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(54) Title: ~~FLAVOURING MATERIALS FROM YEAST EXTRACTS~~ FLAVOURING MATERIALS

(57) Abstract

Flavouring materials may be produced from filamentous fungi by contacting them with water at a temperature sufficient to reduce their nucleic acid content and concentrating or separating solids from the resulting aqueous solution. The materials may be further subjected to a chemical reaction for example with a sulphur containing amino acid.

FLAVOURING MATERIALS

THIS INVENTION relates to flavouring materials.

Throughout the description and claims of this specification, the word "comprise" and variations of the word, such as "comprising" and "comprises", is not intended to exclude other additives, components, integers or steps.

The discussion of documents, acts, materials, devices, articles and the like is included in this specification solely for the purpose of providing a context for the present invention. It is not suggested or represented that any or all of these matters formed part of the prior art base or were common general knowledge in the field relevant to the present invention as it existed in Australia before the priority date of each claim of this application.

It is known to use hydrolysed yeast extracts as flavouring materials. Yeast is non-toxic to humans and is normally cultured at high density (high dry cell weight per litre).

The nucleic acid content of filamentous fungi may be reduced by contacting them with water at high temperatures and separating them from the water, and such a process is described for Fusarium in PCT patent Application WO95/23843. We have discovered that the water from which the fungus is separated contains materials which can be used as or converted to flavouring materials for foods, especially if the fungus is Fusarium, for example Fusarium IMI 145,425.

The current invention comprises a method whereby the soluble components lost from filamentous fungal cells as a result of this heat treatment can be isolated and used as, or converted into, flavouring substances for foods.

This invention comprises a method of processing a filamentous fungus to improve its suitability as food which comprises subjecting it in the presence of water to a temperature sufficient to reduce its nucleic acid content substantially characterised by using materials removed from the fungus in the said method directly or after chemical reaction to flavour food.

The invention also comprises a flavouring material for food which is an aqueous solution which comprises nucleic acids removed from a filamentous fungus by contacting it with water at an elevated temperature in which the concentration of dissolved solids is sufficiently high to render the material stable to storage at a temperature of 20°C for a period of one month or is a solid comprising such nucleic acids or is a flavouring material comprising a reaction product of such nucleic acids with a sulphur containing amino acid, hydrogen sulphide or ammonium sulphide.

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The flavouring materials when in the form of an aqueous solution preferably comprises at least 30% by weight and more preferably 45 to 60% by weight of solids.

Whilst taste is an important factor in food flavours, the odours of flavouring materials are also important.

The soluble components are preferably concentrated from the aqueous solution arising from the nucleic acid reduction step by removing water for example by evaporation, distillation (preferably at reduced pressure) reverse osmosis, freeze drying or freezing out the water as ice leaving an aqueous concentrate. It may suitably be removed by evaporation at reduced pressure for example at a temperature of 40 to 70°C

The dissolved solids may be separated as such or left as a concentrated solution where the Aw (water activity) is reduced sufficiently to ensure biostasis at a range of storage temperatures.

If the nucleic acid content of the filamentous fungus is reduced by raising the temperature of its growth medium the water recovered will contain salts and other nutrients,

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for example glucose and/or complex nitrogen nutrients in addition to the nucleic acids and other materials derived from the fungus. If the flavour imparted by such materials is required they may be left in the materials, but if not they may either be removed, for example by osmosis or ultrafiltration, or the fungus may be washed before its nucleic acid content is reduced thereby avoiding their presence. In WO95/23843 the removal of nucleic acid from a filamentous fungus in its growing state is described; such a process is an improvement over the treatment of fungus in its resting state, for example in pure water. We have found however that the organism takes a short time to adjust from its growing to its resting state and that providing the nucleic acids are removed soon after it is separated from its growth medium the nucleic acids may be satisfactorily removed according to the procedure of WO95/23843.

We have found that after partial or complete removal of water as aforesaid the concentrate can be used as an alternative to hydrolysed vegetable proteins, yeast autolysates or yeast extract as an additive for food. The materials removed from the fungus of value in the production of savoury flavouring preparations and process flavourings. Because of the savoury nature of the flavour it may be used directly in the flavouring of Snacks, Biscuits, Stocks, Soups, Stews, Sauces and Gravies at inclusion levels of preferably between 0.1 and 15 for example 1 to 10 dry weight %.

We have also found that on heating it produces an attractive roast-type aroma. If desired it may be partially hydrolysed before heating, for example by hydrolysis with acetic acid, to produce modified roast flavours.

It may also be reacted, optionally after at least partial hydrolysis, with sulphur containing amino acids, preferably cysteine or optionally with H_2S and/or $(NH_4)_2S$ to produce savoury flavours.

The savoury nature of the material may be altered by chemical reaction to provide a different flavour profile in that the meaty/roasted flavour notes are increased. Such "reaction flavourings" may be used in flavouring Meat (beef, chicken, lamb, pork, etc), meat alternatives (e.g. based on soy, wheat, pea protein, myco-protein), prepared meals, snacks and drinks at inclusion levels of preferably between 0.1 and 10 for example 1 to 8 dry weight %.

The flavourings may be produced by reacting materials removed from the fungus as aforesaid with cysteine. This may be carried out in the presence of water if desired; for example a 1.5 to 75 and preferably a 5 to 50 weight % solution of such materials may be reacted with cysteine in quantities of up to 10%, for example 1 to 5% of cysteine by weight based on such materials. The reaction may be carried out at a temperature of for example 110 to 140°C at a pH of 5.5 to 9. Reaction is suitably continued for 0.5 to 7.5 hours.

It is believed that hydrolysis increases the free ribose content of the concentrate and this may be appropriate if certain flavours are desired. It is desirable to avoid treatment with

hydrochloric acid for regulatory reasons (possible production of chloro-propanol derivatives), but hydrolysis with for example acetic acid may be desirable.

In the following descriptions the term "Centrate" is used for the extracellular liquid recovered by the heat shock treatment of a suspension of Fusarium at about 70°C in the presence of its growth medium after separation of the cellular material. The term FDC means "freeze dried centrate".

EXPERIMENTAL PROCEDURES

Material Preparation

The liquid centrate was freeze dried in order to:

- reduce the water content and therefore inhibit microbial growth;
- carry out studies at a high concentration of centrate;
- facilitate the handling of the product.

All further analyses described in this report deal with the freeze dried centrate abbreviated FDC.

Methods - Compositional Analyses

Moisture

The moisture content was determined by measuring the weight decrease of the FDC, until constant weight, while placed in an oven at 100°C.

Ash

The ash content was determined by placing the FDC in an oven at 600°C until constant weight was obtained.

Organic Nitrogen

Kjeldahl nitrogen determination was carried out; sucrose was used as blank and glycine as standard. The results are shown in Table 1.

Table 1 - Moisture, Ash and Organic Nitrogen Content of FDC

	Replicates 1	2	3	Mean
Moisture (%)	13	13	13	13
Ash (%)	18	18	17	18
Organic Nitrogen (%)	6	6	6	6

Amino Acids

The amino acids determination was done with a 6300 Beckman auto-analyser. The free amino acids present in the FDC were analysed using a 0.06% solution of the FDC. It was possible to measure the total amino acid content by a prior hydrolysis (HCl 6N, 24h, oven 110°C) of the FDC. However, the acid is known to hydrolyse tryptophan and the sulphur amino acids. The hydrolysis of the sulphur amino acids can be avoided by a prior oxidation of cysteine into cysteic acid, and methionine into methionine sulfone. This was

carried out by treating the FDC with a solution of formic acid/hydrogen peroxide/methanol (48.5/1/0.5) during 4h at 0°C in the dark. The results are shown in Table 1a.

