophanyl)-1-deoxy-D-fructose (0·0092 g, 0·02 mmol) were dissolved in water (10 ml) and placed in the high-pressure sampling apparatus at 80 °C and at pressures ranging from 1 to 600 bar.

Samples were removed at intervals and analysed by HPLC for the starting material and tryptophan, permitting rate constants and the volume of activation to be determined. Minor products also appeared, some of which could be identified.

RESULTS AND DISCUSSION

The data obtained for the various reactions are given in Tables 1–4.

Table 1. Reaction between 3-hydroxybenzaldehyde ($c_0 = 0{\cdot}1$ M) and L-tryptophan ($c_0 = 0{\cdot}01$ M) at 70 °C in water

p (bar)	$10^5 k$ (min^{-1})	Ln k_{rel}
1	6·92 ± 0·05	0
200	7·15	0·032
400	7·57	0·090
600	8·39	0·192
800	9·13	0·277

$\Delta V^* = -10$ cm^3 mol^{-1}; $\Delta V^*(50) = -7$ cm^3 mol^{-1}.

Table 2. Reaction between 3-hydroxybenzaldehyde ($c_0 = 0{\cdot}1$ M) and tryptophan methyl ester ($c_0 = 0{\cdot}01$ M) at 20 °C in water

p (bar)	$10^4 k$ (min^{-1})	Ln k_{rel}
1	2·16 ± 0·05	0
200	2·59	0·181
400	2·87	0·284
600	3·26	0·412

$\Delta V^* = -16$ cm^3 mol^{-1}; $\Delta V^*(50) = -21$ cm^3 mol^{-1}.

Table 3. Reaction between glucose ($c_0 = 0{\cdot}025$ M) and tryptophan ($c_0 = 0{\cdot}0025$ M) at 70 °C in water to form the Amadori rearrangement product

p (bar)	$10^5 k$ (min^{-1})	Ln k_{rel}
1	4·53 ± 0·07	0
100	4·79	0·056
200	5·12	0·122
600	6·43	0·350

$\Delta V^* = -17$ cm^3 mol^{-1}; $\Delta V^*(50) = -14$ cm^3 mol^{-1}.

Table 4. Thermal decomposition of 1-(*N*-L-tryptophanyl)-1-deoxy-D-fructose at 95 °C

p (bar)	$10^5 k$ (min^{-1})	Ln k_{rel}
1	3·283 ± 0·05	0
100	3·13	−0·05
200	2·62	−0·22
600	2·35	−0·33

$\Delta V^* = +17$ cm^3 mol^{-1}; $\Delta V^*(50) = 20$ cm^3 mol^{-1}.

The initial step in the Maillard reaction must be the condensation of the amino acid in its neutral form with the sugar, presumably as the free aldehyde, to form an imine. Since all stages of this reaction cannot be directly observed, volume changes were estimated from model reactions. The discussion below relates to volumes of reaction estimated at 50 °C. Thus, the volume of activation for ring opening of the pyranoside (**1**⟶**2**) may be taken as that measured for the mutarotation of glucose, $\Delta V^* = -15$ cm^3 mol^{-1}.[9] Condensation between an aldehyde and an amine is an associative reaction, a polar carbonyl addition process followed by fast elimination, and consequently would be expected to show a negative volume of activation such as has been observed for oxime formation for which a value $\Delta V^* = -16$ cm^3 mol^{-1} has been recorded.[10] For this stage of the Maillard reaction, the volume of activation was estimated using as a model system the condensation between 3-hydroxybenzaldehyde and tryptophan methyl ester, for which $\Delta V^* = -21$ cm^3 mol^{-1}. This may be compared with the less negative volume of activation for the reaction of 3-hydroxybenzaldehyde with tryptophan, $\Delta V^* = -7$ cm^3 mol^{-1}, the difference (+14 cm^3 mol^{-1}) being attributable to the internal proton transfer of the zwitterionic form of the amino acid, a value which accords with charge neutralization[11].

