EFFECT OF LIPID ON THE MAILLARD REACTION BETWEEN METHIONINE AND RIBOSE : A MODEL SYSTEM NVESTIGATE THE FORMATION OF MEAT FLAVOUR.

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mary.

del system containing methionine and ribose was prepared in buffered solution (pH 5.5). Linoleic acid and/or ethanolamine were then d to this solution. The reaction mixtures were cooked under pressure at 140°C for one hour., the volatile compounds were extracted by ltaneous distillation extraction with diethylether as solvent and, finally, qualitively and quantitatively analyzed by GC and GC-MS. esults show that without fatty acid, the major compounds were 3-methylthiopropanal (494 µg/ 100mg ribose), furfural (100 µg /100 ibose) and dimethyl disulphide (94 µg/ 100mg ribose). The addition of linoleic acid modified the chromatogram both quantitatively ualitatively. The oxidation products of linoleic acid such as aldehydes, alcohols and furans were formed. Moreover, the quantities of llard reaction products were modified and some compounds such as 2-pentylpyridine were formed by reaction of Maillard and lipid ation compounds. The addition of ethanolamine caused a reduction in volatile concentration. The most drastic reduction was observed ldehydes formed by Maillard reaction or linoleic acid oxidation. This work establishes that in model system, lipid components are able odify the reaction equilibria and consequently change the overall aroma which is perceived.

oduction.

development of meat flavour involves complex reactions, such as the Maillard reaction, and phospholipids can play a key role tram and Edwards, 1983 ; Farmer and Mottram, 1990). A better knowledge of the role of phospholipids in meat flavour has required stigations in model systems. Among all the amino acid precursors of flavour, sulphur-contanining compounds have been reported to be important in meat (Mottram, 1991; Werkhoff *et al*, 1990). Concerning meat phospholipids, it has been well established that phatidylethanolamine was more degraded during cooking than other lipids (Keller and Kinsella, 1973). The fatty acid composition of phosphatidylethanolamine has been reported by Fogerty *et al* (1989). To determine the relative contribution of fatty acid chain and moity of phospholipid, linoleic acid and/or ethanolamine was added to the base Maillard reaction model system containing ionine and ribose. The effects on Maillard reaction products were examined quantitatively and qualitatively.

erials and methods.

paration of the reaction mixtures and extraction of volatile compounds.

L-Methionine, D(-)Ribose, linoleic acid and ethanolamine were purchased from Sigma Chemical Company. Phosphate buffer M pH 5.6) was prepared from Na_2HPO_4 and NaH_2PO_4 (BDH Chemicals ltd) in glass distilled water. Methionine (10mg/ml) and ribose ng/ml) were dissolved in phosphate buffer. Linoleic acid (10mg/ml) and ethanolamine (2mg/ml) were added to the solution if required. e reaction mixtures were heated under pressure at 140°C for 1 hour. Aliquots (2ml) of each solution were diluted in 50 ml of glass illed water. Then, the volatile compounds were extracted in a Likens-Nickerson apparatus with 20 ml of diethylether as solvent taining 5µg of octadecane (Internal standard). The dried extract was concentrated by fractional distillation with a Vigreux column to a al volume of approximately 100µl.

Chromatography.

Gas Chromatography analyses were performed using a Hewlett-Packard 5890 gas chromatograph fitted with a WCOT fused si capillary column (30m x 0.32mm id 1µm film thickness) coated with DB5 (J&W Scientific, Folsom California). The on-column injec and the flame ionisation detector were used for these analyses. The helium carrier gas was set at a flow of 1.5 ml/min. The ini temperature 40°C was maintained for 5 minutes and then increased to 220°C at 4°C/min, where it was held for 15 minutes. To calculate Linear Retention Index (LRI), a solution of C_8 to C_{20} alkanes in diethylether was injected. Individual components were quantified comparison of GC peak area with the area of internal standard. Since the response factors of each component were not determined, provided only approximate concentration.

Gas chromatography-mass spectrometry.

