

Flavouring

The present invention relates to a precursor mixture of flavourings and to its use in the manufacture of food compositions.

It is known that sulphur-containing compounds are important constituents in the field of food flavourings.

Indeed, it is known, in "BACIS, VFC96, database of volatile compounds in food 1996" that thiol groups are present in food flavourings, giving off a roast odour or a grilled odour formed during the cooking of certain food compositions such as, in particular, meats, eggs or vegetables, during the process of beer-making or the roasting of coffee, for example.

Unfortunately, thiols are unstable compounds which are often lost by evaporation or by oxidation. Hofmann et al. in J. Agric Food Chem., 1996, 44, 251-255 have shown that thiols are oxidized to the corresponding disulphides after storage at a temperature of 60°C.

Moreover, Mottram et al., in J. Agric Food Chem., 1996, 44, 2349-2351, maintain that thiol groups can be formed by reaction, in a hot aqueous solution, of disulphide compounds with proteins comprising sulphydryl groups, in particular cysteines, or disulphide bridges, in particular cystines. In that document, they in particular reveal the fact that the cooking of an aqueous solution containing a disulphide and albumin allows the release of thiol groups, to a yield of 44.8%. That article is entirely theoretical: they do not mention either the possibility of starting with natural products, or the formation of a complex, and there is no application for the manufacture of flavouring.

Patents US 3,706,577, FR 1,423,176, EP 571,031, FR 2,222,030 and FR 2,205,280 relate to processes for manufacturing ready-to-use flavourings: in these

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patents, the aim is to manufacture an overall flavouring, i.e. a non-specific flavouring.

The aim of the present invention is to propose a precursor mixture of flavourings which makes it possible to promote the release of a grilled odour or of a roast odour specifically during the cooking or heating of food compositions.

To this end, a subject of the present invention is a precursor mixture of flavourings which generates, during a heating step, an aromatic note due to the formation of thiols. The preparation of a more specific flavouring is thus achieved according to the invention from a mixture which forms a complex and, as a result, induces a protective effect. Moreover, since the thiols are formed in situ, the phenomenon of instability of the said thiols is minimized. Finally, natural thiols are prepared from starting compounds which are also natural.

The present invention makes it possible to demonstrate the need for the presence of one or more sulphydryl groups which are reacted with one or more polysulphides, so as to allow the release of an aromatic note.

In the remainder of the description, the expression "aromatic note" or the term "odour" will be used, indifferently to denote the formation of a flavouring which is perceptible in sensory terms, by means of the sense of smell or via the retronasal route.

Preferably, the precursor mixture of flavourings consists of a polysulphide and of a non-volatile source of sulphur comprising at least one sulphydryl group.

Preferably, the polysulphide is of $R-(S)_n-R'$ type in which R and R' are identical or different and n is greater than or equal to 2. It is possible in particular to use, as polysulphide, a dimethyl disulphide, a bis(2-furfuryl) disulphide, a bis-(3-methyl-2-furyl) disulphide, a bis(3-methyl-2-buten-

1-yl) disulphide or a thioketone disulphide, such as bis(2-oxopropyl) disulphide.

The present invention also relates to an aromatic system in which the polysulphide is obtained
5 by bioconversion of a cysteine-aldehyde conjugate using a baker's yeast in a phosphate buffer at pH 7-9, preferably at pH 8, containing 10-30 mM of cysteine-aldehyde conjugate.

The non-volatile source of sulphur, which may
10 or may not be combined with other constituents, can be a sulphur-containing amino acid, a peptide containing at least one sulphur-containing amino acid, a protein containing at least one sulphur-containing amino acid and/or a protein hydrolysate containing at least one
15 sulphur-containing amino acid, for example.

Cysteine, glutathione, egg albumin, gluten, BSA, milk proteins or milk protein hydrolysates, for example, can be used as non-volatile source of sulphur, according to the present invention.

20 Preferably, the mixture according to the invention is heated at neutral pH, so as to allow the formation of thiols in a yield of 48-90%.

A subject of the present invention is also the use of such a precursor mixture of flavourings in the
25 manufacture of a food composition, so as to promote the development of a roast or grilled aromatic note when the food composition is heated in aqueous medium or under dry conditions.

It is possible in particular to use such a
30 mixture in the manufacture of sauces, soups, refrigerated or frozen cooked meals and/or freeze-dried food compositions, such as products based on soluble coffee, for example. The mixture according to the invention can be added in a proportion of 0.07-0.50% relative to the
35 dry weight of the food composition, for example.