Table 1a - Amino Acid Content of FDC

mean results in g/100g FDC

Amino Acids	FDC (free AA)	Hydrolysed FDC (total AA)
CYSTEIC ACID	0.09	0.32
ASP	0.14	0.50
THR	0.02	0.24
SER	0.12	0.16
GLU	2.02	1.70
CYSTEINE	0.00	0.21
PRO	0.00	0.20
GLY	0.05	0.24
ALA	2.05	1.73
VAL	0.14	0.37
CYSTINE	0.00	0.01
METH	0.00	0.13
ILE	0.00	0.21
LEU	0.00	0.30
TYR	0.00	0.06
PHE	0.00	0.17
TRYPTOPHAN	0.00	0.00
NH3	1.49	4.56
LYS	0.15	0.26
HIS	0.00	0.08
ARG	0.71	0.65
TOTAL	6.98	11.80

Total Carbohydrates

The carbohydrate content of the FDC was assessed by the phenol-sulphuric acid assay method (Carbohydrate analysis: a practical approach, ed. Chaplin, Kennedy, IRL Press). Solutions of FDC and glucose (standard for calibration) were mixed with a solution of phenol in water (5% w/v). Concentrated sulphuric acid (1 ml) was added rapidly and directly to the solution surface without allowing it to touch the sides of the tube. The solutions were left undisturbed for 10 min before shaking vigorously. The absorbencies were read at 490 nm after a further 30 min.

Sugars

The sugar analysis was performed using a Dionex System of High Pressure Liquid Chromatography (HPLC), in which an eluent of HPLC grade water (1 ml/min) was used with an anion-exchange column (column Dionex PA-1) and a pulsed amperometric detector. Pure compounds were used as standards for retention time determination and quantitation. Free sugars were analysed using a 0.15% solution of FDC after filtering the solution through a 0.45 μm Minisart 25 membrane. Total sugars were also evaluated after a preliminary acid hydrolysis of the FDC (solution 0.15% in HCl 1N, 2h, oven 110°C) and filtration through first an Ag filter (precipitate of AgCl) and second a 0.45 μm Minisart 25 filter. The results are shown in Table 2.

Table 2 - HPLC Analysis of Sugar Content of FDC

g sugar per 100g of FDC

	FDC mean	HYDROLYSED FDC mean
arabinose	0.02	0.04
galactose	0.10	0.10
glucose	8.62	23.01
sucrose	0.09	0.23
xylose	0.00	0.00
mannose	0.00	0.10
fructose	0.01	0.00
ribose	0.00	0.06
maltose	2.08	
TOTAL SUGARS	12.66	25.10

Nucleic acid Derivatives

A Perkin Elmer Binary HPLC pump 250 equipped with a Spectroflow 757 ABI Analytical Kratos Division was used. Standards and samples were filtered through Acrodisc 0.45 μm Gelman Sciences membranes filters and injected by means of an injector valve equipped with a 20 μl injection loop into a reverse-phase μBondapack C18 (3.9 x 300 mm) Waters analytical column, protected by a μBondapack C18 guard column. A wavelength of 254 nm was used. A gradient programme with two mobile phases was used: mobile solvent A was a 60/40 methanol/water mixture and mobile solvent B was 0.02M KH_2PO_4 (pH 5.5) prepared from potassium dihydrogen orthophosphate in distilled water and pH adjusted with IM KOH. All mobile solvents were filtered (Nylaflo 0.2 μm Gelman Sciences membranes filters) and degassed with Helium before use. The total run time was 51 min and the flow rate 1 ml/min which consisted of 100% solvent B during 5 min, followed by a gradient from 0% to 36% solvent A in 36 min and 36% solvent A for 5 min. Then a reverse

gradient of 36% to 0% A was set for 5 min and the HPLC was ready for further injection after 15 min equilibrium.

Identification of the compounds was made by comparison with the retention time obtained from standards analysed in the same HPLC conditions. Standards were analysed separately to know their individual retention time and then all together to check any elution over lap that may occur in the sample case. These standards are presented in Table 3.

Table 3 - HPLC Retention Times of Nucleic Acid Standards

Bases			Cytosine		Uracil				Hypox guanine	
Ribonucleosides								C	U	
2'Deoxy-ribonucleosides										
Ribonucleotides 3'MP				CMP		UMP			GMP	
Ribonucleotides 5'MP	CMP	UMP				GMP	IMP			
2'Deoxyribo-nucleotides 3'MP										DGMP
2'Deoxyribo-nucleotides 5'MP										
Retention times	3'73	4'29	4'69	5'20	5'62	6'32+6'32	6'70	7'52	9'79+9'79+ 9.79+9.79	10'74

Continued below:

Bases	Xanthine		Purine		Adenine					
Ribonucleosides				G		I			A	
2'Deoxy-ribonucleosides							DG	DI		DA
Ribonucleotides 3'MP						AMP				
Ribonucleotides 5'MP		AMP								
2'Deoxyribo-nucleotides 3'MP						DAMP				
2'Deoxyribo-nucleotides 5'MP	DIMP	DGMP				DAMP				
Retention times	11'49+ 11'49	13'33+ 13'33	17'36	21'05	21'56	22'58+22'58 +22'58	23'99	26'08	32'90	35'81

Hydrolysis of FDC

Since free ribose is highly reactive in the Maillard reaction, the effect of gentle hydrolysis conditions on FDC and the subsequent effects on flavour generation were investigated. Acid hydrolysis was carried out with sodium acetate 0.01M, pH4 adjusted with acetic acid. Standards (inosine, adenosine 5'mono phosphate -AMP5', guanosine and guanosine 5'mono phosphate -GMP5') were prepared at 4000 µM in duplicate and an aliquot of each solution was taken and run under the same HPLC conditions as were adapted for the analysis of nucleic acids derivatives above. The solutions were then subjected to hydrolysis for 7.5 h in an oven 100°C (GC oven Carlo Erba). The reaction was stopped by placing the tubes in an ice bath and kept in freezer until analysis.

FDC at 2% w/v was subjected to similar hydrolysis conditions.

Flavour mixture preparation

As indicated previously, FDC is considered to have the potential of being either a flavouring in its own right, or a precursor in the generation of reaction - product flavours. Therefore a range of reaction mixtures were prepared and are presented in Table 4.

Table 4 - Flavoured mixtures analysed by sniffing panel
Selected mixtures for GS-MS analysis are underlined
C = Cysteine

Samle Name Sample Composition	**Aqueous Centrate** 1.7%(w/v, solids of centrate/water)	**Heated Aqueous Centrate** 1.7%(w/v, solids of centrate/water) 0.5h 140°C	
Sample Name Sample Composition	**Heated Buffered (pH 5.5) Centrate** 1.7% (w/v, solids of centrate/sodium acetate 0.01M) 0.5h, 140°C	**Heated Hydrolysed Buffered (pH 5.5 Centrate** 1.7% (w/v, solids of centrate/sodium acetate 0.01M) 7.5h, 110°C 0.5h, 140°C	**Heated Hydrolysed Buffered (pH 5.5) Centracte + C** 1.7% (w/v, solids of centrate/sodium acetate 0.01M) 1g cysteine/17g solids of centrate 7.5h, 110°C 0.5h, 140°C
Sample Name Sample Composition	**Heated Aqueous Centrate 12% pH 5.5** 12% (w/v, solids of centrate/water) 0.5h, 140°C	**Heated Aqueous Centrate 20% pH 5.5** 20% (w/v solids of centrate/water) 0.5h, 140°C	**Heated Aqueous Centrate 30% pH 5.5** 30% (w/v, solids of centrate/water) 0.5h, 140°C
Sample Name Sample Composition	**Heated Aqueous Centrate 50% pH 5.5** 50% (w/v, solids of centrate/water) 0.5h, 140°C	**Heated Aqueous Centrate 75% pH 5.5** 75% (w/v, solids of centrate/water) 0.5, 140°C	**Heated Aqueous Centrate 87% pH 5.5** 87%(w/v, solids of centrate/water) 0.5h, 140°C
Sample Name Sample Composition	**Heated Aqueous Centrate 20% pH 7.5** 20% (w/v, solids of centrate/water) 0.5h, 140°C	**Heated Aqueous Centrate 30% pH 7.5** 30% (w/v, solids of centrate/water) 0.5h, 140°C	
Sample Name Sample Composition	**Heated Aqueous Centrate 20% pH 9** 20% (w/v, solids of centrate/water) 0.5h, 140°C	**Heated Aqueous Centrate 30% pH 9** 30% (w/v, solids of centrate/water) 0.5h, 140°C	
Sample Name Sample Composition	**Heated Aqueous Centrate pH 5.5 + C 1/20** 20% (w/v, solids of centrate/water)	**Heated Aqueous Centreate pH 5.5 + C1/10** 20% (w/v, solids of centrate/water)	**Heated Aqueous Centrate pH 5.5 + C1/5** 20% (w/v, solids of centrate/water)

