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Process for converting elemental sulphur into sulphide

The present invention concerns a process for the
5 bioconversion of elemental sulphur into sulphide and a
process for preparing a flavouring agent, in particular
producing a flavour of the meat type.

Given the central part played by hydrogen sulphide in the
10 generation of flavours of the meat type, there is an
interest in finding alternative sources of sulphur
generating sulphide. One particularly interesting route
consists of microbial fermentation. For example, in Amer.
J. Enol. Vitic., 1977, 28(3), 137-144, Schütz M et al
15 describe the presence of hydrogen sulphide responsible for
the production of bad odours in vinification residues.

We have found that certain micro-organisms which can be
used in food, in particular baker's yeast, *Saccharomyces*
20 *cerevisae*, has the capacity to form hydrogen sulphide from
elemental sulphur by biological fermentation. Such a source
is particularly interesting since it is abundant and
economical.

25 The process according to the invention is characterized in
that a micro-organism which can be used in food, in
particular baker's yeast, is incubated with elemental
sulphur in the presence of a reducing sugar.

30 The invention also concerns a process for preparing a
flavouring agent, characterized in that a culture medium of
the micro-organism, in particular baker's yeast, is
incubated in the presence of elemental sulphur and a
reducing sugar at a temperature where it is active, which
35 produces an evolution of gas, in that incubation continues
until gas ceases to be evolved, and in that the reaction
medium is subsequently heat treated so as to develop the
flavour.



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In order to put the present process into operation, a mixture is prepared containing the micro-organism, for example a cream or an extract of baker's yeast, the pH of which is adjusted for example to 5-9, preferably 6-8, by means of a buffer solution. Elemental sulphur is added and, progressively, a reducing sugar, with stirring, preferably vigorous stirring.

The reducing sugar may be, for example, a monosaccharide and in particular a pentose such as xylose, ribose or arabinose, or preferably hexose such as glucose or fructose. The latter is preferably added progressively, for example in about 60 to 90 min.

As soon as incubation starts, a gaseous evolution of hydrogen sulphide is produced. The incubation conditions are chosen for optimum activation of the micro-organism, for example at a temperature of 25 to 40°C, at a pH of 5 to 9 and for 1 to 7 h, with stirring until gas ceases to be evolved.

After incubation, the reaction medium may be heat treated, preferably under reflux at 100°C for 20 to 40 min. It is through this treatment that the desired flavour develops in the supernatant which can be separated, for example by centrifuging, and then, optionally concentrated and dried, for example by spraying or lyophilisation in the presence of a support, for example maltodextrin or cyclodextrin.

The invention also concerns the use of reaction media containing the flavouring agent or its reactive precursor as the raw material for the preparation of the process flavourings, mixed with other materials rich in precursors and/or flavour enhancers.

These other materials may for example be a soy sauce, a yeast autolysate, sodium chloride, certain sugars, fats and spices.

- 5 In order to prepare such flavourings by reaction, the mixture may be reacted by heating at 80-150°C, preferably at around 100°C for 30 min to 4 h.

It is then possible to add a support to the reaction
10 product, for example maltodextrin or cyclodextrin, and then to dry the mixture at a moderate temperature, for example at 60-70°C under vacuum.

Such flavourings may be incorporated in foods intended for
15 human or animal consumption.

The following examples illustrate the invention. In these, the percentages and parts are by weight, unless stated to the contrary.

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Examples 1-3

Commercial creamed baker's yeast, *Saccharomyces cerevisiae* with 22-28 % dry matter was clarified by centrifuging and
25 the supernatant was discarded. The residue was then mixed with a 0.1 M aqueous solution of phosphate buffer of the chosen pH.

300 ml of this cream of whole cells was then placed in a
30 glass reactor which was fitted with a temperature probe set to 30°C, a stirrer revolving at 600 rpm and a condenser, and which was attached to a pH-stat maintaining the pH at the chosen value by addition of 2N aqueous solution of sodium hydroxide. To this was added 0.8 g (25 mmol) of
35 elemental colloidal sulphur and then, drop-wise, an autoclaved glucose solution (18 g, 100 mmol, dissolved in

30 ml of water), with the same pH, the latter addition taking 90 min.