The food composition can be heated with microwaves, in aqueous medium or under dry conditions, in a conventional oven and/or in boiling water, for

example. It then releases a pronounced roast odour or a pronounced grilled odour.

Lastly, a subject of the invention is a precursor mixture of flavourings, in which the non-volatile source of sulphur includes one or more proteins containing at least one sulphur-containing amino acid, so as to allow the formation of a hydrophobic pocket which imprisons one or more polysulphides in the form of a complex, and thus allowing them to be protected from degradation. This complex can be dried so as to obtain a stable aromatic powder.

In particular, it can be dried by spraying or by freeze-drying, for example.

The present invention is described in greater detail with the aid of non-limiting tests and examples below. In these tests and in these examples, the percentages are given in moles, except where otherwise indicated.

Test 1: Reaction between bis(2-furfuryl) disulphide and a protein source comprising at least one sulphhydryl group

Bis(2-furfuryl) disulphide is reacted, in aqueous medium, under hot conditions, with a protein source, so as to produce 2-furfurylthiol isolated with the aid of a machine for simultaneous distillation-extraction, SDE, at atmospheric pressure or under vacuum. The identity of the furfurylthiol obtained (as for all the thiols generated in the other examples) was confirmed by means of gas chromatography coupled with mass spectrography, relative to the retention index and to the spectrum of a reference sample.

The yield for formation of thiol groups is then calculated by internal calibration on gas chromatography. This yield is expressed as a percentage and is calculated on the basis of the amount of thiol groups which should be formed, i.e. twice the molar amount of disulphide.

The combined data are given in Table 1 below.

Table 1

	(a)	Protein source	Yield	Isolation method
Control			<1%	SDE/atmospheric pressure (100°C)
Test No.1	10 ppm	Egg albumin	87%	SDE/atmospheric pressure (100°C)
Test No.2	10 ppm	Denatured egg albumin	61%	SDE/atmospheric pressure (100°C)
Test No.3	10 ppm	Egg albumin	<1%	SDE/under vacuum (37°C)
Test No.4	100 ppm	Gluten	45%	SDE/atmospheric pressure (100°C)
Test No.5	100 ppm	BSA	51%	SDE/atmospheric pressure (100°C)
Test No.6	100 ppm	β -lactoglobulin	60%	SDE/atmospheric pressure (100°C)
Test No.7	1000 ppm	β -lactoglobulin + 1% cysteine	80%	SDE/atmospheric pressure (100°C)
Test No.8	100 ppm	β -lactoglobulin hydrolysate	20%	SDE/atmospheric pressure (100°C)

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Key: (a) amount of bis(2-furfuryl) disulphide/amount of protein source

10 The results given in Table 1 reveal the fact that the reaction, in aqueous medium under hot conditions, between a polysulphide, such as bis(2-furfuryl) disulphide, and a protein source comprising at least one sulphhydryl group allows the formation of corresponding thiols, in an advantageous
15 yield.

Moreover, test No. 3 reveals the fact that if bis(2-furfuryl) disulphide is reacted with albumin under vacuum at room temperature, no corresponding thiol is formed. This makes it possible to confirm the

fact that the disulphide is complexed in the hydrophobic pocket formed by the protein. It is thus possible to avoid the loss of polysulphide by evaporation and to limit the dispersion of its odour before the heating of the food composition to which the aromatic mixture according to the invention has been added.

In order to increase the yield of thiol, it is also possible to form a ternary complex comprising a disulphide, cysteine and a protein, as illustrated in test No. 7.

Test 2: Effect on the reaction yield of the amount of polysulphide relative to the amount of protein source containing at least one sulphhydryl

The reaction yield is calculated as a function of the amount of polysulphide relative to the amount of protein source containing at least one sulphhydryl.

To do this, several reactions are carried out, in an aqueous medium under hot conditions, with, in each test, an increasing amount of bis(2-furfuryl) disulphide and a constant amount of egg albumin.

The yield for formation of thiol groups is then calculated. This yield is expressed as a molar percentage and is calculated on the basis of the amount of thiol groups which should be formed, i.e. twice the molar amount of disulphide.

The results are given in Figure 1 below.

The x-axis shows the content in ppm of disulphide relative to the protein. The left-hand y-axis gives the yield of thiols relative to the disulphide: this is represented by the plots. The two straight-line plots at concentrations of 1000 and 10,000 ppm show the amount of unconverted disulphide. Finally, the triangles on the curve relate to the right-hand y-axis and represent the amount in ppm of thiols formed relative to the protein.