	1g cysteine/20g solids of centrate 0.5h, 140°C	1g cysteine/10g solids of centrate 0.5h, 140°C	1g cysteine/5g solids of centrate 0.5h, 140°C
Sample Name Sample Composition	**Heated Aqueous Centrate pH 9 + C 1/20** 20% (w/v, solids of centrate/water) 1g cysteine/20g solids of centrate 0.5h, 140°C	**Heated Aqueous Centrate pH 9 + C 1/10** 20% (w/v, solids of centrate/water) 1g cysteine/10g solids of centrate 0.5h, 140°C	**Heated Aqueous Centrate pH 9 + C 1/5** 20% (w/v, solids of centrate/water) 1g cysteine/5g solids of centrate 0.5h, 140°C
Sample Name Sample Composition	**175°C Heated Aqueous Centrate pH 5.5 + C 1/20** 20% (w/v, solids of centrate/water) 1g cysteine/20g solids of centrate 5 min, 175°C	**100°C 1h Heated Aqueous Centrate pH 5.5 + C 1/20** 20% (w/v, solids of centrate/water) 1g cysteine/20g solids of centrate 1h, 100°C	**100°C 1.5h Heated Aqueous Centrate pH 5.5 + C 1/20** 20% (w/v, solids sof centrate/water) 1g cysteine/20g solids of centrate 1.5h, 100°C
Sample Name Sample Composition	**175°C Heated Aqueous Centrate pH 9 + C 1/5** 20% (w/v, solids of centrate/water) 1g cysteine/5g solids of centrate 5 min, 175°C	**100°C 1h Heated Aqueous Centrate pH 9 + C 1/5** 20% (w/v, solids of centrate/water) 1g cysteine/5g solids of centrate 1h, 100°C	**100°C 1.5h Heated Aqueous Centrate pH 9 + C 1/5** 20% (w/v, solids of centrate/water) 1g cysteine/5g solids of centrate 1.5h, 100°C

Reaction mixtures (2 ml) were prepared by mixing appropriate quantities of stock solutions in glass tubes and then transferring to 20 ml Kimble ampules that were sealed in hot flame. The ampules were then placed in a metal cover and heated in a Carlo Erba 4200 gas chromatograph oven.

The reaction mixtures were stored in the freezer at -20°C before analysis. The ampules were broken for analysis after bringing the reaction mixtures to room temperature.

Sensory evaluation of Aroma volatiles

An informal panel of 6 persons (3 females, 3 males) experienced in flavour evaluation was recruited.

For sensory evaluation, 1 ml aliquots of the samples under investigation were transferred into brown screw-cap bottles and diluted 10 times (except for the concentration study where no dilution was applied). The coded samples were presented to one panellist at one time at room temperature and the panellists were asked to describe the aromas using their own terms.

Instrumental evaluation of Aroma volatiles determination

A dynamic headspace collection procedure was used. Each sample (1.7 ml of reaction mixture so that it was equivalent to 0.4 g of FDC) was placed in a 250 ml conical flask fitted with a Drechsel head. Distilled water was added to a final volume of 10 ml and the mixture shaken gently. Oxygen-free nitrogen was passed over the sample for 1 h at a rate of 40 ml/min. The volatiles were swept onto a preconditioned glass-lined stainless-steel trap (105 mm x 3 mm i.d.) packed with 85 mg Tenax GC (CHIS system, SGE Limited). Throughout the collection, the sample was maintained at 37°C using a water bath. The internal standard was 1,2-dichlorobenzene in ether (130 µl/ml) and 1 µl was injected onto the trap at the end of the collection time, the trap was then flushed with nitrogen for 10 min.

A Hewlett-Packard (HP) 5890/5972 gas chromatrograph-mass spectrometer (GC-MS), fitted with a 50 m x 0.32 mm i.d. fused-silica capillary column coated with BPX-5) SGE Limited) at 0.5 µm film thickness, was used to analyse the collected volatiles. These were thermally desorbed at 250°C in the CHIS injection port (SGE Limited) and cryofocused directly onto the front of the GC column, while the oven was held at 0°C for 5 min. The oven temperature was then raised to 40°C over 1 min and held for 5 min before raising the temperature to 250°C at a rate of 4°C/min and holding for a further 10 min. The helium carrier gas flow rate was 1.5 ml/min. Mass spectra were recorded in the electron impact mode at an ionisation voltage of 70 eV and source temperature of 200°C. A scan range of 29-400 m/z and a scan time of 0.69 s were used. The date were controlled and stored by the HP G1034C Chemstation data system.

Volatiles were identified by comparison of their mass spectra with the spectra from authentic compounds in the Reading Laboratory or in the NIST/EPA/MSDC Mass Spectral Database or other published spectra. The linear retention index (LRI) was calculated for each component using the retention times of a homologous series of C_6-C_{22} n-alkanes.

Nucleic Acid Derivatives

The nucleic acid composition of the centrate was determined from 3 replicates and is presented in Table 5. It explains most of the HPLC eluted peaks. A number of compounds co-eluted, but it was not possible to investigate alternative analysis conditions and, therefore, for co-eluting compounds it was not possible to determine which of the compounds contributed to the peak obtained in the centrate analysis.

As expected, there were few deoxyribonucleic acid derived compounds compared with the ribonucleic acid derived compounds, which are more abundant in nature. The major nucleic acid components are cytosine 5' monophosphate (26% of the total nucleic acid content), uridine 3' monophosphate and/or guanosine 5' monophosphate (18%), adenosine 5' monophosphate and/or deoxyribo guanosine 5' monophosphate (16%). All of them are potential sources of ribose and ribose phosphate which are good reactive precursors the Maillard reaction. Excluding the bases, the potential source of ribose or ribose phosphate represents 96% of the nucleic acid content of the centrate, which is equivalent to 202 ppm of the content of the centrate.

Table 5 - Centrate Nucleic Acids HPLC Analysis Results

Nucleic Acid Type	CMP'5	UMP5'	Uracil'	UMP3' + GMP5'	IMP5'	Cytidine	Hypox+ guanine + uridine + GMP3'	AMP5' + I + DAMP3' + DAMP5'	Purine	AMP5' + I + DAMP3' + DAMP5'	DG	DI	Adeno-sine	Total
nucleic acid (ppm in centrate	54	23	1	38	5	5	7	34	2	6	2	15	19	212
nucleic acid (% of total)	26	11	0	18	3	2	3	16	1	3	1	7	9	100
Standard deviation (ppm in centrate)	3	1	0	1	0	1	0	2	0	0	0	1	2	

hypox = hypoxanthine
I = inosine
C = cytidine
U = Uridine
G = Guanosine
A = Adenosine
MO = mono phosphate
D = deoxyribose

Effects of Acid Hydrolysis on Nucleic Acid Derivatives

Table 6 presents the results of hydrolysis of solutions of inosine, guanosine and their respective 5' phosphate ribonucleotides. The method is based on that used by Matoba et al (J. Food Science, vol. 53, n.4, 1988, p1156). The last column gives an indication of quantity recovery and it can be seen that the results of the hydrolysis on the guanosine showed a significant loss, which suggests that the guanine molecule is unstable.

The most interesting model systems are the ribonucleotides since they are major components in the centrate. They were hydrolysed by half or less, producing their respective nucleosides which were further hydrolysed into their bases. Although it is possible to hydrolyse the ribonucleotides into their bases and consequently produce ribose and/or ribose phosphate, relatively low yields of bases were obtained and an optimisation of this process should be carried out.