As soon as the glucose began to be added a spontaneous evolution of gas was observed which ceased after 5 h of incubation. It was possible to quantify this evolution by means of a trap in the form of a flask mounted at the outlet from the condenser and containing 60 ml of 0.5 M aqueous solution of lead (II) nitrate.

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After 6 h of fermentation, the pH of the culture medium was adjusted, if necessary, to 6 and the black precipitate of lead sulphide formed in the trap was then collected and dried in an oven at 100°C for 12 h. The respective quantities by weight of hydrogen sulphide produced, calculated as lead sulphide, as well as the yields of hydrogen sulphide are shown in Table 1 below.

Table 1

Example, pH	1, pH 6	2, pH 7	3, pH 8
PbS (mg)	1420	1765	2548
PbS (mmol)	5.94	7.38	10.7
Hydrogen sulphide yield (%)	23.8	29.5	42.6

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Example 4

After incubation according to Example 1, the culture medium was heated at 100°C for 30 min. After cooling to room temperature, the supernatant was separated by centrifuging for 15 min at 5000 rpm, the residue was then extracted with water, and the supernatant was once again separated by centrifuging and the two supernatants were combined.

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After having adjusted the pH of the supernatant to 4 with a 2N aqueous solution of hydrochloric acid, it was saturated with sodium chloride and the sample was extracted with

300 ml of diethyl ether by means of a liquid-liquid extractor, the organic phase was separated off, dried over sodium sulphate, concentrated in a Vigreux column to a volume of 5 ml, and the concentrated solution obtained was placed in a freezer at - 40°C until analysed.

The concentrated extract was analysed at the olfactory level as well as chemically.

10 Analysis by gas chromatography was carried out on a Carlo Erba Mega 2 chromatograph fitted with a cold injector and a flame ionization detector (GC-FID). A flame photometric detector was used (GC-FPD) for the sulphur compounds. The capillary columns were DB-5 and DB-FFAP, 30 m x 0.32 mm, film thickness 0.25 micrometres, from J & W Scientific, Folsom, USA. The carrier gas was helium (65 kPa) with an addition of nitrogen (40 kPa) for the GC-FID. Olfactory analysis was carried out with the aid of the technique of gas chromatography coupled to olfactometry (GC/O). For this purpose, the gaseous effluent was divided into two equal portions. Half went to the FID detector and the other half was led to a device where the volatile constituents were sniffed and the nature of their odour described by the analyst. This olfactometric analysis was carried out by two analysts.

The retention indices (RI) were calculated by linear interpolation.

30 The analyses were confirmed by establishing spectra produced by coupling a gas chromatograph to an electron impact mass spectrograph (GC/EI-MS, HP 5890/HP 5971) under the same operating conditions as for GC-FID.

35 The results for the main sulphur compounds identified are shown in Table 2 below.

Table 2

<u>Compound</u>	RI (FFAP), FID	RI (FFAP), FPD	RI (FFAP), Sniffing	Odour	FFAP- FPD Area of peaks (%)
O-methyl thioacetate	-	1005	1002	Fruity, sweet, buttery	1.87
S-methyl thioacetate	1053	1060	1056	Fruity	2.02
S-ethyl thioacetate	1072	1100	1085	Fruity, sweet	0.18
Thioacetic acid	1107	1130	1118	Acid, meat-like, roasted	78.38
S-methyl-thio thioacetate	1464	1466	1470	Toasted cereal	2.33
S-methyl- thiomethyl thioacetate	-	1535	1545	Meat-like, stock	1.33
3-methyl-thio- 1- propanol	1718	1722	1729	Vegetable, potato	2.87

-: not measured

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Other different sulphur compounds not identified represented 7.9 % of the total area of the peaks and contributed to the odour of vegetable stock/cereals.

10 It was noted that the concentrated extract had overall a strong flavour resembling toasted cheese similar to that of toasted rind produced in a cheese fondue or a raclette.