Figure 1 reveals the fact that the degree of conversion of the disulphide into furfurylthiol formed decreases when the level of bis(2-furfuryl) disulphide complexed in the hydrophobic pocket of the protein increases. The straight-line plots at 1000 and 10,000 ppm show that a fraction of disulphide is not converted. The triangles show that the thiol content is nevertheless increased if the content of starting disulphide is increased.

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Test 3: Reaction in an aqueous medium under hot conditions between egg albumin and different polysulphides

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Egg albumin is reacted, in an aqueous medium under hot conditions, with different polysulphides and the yield for formation of thiol is calculated as described in Test 1.

The combined results are given in Table 2 below.

Table 2:

	Polysulphide	(a)	Yield	Method of isolation
Test No.9	bis(2-furfuryl) disulphide	10 ppm	87%	SDE/atmospheric pressure
Test No.10	bis(3-methyl-2-buten-1-yl) disulphide	10 ppm	30%	SDE/atmospheric pressure
Test No.11	2-furfurylthiol	100 ppm	61%	SDE/atmospheric pressure
Test No.12	dimethyl trisulphide	10 ppm	7%	SDE/atmospheric pressure

25 Key: (a) amount of polysulphide/amount of egg albumin

The results given in Table 2 reveal the fact that the reaction, in aqueous medium under hot

conditions, between a polysulphide and a protein source comprising at least one sulphydryl group allows the formation of corresponding thiols.

Moreover, these results reveal the fact that
5 this reaction between a polysulphide, bis(2-furfuryl) disulphide, and a protein source comprising at least one sulphydryl group (Test No. 9) allows the formation of corresponding thiols, in a better yield than in the case in which a simple complexation is carried out
10 between a thiol group, 2-furfurylthiol, and a protein source comprising a sulphydryl group (Test No. 11). It can be assumed that this difference in yield is due to the fact that there is an irreversible reaction (Test No. 11) between the thiol group and the protein with
15 formation of disulphides, furfuryl-S-S-proteins, which are not volatile.

Furthermore, there is no loss in yield during the reaction between a polysulphide and a protein source comprising at least one sulphydryl group, since
20 the corresponding thiols are released progressively as they are formed.

Test 4: Amino acid or peptide as non-volatile source of sulphur comprising at least one sulphydryl group

25 Bis(2-furfuryl) disulphide and an amino acid or a peptide, as non-volatile source of sulphur comprising at least one sulphydryl group, are reacted together in an aqueous medium under hot conditions. The ratio by
30 mass of bis(2-furfuryl) disulphide/non-volatile source of sulphur comprising at least one sulphydryl group is 1/10. The yield for formation of thiol is then calculated as described in Test 1.

The combined results are given in Table 3
35 below.

Table 3:

	(b)	Yield	pH value
Test No.13	cysteine	10-59%	-
Test No.14	cysteine	87%	7.6
Test No.15	glutathione	80%	7.6
Test No.16	cystine	2.8%	7.6

Key: (b) non-volatile source of sulphur containing at
5 least one sulphydryl group or one disulphide bridge

The results given in Table 3 reveal the fact that the precursor mixture of flavourings according to the present invention consisting of a volatile
10 disulphide, bis(2-furfuryl) disulphide, and of a sulphur-containing amino acid, cysteine, or of a peptide containing at least one sulphur-containing amino acid, glutathione, allows the formation of thiols, during a step of heating in aqueous medium, and
15 thus generates an aromatic note.

On the other hand, as illustrated by Test No. 16, if the non-volatile source of sulphur is a source of disulphide bridges, such as cystine, without free sulphydryl, a negligible yield for formation of thiols
20 is obtained. The results given in Table 3 furthermore reveal the fact that the reaction is pH-dependent since, if cysteine is reacted in distilled water, under hot conditions, with bis(2-furfuryl) disulphide, as in Test No. 13, the yield for formation of thiol ranges
25 between 10 and 59%. On the other hand, if the same reaction is carried out in water at a pH of 7.6, as in Test No. 14, a yield for formation of thiols of 87% is obtained.

30 **Test 5: Formation of natural thiols**

The process is performed by bioconversion of disulphides.

To do this, a baker's yeast is suspended in a phosphate buffer at pH 8 containing a cysteine-aldehyde conjugate at 20 mM.