Table 6 - Acid hydrolysis results of model systems

	Quantity (%) initial	After hydrolysis hypoxanthine	inosine	IMP5'	total
inosine	100	13	85		98
IMP5'	100	6	15	71	92
		guanine	guanosine	GMP5'	
guanosine	100	2	42		44
GMP5'	100	2	35	51	88

Conclusion

The nucleic acid composition of the FDC has been characterised. It comprises mainly ribonucleotides with relatively small amounts of deoxyribonucleotides. Hydrolysis of nucleotides releases free ribose or ribose phosphate only occurs to a relatively small extent in acetate buffer at pH 4.

Results - Sensory Evaluations of Aroma Volatiles

It was decided to present the individual results of each panellist and not to group them under specific common descriptors because of the too large diversity of the terms described.

Tables 7 and 8 present the effect of heating and the impact of the hydrolysis, with or without the addition of cysteine.

Table 7 - Aroma panel results on centrate - Study of the effect of cooking

Sample name	Aqueous Centrate	Heated Aqueous Centrate
Sample Composition	1.7%(w/v, solids of centrate/water	1/7%(w/v, solids of centrate/water) 0.5, 140°C
Panellist 1	scrumpy, glucose, syrup, molasses	molasses, caramel
Panellist 2	caramel, sweet	burnt
Panellist 3	whey, old yoghurt, sharp, creamy	raw celery, braised celery, weird smell
Panellist 4	wet cloth/ironing/scorching auto-claving media, slightly acrid	fermenting cereal, ironing/wet cloth, cotton/wool, treacle, golden syrup
Panellist 5	honey, urine	caramel, fatty
Panellist 6	caramel, slightly fruity	slightly fruity caramel, burnt, sharp

Table 8 - Aroma panel results on heated buffered centrate - Study of the effect of hydrolysis and of addition of cysteine

Sample	Heated Buffered (pH 5.5) Centrate	Heated Hydrolysed Buffered (pH 5.5) Centrate	Heated Hydrolysed Buffered (pH 5.5) Centrate + Cysteine
Sample Composition	1.7% (w/v solids of centrate/sodium acetate 0.01M) 0.5h, 140°C	1.7% (w/v, solids of centrate/sodium acetate 0.01M) 7.5h, 110°C 0.5h, 140°C	1.7% (w/v, solids of centrate/soldium acetate 0.01M) 1g cysteine/17g solids of centrate 7.5h, 110°C 0.5h, 140°C
Panellist 1	burnt, caramel, toffee, acrid, acid	resinous, burnt, acrid	burning paper/plastic/hair, putrid, acrid
Panellist 2	burnt, caramel, celery, slightly sweet	stale shall, burnt, acidic	burnt, celery, acid
Panellist 3	braised celery, marmite, jammy, cooked apple	varnish, paint, biscuit	sweet, grassy, herbal, raw onion, vinegar
Panellist 4	rotting vegetable, sulfur, autoclaving malted barley, burnt, nutty, treacle	wet cloth, autoclaving, fermenting cereal, nose catching/sharp	onion rotting, hard boiled egg, malty/barley/fermenting cereal
Panellist 5	treacle, honey, fatty	nicotine	burning tyres
Panellist 6	caramel, toffee, slight fruity, slightly burnt	chemical, slightly burnt rubbery	caramel, toffee, sweet, floral

The study of the effect of concentration of centrate involved the range of concentrations likely to be reached in commercial practice, i.e., within the range 12% and 30% of solids. These were compared with the non diluted freeze dried centrate powder (87% solids). The other preparation conditions were kept constant. viz. pH 5.5 and heating at 140°C for 30 min. The results are presented in Table 9. The odours were very strong and the reproducibility of the results within 2 replicates for each panellist was fairly poor. However, there was a noticeable trend within the sample set from low to high concentration: at 12%, the odours were mainly sweet, vegetable and molasses. These notes became associated with burnt and sharp as well as savoury at 20 and 30% solids. At 50%, the sample had roasted and paint smells that became dominant at 75%. Some extra metallic, burnt rubbery, and sulphur notes were detected with the 87% sample. The 20 and 30% solid samples seemed the most interesting because of their meaty savour smells and therefore they were selected for further analysis. it was also decided to dilute the original flavoured reaction mixtures before sniffing further reaction mixtures.

Table 9 - Aroma panel results on heated centrate pH 5.5 - Study of the effect of concentration

Sample Name	Heated Aqueous Centrate 12% pH 5.5	Heated Aqueous Centrate 20% pH 5.5	Heated Aqueous Centrate 30% pH 5.5
Sample Composition	12% (w/v, solids of centrate/water) 0.5h, 140°C	20% (w/v, solids of centrate/water) 0.5h, 140°C	30% (w/v, solids of centrate/water) 0.5h, 140°C
Panellist 1	burnt, caramel, slightly sweet	sweet, slightly meaty, nutty	soja sauce, caramel, toffee, burnt, sweet
Panellist 2	sickly molasses	sharp, acrid, cloying, burnt paper	molasses, black treacle
Panellist 3	celery, burnt roast coffee, bovril	marmite	marmite, emulsion paint
Panellist 4	honey, caramel, stir fry	honey, caramel, stir fry, vegetable	caramelised vegetables
Panellist 5	celery, rancid, maggi, bovril	sharp, marmite, yeasty, bovril+celery undertones	sharp, bovril, vinegary top note
Panellist 6	malty, brewery, virol (malt extract), v.concentrate digestive biscuit, black treacle, autoclaving	honey, malty	autoclaving, honey, digestive biscuits
	REPLICATE	REPLICATE	REPLICATE
Panellist 1	sweet, caramel, soja sauce	burnt, toffee, acrid	soja sauce, caramel, burnt, sweet
Panellist 2	burnt, caramel, burnt skin/hair	sharp, acrid, cloying, burnt paper	molasses, resinous
Panellist 3	malt	marmite, celery	marmite, celery, slight roast coffee
Panellist 4	honey, caramel, stir fry	honey, earthy, uncooked potato	soy sauce, wood smoke
Panellist 5	celery, meaty	burnt celery	bovril, strange top note
Panellist 6	black treacle, virol, autoclaving	concentrated honey, biscuity digestives, autoclaving	biscuits, honey, green/fruity, green bananas

Sample Name	Heated Aqueous Centrate 50% pH 5.5	Heated Aqueous Centrate 7.5% pH 5.5	Heated Aqueous Centrate 87% pH 5.5
Sample Composition	50% (w/v, solids of centrate/water) 0.5h, 140°C	75% (w/v, solids of centrate/water) 0.5h, 140°C	87% (w/v, solids of centrate/water) 0.5h, 140°C
Panellist 1	burnt, caramel, bitter chocolate	burnt, very strong, chocolate	burnt sugar
Panellist 2	molasses, black treacle, solvent	burnt, solvent, sickly	savoury, burnt, burnt flesh/skin
Panellist 3	paint, marmite, slight celery	burnt	slightly sweet, burnt, marmite
Panellist 4	raw vegetables, caramel	caramel, roasted slight treacle	soy sauce

Panellist 5	strange top note of sharp green/ fruity note/household or dry smell	very burnt sugar + top note	burnt rubber, sulphur, very burnt sugar
Panellist 6	green banana, honey, slight autoclaving, slight virol	burnt milk, burnt sugar (not caramel - really burnt)	dry, dusty sensation, like something burnt black in oven, sharp, faint marmite, black treacle, slightly stale, metallic, rusty steel
	REPLICATE	REPLICATE	REPLICATE
Panellist 1	burnt, caramel	burnt, slightly sweet, caramel	burnt sugar
Panellist 2	burnt, black treacle, slightly acrid	very strong marmite, meat extract, burnt, caramel	molasses, burnt, savoury
Panellist 3	paint, marmite, cereal	marmite, burnt	burnt, marmite
Panellist 4	vegetable, roasted, woodland	damp wood, syrup, honey	vegetable
Panellist 5	strong strange top note, bovril	strong strange top note, burnt sugar	marmite, yeasty, bovril, very concentrated
Panellist 6	black treacle, acrid/burnt honey, virol/malt	fresh sensation, honey, malt	same as other replicate

The pH effect was studied on the centrate at 3 different values: 5.5, 7.5 and 9, with the heating conditions kept at 140°C for 30 min. The results are in Table 10. There was not much difference in the results between the 20% and the 30% solids samples. The results at pH 7.5 were similar to those at pH 5.5 and the smells were mainly autoclave and caramel. The odour became burnt with pH 9. Therefore it was decided to carry on the sniffing experiments by selecting the two extreme pHs and to keep only one concentration (20% solids).