A Carlo Erba 4200 gas chromatograph, equipped with a split-splitless injector and fitted with a WCOT fused silica capillary colu (30m x 0.32mm id) coated with DB5 (J&W Scientific) was used for all analyses. The gas chromatograph was coupled to a Finnigan 40 mass spectrometer. The end of the capillary column was connected directly into the ion source heated at 250°C. The flow rate of the carri gas (helium) was set at 1 ml/min. The initial column temperature was set at 60°C, maintained for 5 min and then increased to 220°C 4°C/min. The final temperature was held for 15 min. The mass spectrometer was operated in the electron impact mode with an electro energy of 40 eV. A continous scan mode with a scan time of 1 s over a mass range 33-400 was used. All GC-MS data were monitore stored and processed using an INCOS 2100 data system. The LRI of each compound was calculated as previously described.

Results and discussion .

The " methionine + ribose" reaction mixture possessed a strong and penetrating aroma of burnt cabbage and sulphur. In presence linoleic acid, the overall aroma was similar but fatty and green notes appeared. With ethanolamine, the intensity of sulphur and burn cabbage aroma decreased both in presence and absence of linoleic acid.

The colour of the reaction mixture, which did not contain ethanolamine, was yellow, while ethanolamine-containing solutions were darker. The pH of solution was 5.2 without ethanolamine and 5.8 with this compound.

The heating of methionine and ribose led to the formation of many volatile compounds (approximately 60), including 12 furans, aliphatic sulphur-containg components, 1 sulphur-containing aldehyde and 2 pyrroles. Selected compounds are listed in table 1. In the methionine and ribose solution, the major components were : 3-methylthiopropanal (methional 494 µg/100mg ribose), furfural (105 µg/100mg ribose) and dimethyldisulphide (94 µg/100g ribose). Other compounds included 2-furanmethanol and dimethyltrisulphide, and furan derivatives but their quantities were much lower (<5 µg/100mg ribose).

The major compound (methional) is a product of the Strecker degradation of methionine. The decomposition of methional to methanethiol and its subsequent oxidation could produce dimethyldisuphide. Furfural is formed by the thermal degradation of ribose.

Effect of linoleic acid In the Maillard reaction.

When linoleic acid was added to the basic solution, it induced a modification of the GC profile. The effect on Maillard reaction products and linoleic acid oxidation are discussed successively.

The thermal oxidation of linoleic acid was studied and the main components are listed table 2.

Linoleic acid had some effect on the Maillard reaction products. Amounts of aldehydes such as methional and furfural tended to increase, while quantities of other compounds were reduced (dimethyldisulphide) and some could no longer detected (2-furfuryl methyl sulphide). The reaction of some linoleic acid oxidation products with methanethiol, a precursor of dimethyl disulphide and 2-furfuryl methyl sulphide, could explain this observation.

In both systems containing 18:2 alone and those with methionine and ribose, the major linoleic oxiadtion products were pentanal and hexanal (see table 2). In presence of amino acid and sugar they were detected in higher amount (2 and 4 fold more) suggesting a prooxidant effect of amino acid and sugar on linoleic acid. This effect could be explained by the formation of free radicals during the early

of Maillard reaction (Namiki and Hayashi, 1983). Such compounds were well known to initiate the fatty acid oxidation (Frankel,

Two compounds were formed by the interaction of amino acid, sugar and fatty acid (table 3). 2-Pentylpyridine could be formed reaction of ammonia (a Strecker degradation products) and 2,4 decadienal (a linoleic acid ovidation product). The pathway of ation was described by Farmer and Mottram (1990). 3-Methylthiobutanal could be formed by reaction of 2-butenal and methanethiol rouk, 1979).

et of ethanolamine.

The addition of ethanolamine in reaction mixture induced a reduction in the quantities of the volatile compounds. The darker colour solutions suggested formation of non-volatile melanoidin pigments. The most affected volatile compounds were aldehydes.