Example 5

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The culture medium produced by incubation according to example 1 was used in a reaction for producing a meat
5 flavour by heating the ingredients except maltodextrin in the proportions shown in Table 3 below for 150 min at 100°C and at pH 5. Following the reaction, maltodextrin was added and the mixture was dried at 65°C under vacuum. As a
10 comparison, the same reaction was carried out with baker's yeast which had not been incubated with sulphur, without added sodium sulphide (comparison 1), with added sodium sulphide (comparison 2) and with cysteine (at the rate of 1 mole of cysteine per 1/5th mole of sulphide produced by incubation) (comparison 3).

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Table 3

	Ingredients (%)	Example 5	Comparison 1	Comparison 2	Comparison 3
5	Salt, onion extract, powdered soy sauce	28.6	28.6	28.6	28
10	Chicken meat meal, chicken fat	6.5	6.5	6.5	6.4
	Sucrose, xylose, glucose	6.1	6.1	6.1	6
	Yeast extract	5.5	5.5	5.4	5.4
15	Creamed yeast incubated with sulphur	42.5	-	-	-
20	Creamed yeast incubated without sulphur	-	42.5	42.5	42.5
	Sodium sulphide (60% crystalline)	-	-	0.2	-
	Cysteine HCl	-	-	-	1
25	Maltodextrin	10.8	10.8	10.8	10.8

When a culture medium containing 6 g of flavouring and 1 g of NaCl dissolved in 500 ml of hot water was tasted by a panel of five experienced persons, the extract prepared according to the invention proved to have a distinctly meaty flavour compared to the general note of stock for comparison 1, but however the comparison 2 had a meaty flavour with a slight accent of sulphur. The flavour of the extract prepared according to the invention was very close to that of the comparison 3.

Claims

1. Process for the bioconversion of elemental sulphur into sulphide, characterized in that a micro-organism which can be used in foods, in particular baker's yeast, is incubated with elemental sulphur in the presence of a reducing sugar.
2. Process according to claim 1, characterized in that a mixture is prepared containing the micro-organism, in particular a cream or an extract of baker's yeast, the pH of which is adjusted to 5-9 by means of a buffer solution, and in that elemental sulphur is added and progressively a reducing sugar with stirring in 60 to 90 min.
3. Process according to claim 2 characterized in that the reducing sugar is a monosaccharide and in particular a pentose such as xylose or arabinose, or a hexose such as glucose or fructose.
4. Process according to claim 2, characterized in that incubation takes place at a temperature of 25 to 40°C, at a pH of 5 to 9 and for 1 to 7 h with stirring until evolution of gas ceases.
5. Process for preparing a flavouring agent, characterized in that a culture medium of a micro organism which can be used in food, in particular baker's yeast, is incubated in the presence of elemental sulphur and a reducing sugar at a temperature where it is active, which produces an evolution of gas, and in that incubation lasts until gas is no longer evolved, and the reaction medium is then heat treated so as to develop the flavour.
6. Process according to claim 5, characterized in that after incubation, the reaction medium is treated under reflux at 100°C, for 20 to 40 min, in that the supernatant

is separated, in particular by centrifuging and in that, optionally, the supernatant is then concentrated and dried, in particular by spraying or lyophilisation in the presence of a support, in particular maltodextrin or cyclodextrin.

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7. Use of the reaction medium containing the flavouring agent or its reactive precursor as a raw material for preparing a process flavour, mixed with other materials rich in precursors and/or in flavour enhancers, in particular soy sauce, a yeast autolysate, sodium chloride, certain sugars, fats and spices.

8. Use according to claim 7, characterized in that the mixture is reacted by heating at 80-150°C, in particular at about 100°C for 30 min to 4 h.

9. Use according to claim 7, characterized in that a support is added to the reaction product, in particular maltodextrin or cyclodextrin, and the mixture is then dried at a moderate temperature, in particular at 60-70°C under vacuum.

10. Food intended for human or animal consumption, characterized in that it contains a product obtained by the process according to claim 5 or 6 or a flavouring composition containing it derived from the use according to one of claims 7 to 9.