Table 10 - Aroma panel results on heated centrate study of the effect of pH

Sample Name	**Heated aqueous centrate 20% pH 5.5**	**Heated aqueous centrate 30% pH 5.5**
Sample composition	20%(w/v, solids of centrate/water) 0.5h 140°C	30% (w/v, solids of centrate/water) 0.5h 140°C
Dilution before sniffing	50 for panellist 1	50 for panellist 1
	10 for panellist 2	10 for panellist 2
Panellist 1	1-slight autoclaving 2-diacetyl then going to caramel, butter scotch, slightly nutty	1-slightly autoclave, "catching" in nose. 2-then quite a lot of caramel
panellist 2	caramel, butter-like	caramel, sweet
Sample name	**Heated aqueous centrate 20% pH 7.5**	**Heated aqueous centrate 30% pH 7.5**
Sample composition	20%(w/v, solids of centrate/water 0.5h 140°C	30%(w/v, solids of centrate/water) 0.5h 140°C
Dilution before sniffing	50	50
Panellist 1	similar to sample 20% pH 5.5 in the way it changes, bit more autoclaving, caramel but not really butterscotch	1-slightly autoclave 2-caramel
Sample name	**Heated aqueous centrate 20% pH 9**	**Heated aqueous centrate 30% pH9**
Sample composition	20% (w/v, solids of centrate/water) 0.5h 140°C	30% (w/v, solids of centrate/water) 0.5h 140°C
Dilution before sniffing	10	10
Panellist 2	burnt, baked, roasted, cereals	slightly burnt, caramel sweet

The effect of addition of cysteine was studied on the centrate in solution at 20% solids at pH 5.5 and 9. There were three concentrations of cysteine tested: ratios 1/20, 1/10 and 1/5 of cysteine (g)/centrate solids (g). The heating conditions were kept the same as previously (104°C for 30 min). The results are presented in Table 11. Within the pH 5.5 sample series, the low concentration of cysteine sample lead to a somewhat pleasant odour of sweet, greasy, meaty sauce, that was progressively replaced by roasted and rubber notes as the cysteine concentration increased. At pH 9, the burnt roasted cereals notes already mentioned in the previous experiment were present again with cysteine at low concentration. When the cysteine content increased, the odour became strong and more nutty and then close to savoury, meaty stock. It was therefore decided to select pH 5.5 with cysteine 1/20 sample and pH9 with cysteine 1/5 sample for the next set of experiments.

Table 11 - Aroma panel results on heated centrate pH 5.5 and pH 9 - Study of the effect of addition of cysteine

Sample Name	Heated Aqueous Centrate pH 5.5 + cysteine 1/20	Heated Aqueous Centrate pH 5.5 + cysteine 1/10	Heated Aqueous Centrate pH 5.5 + cysteine 1/5
Sample Composition	20% (w/v, solids of centrate/water) 1g cysteine/20 g solids of centrate 0.5h, 140°C	20% (w/v, solids of centrate/water) 1g cysteine/10g solids of centrate 0.5h, 140°C	20% (w/v, solids of centrate/water) 1g cysteine/5g solids of centrate 0.5h, 140°C
Dilution before Sniffing	10	10	10
Panellist 1	celery, cooked vegetables, burnt wool, sweaty socks	bacon fat, crispy duck (chinese style), sl.sweaty, roasted	sweet, caramel, roasted
Panellist 2	celery (strong), sl. burnt coffee, treacle toffee (sl.)	puffed wheat, sweet+sour	celery, sweet+sour, puffed wheat (strong)
Panellist 3	strong chicken stock, greasy, acidic, rubber	burning rubber, savoury, marmite, malt extract	weak rubbery, acrid
Panellist 4	strong celery (main odour) + other notes - meaty, pork and apple, sweet and sour sauces	medicinal smell, also watery stock (weak) faint celery/sweet'n sour	(sweet and sour), rubber, magi
Panellist 5	black treacle, buttery/caramel, burnt sugar, burnt fried boiled cabbage, marsala, sweet, nutty	buttery, golden syrup, something burnt? (wet wood or toasted nuts, almond, hazelnut)	golden syrup, caramel, toasted nuts (not burnt) sl. sulphur.

Sample Name	Heated Aqueous Centrate pH 9 + cysteine 1/20	Heated Aqueous Centrate pH 9 + cysteine 1/10	Heated Aqueous Centrate pH9 + cysteine 1/5
Sample Composition	20% (w.v, solids of centrate/water) 1g cysteine/20g solids of centrate 0.5h, 140°C	20% (w/v, solids of centrate/water) 1g cysteine/10g solids of centrate 0.5h, 140°C	20% (w/v, solids of centrate/water) 1g cysteine/5g solids of centrate 0.5h, 140°C
Dilution before Sniffing	10	10	10
Panellist 1	burnt, roasted, nutty	sweet caramel, roasted, nutty, burnt	meaty, pork crackling, nutty
Panellist 2	sweet (strong), toffee, biscuity, marzipan	celery (strong), sl. sweet, biscuity	meaty (strong)
Panellist 3	savoury, burnt skin, stock	burning rubber, strong savoury, malt extract	strong chicken stock, chicken fat, sl. acrid
Panellist 4	v.strong weird note, stale biscuit crumbs, Farleys rusks, faint sweet'n sour note	fruity, sweet, burnt sugar, vinegar	sulphury, weak stock, nasty note, burnt meat?
Panellist 5	burnt, scorched/burnt wet wool, golden syrup	same as sample 1/20 but stronger burnt, less golden syrup	meaty (beef gravy?), burnt, burnt sugar, plus caramel

The results of the temperature/duration heating conditions study are presented in Table 12. They involve the two selected samples previously described which were then cooked at a lower temperature and longer time: 100°C for 60 min and 90 min, and at a higher temperature but for a shorter time: 175°C for 5 min. Compared to the original heating conditions, similar results were obtained for the pH 5.5 cysteine 1/20 sample by heating 100°C for 60 min. Longer time of heating at 100°C resulted in more roasted burnt notes and a similar type of odour was obtained after 5 min at 175°C. Regarding the pH 9 cysteine 1/5 sample, the meaty notes obtained by 30 min at 140°C were reached by the treatment 5 min at 175°C. At 100°C, some very strong odours of urine and boiled eggs were described.

Table 12 - Aroma panel results on heated centrate pH 5.5 and pH 9 + Cysteine
Study of the effect of temperature and duration of the cooking

Sample Name	**175°C Heated Aqueous Centrate pH 5.5 + cysteine 1/20**	**100°C 1h Heated Aqueous Centrate pH 5.5 + cysteine 1/20**	**100°C 1.5h Heated Aqueous Centrate pH 5.5 + cysteine 1/20**
Sample Composition	20% (w/v, solids of centrate/water) 1g cysteine/20g solids of centrate) 5min 175°C	20% (w/v, solids of centrate/water) 1g cysteine/20g solids of centrate 1h, 100°C	20% (w/v, solids of centrate/water) 1g cysteine/20g solids of centrate 1.5h, 100°C
Dilution before sniffing	10	10	10
Panellist 1	burnt-strong, caramel-moderate, roasted-moderate	fruity-moderate, nutty-slight	celery-moderate, roasted-light
Panellist 2	puffed wheat-strong, marmite-medium, celery-light	celery-strong	celery-medium, cereal-slight
Panellist 3	rancid-v.strong, acrid-strong, burnt fat/skin-medium, molasses-medium/weak	chicken fat-medium, rubber-medium, burnt rubber-medium	burnt rubber-weak, molasses-weak medium, burnt/cold wood-weak