The formation of the Amadori product, **4**, from tryptophan and glucose was directly observed and gave an apparent $\Delta V^* = -14$ cm^3 mol^{-1}. This is a much slower reaction than either ring opening of glucose or proton transfer within the amino acid and therefore these two steps may be treated as pre-equilibria and, using volume changes quoted above, it may be inferred that the rearrangement step, the conversion of imine to aminoketose (**3**⟶**4**), is characterised by a small positive activation volume, $\Delta V^* \approx 8$ cm^3 mol^{-1}. Although there is considerable uncertainty in this value owing to the approximations used in its assessment, it is reasonable that the volume change should be very small for such a rearrangement. Overall, therefore, pressure favours the formation of the Amadori product. The rates of this reaction are sensitive to pH, especially at lower temperatures, but in our system the initial and final values of pH remained close to 7·0. In contrast, the decomposition of the preformed aminoketose in water at 100 °C was found to be retarded by pressure, $\Delta V^* = 17$ cm^3 mol^{-1} at 95 °C (it is not clear which temperature coefficient would be appropriate for this reaction). This is a complex process and must represent a mean value for several decomposition modes. However, whether the decomposition of the Amadori compound leads to the formation of 5-hydroxymethylfurfural, maltole or norharman (**4**⟶**5**, **6**, etc.) in addition to some tryptophan, similar volume changes would be predicted since all are fragmentation reactions. A comparison might be made with the fragmentation of 2-methyl-3-bromobut-2-enoate,[12] $\Delta V^* = 18$ cm^3 mol^{-1}.

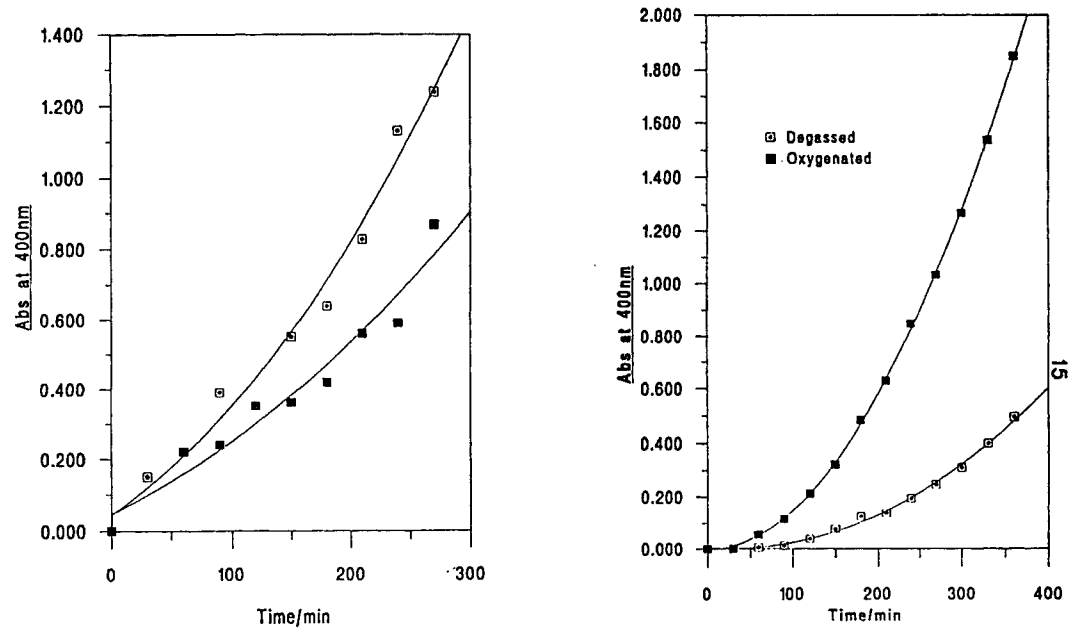

Figure 1. (a) Meloidin formation from trytophan and xylose at 80 °C and (■) 800 and (⊡) 50 bar. (b) Melanoidin formation from trytophan and xylose at 80 °C and 1 bar under (■) oxygen and (⊡) argon atmospheres

As regards the formation of the melanoidins, we have confirmed that pressure retards the development of the brown colour measured by absorption around 400 nm in the reaction between tryptophan and xylose, a more reactive sugar than glucose. However, oxygen provides a complication. We observe that the development of brown colour is greatly accelerated by the presence of oxygen, presumably due to radical oxidations promoted in the presence of air. When carried out in carefully degassed solutions under an argon atmosphere, browning is much slower (Figure 1). Nevertheless, further retardation is brought about by high pressure. Previous workers[13] had ascribed rate constants to these processes and also a volume of activation. We find, however, under either oxygen-free or oxygenated conditions, that the rate of reaction increases with time and leads eventually to precipitation of dark-coloured solids and therefore no specific rate coefficient can be attached to this process and no volume of activation other than to affirm that it must be positive. It is not unrealistic to consider anaerobic processes in relation to food processing since, if high-pressure conditions were to be used, it is likely that access to oxygen would be very restricted in comparison with normal thermal processing

In conclusion, the retarding effect of pressure on the Maillard reaction as estimated by the formation of the brown colour appears to stem from a retardation of the decomposition of the Amadori rearrangement product whose production is actually facilitated and which should therefore tend to accumulate. The volume profile of the sequence of reactions is as shown in Scheme 1.

Further work is in progress to determine the kinetic effect of pressure on the individual low molecular weight products which determine flavour.

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