5 This bioconversion is carried out for 48 h, for half of the preparation in anaerobic medium and, for the other half, in aerobic medium, so as to compare the effect of oxygen on the formation of 2-furfurylthiol.

The reaction yield is then measured. The formation of 2-furfurylthiol is at an optimum yield of 10 30-40% in anaerobic medium, and at an optimum yield of 10-25% in aerobic medium.

After incubation for 144 h, the formation of bis(2-furfuryl) disulphide at an optimum yield of 24% in anaerobic medium and at an optimum yield of 4% in 15 aerobic medium, are obtained.

Natural bis(2-furfuryl) disulphide, thus prepared over 144 h in anaerobic conditions, is then reacted, in aqueous medium, under hot conditions, with egg albumin, so as to produce, in a yield of 60%, 20 2-furfurylthiol isolated using a simultaneous distillation-extraction machine, SDE, at atmospheric pressure.

Example 1

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The flavouring of soluble coffee is carried out with the precursor mixture of flavourings according to the invention, so as to develop a very pronounced grilled note.

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To do this, the mixture according to the invention, comprising 100 ppm of bis(2-furfuryl) disulphide and β -lactoglobulin are reacted, under hot conditions in 100 ml of water at pH 7.5, with 1.5 g of soluble coffee.

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A mixture having a coffee taste with a very pronounced grilled note is obtained.

Example 2

A precursor mixture of flavourings in powder form is prepared, which can readily be used to give a pronounced aromatic note to foods.

To do this, a stable precursor mixture of flavourings is formed by mixing 100 ppm of bis-(2-furfuryl) disulphide with albumin in water. The precursor mixture of flavourings thus prepared is freeze-dried.

A precursor mixture of flavourings in powder form is thus obtained, which can readily be used dry, as a suspension or as a solution, in particular, with microwaves, such that it develops a very pronounced grilled aromatic note.

Example 3

A roast chicken liquid stock is prepared by adding the mixture according to the invention thereto.

To do this, a precursor mixture of flavourings as described in Example 2 is prepared and is added to a stock in a proportion of 0.35% relative to the dry weight of this stock.

A roast chicken liquid stock with a very pronounced meat note is thus obtained.

Example 4

A hamburger is prepared by adding the aromatic system according to the invention to the minced meat burger.

To do this, a precursor mixture of flavourings as described in Example 2 is prepared and is added to the burger in a proportion of 0.45% relative to the dry weight of the minced meat burger.

The burger thus prepared is placed on one half of a bread roll with a filling composed of lettuce,

tomatoes and gherkins cut into rings, then the other half of the bread roll is placed on top.

A hamburger with a very pronounced meat note is thus obtained when heated with microwaves.



Claims

1. Precursor mixture of flavourings, characterized in that it generates, during a heating step, an aromatic note due to the formation of thiols.
- 5 2. Mixture according to Claim 1, characterized in that it consists of a polysulphide and of a non-volatile source of sulphur comprising at least one sulphydryl group.
3. Mixture according to Claims 1 and 2, in which
10 the polysulphide is of $R-(S)_n-R'$ type in which R and R' are identical or different and n is greater than or equal to 2.
4. Mixture according to Claims 1 to 3, in which
15 the polysulphide is obtained by bioconversion of a cysteine-aldehyde conjugate using a baker's yeast.
5. Mixture according to Claims 1 to 4, in which the non-volatile source of sulphur, which may or may not be combined with other constituents, is a sulphur-containing amino acid, a peptide containing at least
20 one sulphur-containing amino acid, a protein containing at least one sulphur-containing amino acid and/or a protein hydrolysate containing at least one sulphur-containing amino acid.
6. Mixture according to Claims 1 to 5, in which
25 the formation of thiol is in a yield of 48-90%, during a step of heating at neutral pH.
7. Mixture according to one of Claims 1 to 6, in which the non-volatile source of sulphur is one or more proteins comprising at least one sulphur-containing
30 amino acid, so as to allow the formation of a hydrophobic pocket which imprisons one or more polysulphides.
8. Mixture according to Claim 7, characterized in that it is dried so as to obtain a stable aromatic
35 powder.
9. Use of the mixture according to one of Claims 1 to 8 in the manufacture of a food composition, so as to promote the development of a roast or grilled aromatic note when the food composition is heated.



10. Use according to Claim 9, in which the aromatic mixture is added in a proportion of 0.07-0.50% relative to the dry weight of the food composition.