Sample Name	**175°C Heated Aqueos Centrate pH 9 + cysteine 1/5**	**100°C 1h Heated Aqueous Centrate pH 9 + cysteine 1/5**	**100°C 1.5h Heated Aqueous Centrate pH 9 + cysteine 1/5**
Sample Composition	20% (w/v, solids of centrate/water) 1g cysteine/5g solids of centrate 5min, 175°C	20% (w/v, solids of centrate/water) 1g cysteine/5g solids of centrate 1h, 100°C	20% (w/v, solids of centrate/water) 1g cysteine/5g solids of centrate 1.5h, 100°C
Dilution before sniffing	10	10	10
Panellist 1	crackers-moderate, stale-slight, baked bread-moderate	yeast extract-weak, vegetables-moderate, wet washing-moderate	yeast, bread dough-moderate, vegetables-weak, nutty-background after other odours decrease
Panellist 2	sulphury-slight, meaty-medium, burnt-medium	hard boiled egg-strong	egg-strong
Panellist 3	burnt paper-weak, chicken stock-weak/medium cereal/malty-weak	wet wall paper-medium, urine	state, wet wallpaper, musty

Results - Instrumental Analysis CF Aroma Volatiles by GC/MS

A selection of the reaction mixtures described above were further studied by GC-MS analysis of the headspace volatiles by the method described previously. These mixtures are underlined in Table 2. A sample of autolysed yeast was analysed under the same conditions for comparison of the volatiles. The results of the study are presented in detail in Table 13.

Table 13 - Volatile compounds analysed by headspace concentration

Identified Compounds	LRI	Approximate quantities (ng/0.4g of Freeze Dried Centrate, or ng/45.7g of Centrate										
		pH5.5 140°C	ph 9 140°C	pH5.5 + C1/20 140°C	pH5.5 + C 1/5 140°C	ph 9 + C 1/20 140°C	pH 9 + C 1/5 140°C	pH5.5 + C 1/20 175°C	pH 9 + C 1/5 175°C	pH5.5 + C1/20 100°C	pH 9 + C 1/5 100°C	Yeast
Pyrazines												
pyrazine	762	-	648	-	-	1108	-	-	-	-	-	-
methylpyrazine	845	78	4607	-	37	2521	813	102	132	-	-	-
2-6-dimethylpyrazine	928	-	-	-	-	465	590	-	-	-	3	-
2,5-dimethylpyrazine	936	104	3859	-	-	382	500	-	-	-	17	4
2,3-dimethylpyrazine	940	-	966	-	-	30	-	-	157	-	-	-
ethenylpyrazine	950	44	239	-	-	-	-	-	-	-	-	-
2-ethylpyrazine	956	-	38	-	-	118	186	-	167	-	-	-
2-ethyl-6-methylpyrazine	1014	-	984	-	-	301	343	-	-	-	-	-
trimethylpyrazine	1018	256	1758	-	63	1453	1758	-	518	-	98	-
propylpyrazine	1025	-	21	-	-	-	-	-	-	-	-	-
6-methyl-2-ethenylpyrazine	1034	20	160	-	-	-	-	-	-	-	-	-
tetramethylpyrazine	1098	-	-	-	46	1009	1121	-	660	-	647	-
dimethylethylpyrazine	1099	416	120	51	-	85	111	74	-	-	9	-
dimethylethylpyrazine	1101	-	1593	-	-	-	35	-	-	-	-	-
methylpropylpyrazine	1101	26	-	-	-	-	-	-	-	-	-	-
3,5-diethyl-2-methylpyrazine	1168	-	137	-	-	-	-	18	-	-	-	4
trimethylethylpyrazine	1168	51	-	-	-	120	195	-	75	-	105	-
dimethylpropylpyrazine	1170	12	63	-	-	98	186	-	51	-	15	-
dimethylpropylpyrazine	1179	32	130	-	-	81	146	-	40	-	9	-
dimethylbutylpyrazine	1321	-	-	-	-	-	-	-	-	-	16	2
trimethylpropylpyrazine	1247	75	168	-	23	166	352	36	150	-	265	-
trimethylpropylpyrazine	1260	16	99	-	-	27	-	-	-	-	-	-
2-(2-methylpropyl)-3-(1-methylethyl)-pyrazine		-	-	-	-	-	-	-	-	-	2	-
methyldiethylpyrazine		-	-	-	-	-	-	-	-	-	7	-

2,5-dimethyl-3.6-dipropyl-pyrazine		-	-	-	-	-	-	-	-	-	5	-
Furans												
2-ethylfuran	707	-	-	29	398	32	2037	-	-	-	-	-
dimethylfuran	711	-	-	-	22	-	-	112	-	-	-	-
2,4-dimethylfuran	723	111	-	104	111	43	85	-	-	-	-	-
dihydro-2methyl-3(2H)-furanone	831	71	69	-	-	-	-	-	-	-	-	-
2-furancarboxaldehyde	857	668	-	253	109	-	-	165	-	-	-	-
2-furanmethanol	881	85	298	153	85	33	-	121	11	-	-	7
2-furanmethanethiol	926	-	-	-	249	-	-	-	-	-	-	-
2-acetylfuran	924	-	-	-	-	-	-	-	406	-	-	-
1-(2-furanyl)-ethanone	929	84	-	629	1193	-	391	494	-	-	7	-
2-pentylfuran	997	113	62	?	-	35	47	-	-	-	-	-
1-(2-furanyl)-1-propanone	1025	21	-	-	-	-	-	-	-	-	-	-
Pyrans									-			
3,4-dihydro-2H-pyran	809	-	-	-	20	-	-	-		-	-	-
tetrahydro-6-methyl-2H-pyran- 2-one	879	-	-	-	-	-	-	-	-	-	8	-
tetrahydro-2H-pyran-2-oll	884	155	-	-	-	-	-	-	-	-	-	-
Thiophenes												
thiophene	677	-	-	-	179	-	-	-	-	-	-	-
methylthiophene	778	-	-	66	63	47	478	62	-	-	-	-
ethylthiophene	875	-	-	-	28	-	58	-	-	-	-	-
2,5 dimethyl thiophene	897	-	-	-	19	-	36	38	-	-	-	-
thiophenethiol	995	-	-	-	205	-	-	-	-	-	-	-
2-methyltetrahydrothiophen-3-one	1014	693	-	1366	-	-	-	-	-	28	-	-
2-thiophenecarboxaldehyde	1026	20	-	99	-	104	-	-	-	-	-	-
2-thiophenemethanethiol	1077	-	-	-	27	-	-	-	-	-	-	-
3-(methylthio)thiophene	1103	-	-	-	37	145	-	-	-	-	-	-
methylthiophene-carboxaldehyde	1143	-	-	43	5	-	-	52	97	-	-	-

methylthiophene-carboxaldehyde	1148	-	-	526	89	134	41	329	-	-	-	-
thienothiophene	1233	-	-	35	49	-	32	16	24	-	-	-
thienothiophene	1276	-	-	-	30	-	-	-	36	-	-	-
Thiazoles												
thiazole	734	-	-	46	51	-	1165	219	870	-	-	-
methylthiazole	821	-	-	-	62	72	2	71	134	-	6	-
5-methylthiazole	857	-	-	-	106	57	112	779	82	-	-	-
methylthiazole	866	-	-	-	39	-	-	-	-	-	-	-
dimethylthiazole	947	-	-	-	29	88	289	-	95	-	-	-
2-ethylthiazole	959	-	-	-	22	-	42	26	78	-	-	-
2,4,5-trimethylthiazole	1016	-	-	-	1499	59	262	-	153	-	-	-
methylethylthiazole	1028	-	-	-	140	-	68	123	290	-	-	-
2-acetylthiazole	1041	-	-	-	-	647	361	-	109	-	-	-
4-methyl-5-ethenylthiazole	1043	57	67	-	-	-	59	43	-	-	20	-
2-isobutylthiazole	1065	31	-	-	-	-	-	-	-	-	-	-
methylpropylthiazole	1128	-	-	-	-	36	-	-	-	-	-	-
dimethylisopropylthiazole	1157	-	-	-	24	29	87	-	55	-	-	-
dimethylisopropylthiazole	1152	-	-	-	-	-	52	-	37	-	-	-
Aliphatic compounds												
1-propanol	616	65	-	-	101	-	-	-	-	1319	-	-
2-butanone	628	1449	-	-	2021	-	3074	-	3755	-	1164	107
ethylacetate	631	-	144	74	92	112	-	97	47	82	110	13
2,3-butanedione	637	1843	1092	2001	-	-	-	1548	-	664	-	-
3-methylbutanal	655	84	3297	1232	-	903	-	1213	752	-	-	1116
pentanal	686	2217	-	-	-	-	-	-	-	-	-	-
2-methylbutanal	691	621	703	1277	-	-	-	1242	151	-	-	582
2-methyl-1-propanol	705	1133	2412	1238	2430	1614	1997	948	1791	1923	3992	17
3-methyl-2-butanone	672	-	-	-	143	-	2205	-	-	42	62	-
2-pentenal	698	-	-	48	22	-	-	34	-	-	-	-
2-pentanone	708	108	-	72	279	93	1190	85	445	34	91	-
3-pentanone	708	-	-	-	72	-	502	-	163	-	-	-
2,3-pentanedione	721	708	487	567	-	240	-	525	309	66	-	-