Furfural (one of the major Maillard reaction products) was mot detected in presence of ethanolamine. Although pH can influence rmation of furfural, its loss can not be totally explained by the higher pH ($\Delta pH = 0.6$) of ethanolamine-containing solution, tram and Leseigneur, 1990). Reaction of carbonyls with amines is well known to form Schiff bases or imines (Tressl, 1979) and this explain the decrease of aldehyde concentration. Other compounds such as dimethyldisulphide, 2-furanmethanol or 2-rylmethylsulphide were also detected in lower quantities in presence of ethanolamine. In this case, a direct reaction of ethanolamine these compounds does not appear likely and a more complex effect is probably involved. Most likely hypothesis is the reaction of nolamine with compound precursors. The Maillard reaction involved many steps and changes in the concentration of one intermediate ld influence the relative of final products.

With the exception of 2-pentylfuran and pentanol, all linoleic oxidation products were detected in lower quantities in presence of nolamine. The most dramatic changes occured for alkanals and 2-alkenals (4 and 10 fold less respectively) and as for Maillard reaction ducts, formation of imines from aldehydes and ethanolamine could be the explanation.

clusion.

The reaction of methionine and ribose generated a complex mixture of aroma compounds. The addition of phospholipid elements oleic acid, ethanolamine) modified the overall aroma and the volatile compounds quantitatively and qualitatively. The presence of oleic acid led to appearance of its oxidation products and of interaction compounds such as 2-pentylpyridine which were formed by tion of oxidation compounds with Maillard reaction products such as ammonia and methanthiol. Ethanolamine reduced the quantities olatile components, especially of aldehydes formed via Maillard reaction and linoleic acid oxidation. Such simple model systems can to understand to key role of phospholipid in meat flavour.

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Table 1 : Effect of linoleic acid on the Maillard reaction compounds
(in µg/100mg ribose)

Compound	Methionine + ribose	Methionine + ribose + 18:2	Methionine + ribose + ethanolamine	Methionine + ribose + 18:2+ethanolamine
Aldehydes				
3-methylthiopropanal	494.4	583.3	183.3	183.3
Alcohols				
2-furanmethanol	1.3	2.3	0.7	1.3
Furans				
2-furfuryl methyl sulphide	2.8	nd	0.6	nd
furfural	105.5	200.0	nd	nd
1-(2-furanyl)-2-propanone	1.3	nd	nd	nd
2-propionylfuran	0.7	trace	nd	nd
1-(2-furanyl)-1,2-propanedione	1.1	nd	nd	nd
Aliphatic sulphur				
dimethyl disulphide	94.4	61.1	22.8	9.4
dimethyl trisulphide	1.4	1.7	1.4	1.7
Pyrroles				
2-formylpyrrole	1.6	trace	nd	nd
1-(2-furfuryl)-2-pyrrolaldehyde	2.3	2.2	nd	nd
Miscellaneous				
2-cyclohexen-1-one	1.4	2.9	1.4	1.7
methylthiocyclohexane	0.9	trace	3.4	0.6
2,4,5-trithiane	0.6	nd	trace	nd

Table 2 : Effect of amino compounds and sugar on linoleic oxidation products
(in µg /100mg ribose)

Compound	18:2	Methionine + ribose + 18:2	Methionine + ribose + ethanolamine	Methionine + ribose + 18:2+ethanolamine
aldehydes				
hexanal	33.3	166.7	nd	0.5
trans-2-heptenal	43.9	33.9	nd	0.6
t-c-2,4 decadienal	3.2	2.2	nd	0.7
t-t-2,4 decadienal	8.9	17.8	nd	0.4
alcohols				
pentanol	38.9	205.5	nd	75.0
1-octen-3-ol	28.3	66.7	nd	15.5
furan				
2-pentylfuran	4.0	5.6	nd	6.7

Table 3 : Compounds from interaction of Maillard reaction with lipid
(in g/ 100mg ribose)

Compound	Methionine + ribose	Methionine + ribose + 18:2	Methionine + ribose + ethanolamine	Methionine + ribose + 18:2+ethanolamine
2-pentylpyridine	nd	trace	nd	trace
3-methylthio-butanal	nd	10.0	nd	2.2