1-butanol	719	-	132	-	70	100	-	-	56	28	77	-
1-methoxy-2-propanone	723	-	-	-	-	67	-	-	58	-	-	-
3-hydroxy-2-butanone	728	-	-	-	1377	332	-	1694	-	142	-	-
2-methyl-2-butenal	753	-	76	-	-	-	-	-	-	-	-	-
3-methyl-3-buten-1-ol	761	511	-	1794	-	-	578	-	-	98	294	-
3-methylbutanol	766	682	2221	954	730	254	1278	1375	564	355	796	94
2-butennitrile	770	-	-	-	-	-	249	-	-	-	-	-
heptanol	770	433	-	-	-	-	-	-	-	-	-	-
4-methyl-2,3,-pentanedione	801	60	607	-	-	-	-	-	-	-	-	-
2,3-hexanedione	811	1686	137	1791	-	434	-	1404	-	591	-	-
hexanal	818	91	-	38	-	144	-	171	57	-	-	-
3-hexanone	822	36	-	-	203	-	1654	-	244	-	58	-
2-hexanone	803	-	-	-	391	-	1502	-	-	-	80	-
3-hydroxy-2-pentanone	831	-	-	75	59	-	-	125	-	-	-	-
2-heptanone	903	99	122	106	111	113	206	161	83	57	71	7
heptanal	910	-	-	-	-	-	-	-		-	-	138
2-hydroxy-3-hexanone	913	138	78	323	234	127	65	432	135	-	10	-
6-methyl-2-heptanone	967	594	722	9465	15413	1397	4781	7347	-	-	3512	-
1,3-cyclopentanedione	973	26	24	-	-	-	-	-	-	-	-	-
5-methyl-2-heptanol	979	31	34	555	684	91	205	572	399	248	247	-
methylheptanone	984	-	-	-	149	11	234	-	2995	4909	110	19
octenol	988	-	-	-	-	-	-	-	-	-	5	40
6-methyl-5-hepten-2-one	997	-	-	114	104	-	-	-	30	25	20	23
2-heptenal	1036	-	-	-	-	-	-	-	-	-	15	24
2-nonenal	1113	-	-	-	-	-	-	-	70	25	7	81
2-octenal	1069	-	-	-	-	-	-	-	-	-	-	25
1-octanol	1078	-	-	-	-	-	-	-	-	-	-	25
nonanal	1116	37	-	56	-	-	-	48	-	-	-	-
decanal	1217	28	-	-	-	-	-	-	-	-	6	-
2-undecanone	1298	-	-	-	-	-	-	-	-	-	4	-
butanoic acid butyl ester	1380	-	-	-	-	-	-	-	-	-	-	3
dodecanal	1417	-	-	-	-	-	-	-	-	-	-	3
unsaturated ketone	1459	-	-	-	9	-	-	-	-	-	-	8

1-tetradecanol	1480	-	-	-	-	-	-	-	-	-	11	
1-hexadecanol	1581	-	-	-	-	-	-	-	-	-	7	
Pyrroles/Benzopyrroles												
pyrrole	765	-	-	-	-	102	238	-	199	-	0	-
2-meth-1H-pyrrole	830	-	-	-	-		12	-	-	-	-	-
tetrahydro-6-methyl-2H-pyran-2-one	879	-	-	-	-	-	-	-	-	-	8	-
2,4-dimethyl-3-ethyl-1H-pyrrole	1055	-	-	-	-	-	-	-	-	-	22	-
indole	1313	-	-	-	-	-	-	-	-	-	5	-
Phenyls												
phenol	727	-	-	-	11	-	-	-	-	-	-	-
benzaldehyde	983	223	126	152	60	175	-	106	-	-	17	267
methylethylbenzene	1025	-	-	-	-	-	-	-	-	-	-	20
benzenaceataldehyde	1059	-	-	-	-	-	-	-	-	-	-	36
2-methylphenol	1065	-	-	-	-	-	-	-	-	-	-	5
methylchlorophenol	1088	-	-	-	-	-	-	-	-	-	-	6
acetophenenone	1080	-	-	-	-	-	-	-	-	-	6	-
2-methylthiophenol	1157	-	-	-	-	-	45	-	226	-	-	-
ethoxybenzaldehyde	1244	-	-	228	10	-	-	-	-	-	-	-
Pyridines												
methylpyridine	833	-	-	-	-	-	96	-	82	-	5	-
2-methylpyridamine	848	52	-	-	-	-	-	-	-	-	-	-
6-methyl-4-(1H)-pyrimidinone	937	-	-	253	252	46	-	339	190	84	-	-
Terpenes												
3,6,6-trimethyl-bicyclo-(3.1.1.)hept-2-ene	934	-	-	-	-	-	-	-	-	-	-	8
1,5-dimethyl-1-5-cyclo-octadiene	1034	-	-	-	-	-	-	-	-	-	-	22
limonene	1037	-	-	-	-	-	41	-	-	-	15	-
cymene	1031	-	-	-	-	-	-	-	-	-	-	10

eucalyptol	1039	-	-	-	-	-	-	-	-	-	-	13
verbenene	1063	-	-	-	-	-	-	-	-	-	-	4
3-7-dimethyl-1,6-octadien-3-0l	1105	-	-	-	-	-	-	-	-	-	-	19
borneol	1184	-	-	-	-	-	-	-	-	-	-	2
terpin-4-ol	1191	-	-	-	-	-	-	-	-	-	-	15
trimethylbicyclo-(2.2.1)-heptan-2-one	1236	-	-	-	38	-	-	-	-	-	-	14
bornyl acetate	1292	-	-	-	-	-	-	-	-	-	-	2
safrole	1305	-	-	-	-	-	-	-	-	-	-	3
terpine	1339	-	-	-	-	-	-	-	-	-	-	3
a sesquiterpene	1383	-	-	-	-	-	-	-	-	-	-	5
a sesquiterpene	1435	-	-	-	5	-	-	-	-	16	12	2
a sesquiterpene (cadinene ?)	1494	20	-	-	36	-	27	24	15	-	14	14
a sesquiterpene	1499	-	-	-	-	-	17	50	-	-	9	2
Oxazoles												
4,5-dimethyloxazole	771	-	-	-	105	-	-	-	-	-	-	-
trimethyloxazole	865	649	540	580	321	174	122	-	68	161	29	-
2,5-dimethyl-4-ethyloxazole	932	256	-	-	-	-	-	-	-	48	-	-
4,4-dimethyl-2-ethyloxazole	946	10	-	-	-	-	-	-	-	-	-	-
4,5-dimethyl-2-isopropyloxazole	986	237	158	-	147	-	68	160	22	47	-	-
4,5-dimethyl-2-propyloxazole	1014	-	-	-	-	-	-	1463	-	369	65	-
2,4-dimethyl-5-propyloxazole	1020	697	-	1448	-	-	-	1555	-	513	-	-
4-methyl-5-ethyl-2-isopropyloxazole	1044	-	-	-	20	-	-	48	-	-	-	-
Aliphatic sulfides												
dimethyldisulfide	754	295	389	280	-	311	-	-	-	-	-	81

dimethyltrisulfide	987	-	-	402	-	92	-	-	-	62	87
2-pentanethiol	841	-	-	-	2403	-	-	102	-	-	-
1(ethylthio)-2-propanone	921	-	-	-	51	-	-	-	-	-	-
2,2-dithio-bis-ethanol	1280	-	-	-	16	-	-	-	-	-	-
Cyclic polysulfides											
3,5-dimethyl-1,2,4-trithiolane	1280	-	-	-	-	21	91	-	36	-	-
Dioxanes											
4-methyl-1,3-dioxane	737	321	46	256	-	-	-	142	-	-	-
TOTAL		18678	29633	28779	33228	16478	32517	25890	17369	11936	12195	2982

The pyrazines, thiazoles and thiophenes content was very much affected by the reaction conditions. The highest levels of pyrazines were found at pH9, as expected, since the formation of N-heterocyclic compounds in the Maillard reaction is favoured by high pH. With a few exceptions, sulphur compounds were formed only in the presence of cysteine, confirming that the content of sulphur amino acids in the freeze dried centrate was very low.

The yeast autolysate aroma volatiles were dominated by terpenes and its composition was very different from the volatiles obtained from the centrate.

Conclusion

The range of flavours was generated from the centrate, showing its potential as a flavouring ingredient or as a source of precursors for reaction product flavourings. The variables that were applied in this study were the centrate concentration, the pH, the presence of added cysteine, and the temperature/duration of the heating conditions. An addition of cysteine was necessary to generate meaty aromas which derive from sulphur-containing volatiles.

CLAIMS

1. A flavouring material for food which:

(a) is an aqueous solution which comprises materials removed from a filamentous fungus by contacting the filamentous fungus in its growing state and/or in the presence of its growth medium with water at an elevated temperature sufficient to reduce the nucleic acid content of the filamentous fungus in which the concentration of dissolved solids is sufficiently high to render the solution stable to storage at a temperature of 20°C for a period of one month; or

(b) is a solid comprising materials so removed from the filamentous fungus; or

(c) is a flavouring material comprising a reaction product of materials removed as described in (a) or (b) with a sulphur containing amino acid, hydrogen sulphide or ammonium sulphide.

2. A flavouring material for food which:

(a) is an aqueous solution which comprises materials removed from a filamentous fungus by contacting filamentous fungal cells in their growing state and/or in the presence of their growth medium with water at an elevated temperature sufficient to reduce the nucleic acid content of the filamentous fungal cells in which the concentration of dissolved solids is sufficiently high to render the solution stable to storage at a temperature of 20°C for a period of one month; or

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(b) is a solid comprising materials so removed from the filamentous fungal cells; or

(c) is a flavouring material comprising a reaction product of materials removed as described in (a) or (b) with a sulphur containing amino acid, hydrogen sulphide or ammonium sulphide.

3. A flavouring material according to Claim 1 or Claim 2 which is an aqueous solution comprising at least 30% by weight of solids.

4. A flavouring material according to Claim 1 or Claim 2 which comprises a reaction product of materials removed as described in (a) or (b) with cysteine.

5. A flavouring material as claimed in any preceding claim, wherein said filamentous fungus is Fusarium.

6. A process of producing a flavouring material for food which comprises the step of improving the suitability of filamentous fungus for food by subjecting it

(a) in its growing state and/or in the presence of its growth medium; and

(b) in the presence of water;

to a temperature sufficient to reduce its nucleic acid content thereby producing an aqueous solution and concentrating the aqueous solution.

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7. A process of producing a flavouring material for food which comprises the step of improving the suitability of filamentous fungus for food by subjecting filamentous fungal cells

(a) in their growing state and/or in the presence of their growth medium; and

(b) in the presence of water;

to a temperature sufficient to reduce the nucleic acid content of said cells thereby producing an aqueous solution and concentrating the aqueous solution.

8. A process according to Claim 6 or Claim 7, wherein said aqueous solution after concentration comprises at least 30% by weight of solids.

9. A process as claimed in any one of Claims 6 to 8 in which water is removed from the aqueous solution by evaporation, distillation, reverse osmosis, freeze drying or freezing out the water as ice leaving an aqueous concentrate.

10. A process as claimed in any one of Claims 6 to 8 in which the soluble components are concentrated by removing water at reduced pressure and at a temperature of 40 to 70°C.

11. A process of producing a flavouring material for food according to any one of Claims 6 to 10 in which nucleic acid is removed from the filamentous fungus in its growing state.

12. A process as claimed in Claim 11 in which the filamentous fungus in its growing state is washed to remove growth medium and immediately thereafter heated in the presence of water to remove nucleic acid.

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13. A process as claimed in any one of Claims 6 to 12 in which materials recovered from the filamentous fungus are reacted with a sulphur containing amino acid, hydrogen sulphide or ammonium sulphide thereby producing savoury flavours.

14. A process as claimed in Claim 13 in which the sulphur containing amino acid is cysteine.

15. A process as claimed in Claim 13 or Claim 14 in which the reaction is carried out in the presence of water.

16. A process as claimed in Claim 14 or Claim 15 in which a solution of 1.5 to 75% of the materials removed from the fungus in water is reacted with cysteine in amounts up to 10% of cysteine by weight based on the said materials at a pH of 5.5 to 9.

17. A process as claimed in Claim 16 in which the temperature of the reaction is 110 to 140°C.

18. A process as claimed in any one of Claims 6 to 17, wherein said filamentous fungus is Fusarium.

19. A food which comprises 0.1 to 15% by weight based on the solids content of a flavouring material as claimed in any one of Claims 1 to 5.

20. A food according to Claim 19 which is a snack, biscuit, stock, soup, stew, sauce, gravy or a drink.

21. A food which comprises 0.1 to 15% by weight based on the solids content of a flavouring material produced by a process as claimed in any one of claims 6 to 18.

22. A food according to Claim 21 which is a snack, biscuit, stock, soup, stew, sauce, gravy or a drink.

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23. A process of flavouring food which comprises the step of improving the suitability of a filamentous fungus as food by subjecting filamentous fungal cells of filamentous fungus

(a) in their growing state and/or in the presence of their growth medium; and

(b) in the presence of water;

to a temperature sufficient to reduce the nucleic acid content of the cells substantially and flavouring food with materials removed in the said step directly or after chemical reaction.

24. A process of flavouring food which comprises the step of improving the suitability of a filamentous fungus as food by subjecting it

(a) in its growing state and/or in the presence of its growth medium; and

(b) in the presence of water;

to a temperature sufficient to reduce its nucleic acid content substantially and flavouring food with materials removed from the fungus in the said step directly or after chemical reaction.

25. A method of preparing a flavouring material for food which comprises subjecting a filamentous fungus in the presence of water to a temperature sufficient to remove materials therefrom and thereby reduce its nucleic acid content; and subjecting materials removed from the fungus to a chemical reaction thereby to prepare a flavouring material for food.

26. A method of processing filamentous fungus to improve its suitability as food which comprises subjecting it in the presence of water to a temperature sufficient to remove materials therefrom and thereby reduce its nucleic acid content; and subjecting materials removed from the fungus to a chemical reaction thereby to prepare a flavouring material for food.

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27. A flavouring material for food when produced by a process according to any one of Claims 6 to 18, 23 or 24.

28. A flavouring material for food when produced by a method according to Claim 25 or 26.

29. A flavouring material for food according to any one of Claims 1 to 5, 27 or 28 substantially as hereinbefore described, with reference to any of the Tables.

30. A food according to any one of Claims 19 to 22 substantially as hereinbefore described, with reference to any of the Tables.

31. A process according to any one of Claims 6 to 18, 23 or 24 substantially as hereinbefore described, with reference to any of the Tables.

32. A method according to claims 25 or 26 substantially as hereinbefore described, with reference to any of the Tables.

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