FORM 25 (12) PATENT granted by (19) AP

AFRICAN REGIONAL INDUSTRIAL PROPERTY ORGANISATION (ARIPO)

674

(11) (A)

(21) Application Number: AP/P/96/00821

(22) Filing Date: 19951025

(24) Date of Grant & (45) Publication: 19980925

(30) Priority Data

(33) Country:	EP		
(31) Number:	PCT/EP95/04199	94117413.8	
(32) Date:	19951025	19941104	

(84) Designated States:

KE LS MW SD SZ UG

(73) Applicant(s):
SOCIETE DES PRODUITS NESTLE S.A.
Vevey
Switzerland

(72) Inventors:
BEAT DENIS ZURBRIGGEN
Wibergstrasse 14
CH-8180 Beulach
Switzerland

(See Overleaf)

(74) Representative
HONEY & BLANCKENBERG
P O BOX 85
HARARE
ZIMBABWE

(51) International Patent Classification (Int.Cl.6): A23L 1/23; 1/227

(54) Title: Flavouring Agent

(57) Abstract:

Flavouring agent obtained by a process in which seeds of an edible plant are germinated, the sprouts obtained are matured under the effect of their endogenous enzymes, an optional fermentation of these sprouts is envisaged with at least one microorganism, the said enzymes and/or microorganism are inactivated and all or part of the matured and/or fermented sprouts is recovered, and use of this flavouring agent as raw material for the preparation of products of the Maillard reaction, alone or mixed with other materials rich in flavour precursors and/or enhancers.

(56) Documents Cited: US - 4371551 A

Inventors Continued

BENGT BENGTSSON
Stadlerstrasse 28
CH-8472 Seuzach
Switzerland

Flavouring agent

Field of the Invention

The subject of the present invention is a process for the preparation of a flavouring agent as well as a use of this agent.

Background of the Invention

The use of germinated seeds in the human diet, especially the use of germinated cereals such as barley in the manufacture of beer has been known since antiquity.

However, more recent uses of the enzymes produced in the newly formed sprout during the germination of the seed are also known.

EP 0,320,717 (ENIRICERCHE S.p.A), for example, describes a process for the preparation of enzymatic hydrolysates of proteins free of bitterness, using enzymes extracted from germinated sorghum seeds.

AP/P/96/00821

Summary of the Invention

The aim of the present invention is to propose the process for the preparation of a flavouring agent which takes a more direct and more complete advantage of the potential flavour content of the seeds of an edible plant, as well as a precise use of the agent thus prepared.

To this end, in the process for the preparation of a flavouring agent according to the present invention, seeds of an edible plant are germinated, the sprouts obtained are matured under the effect of their endogenous enzymes, the enzymes are inactivated and all or part of the matured sprouts is recovered.

Likewise, within the framework of the present invention, the said flavouring agent is used as raw material for the preparation of products of the Maillard reaction, alone or mixed with other materials rich in flavour precursors and/or enhancers.

Detailed description of the Invention

In order to implement the present process, seeds of a plant containing proteins rich in cysteine or methionine, and/or containing hemicelluloses rich in reducing sugars such as xylose, arabinose or glucose, and/or rich in galacturonic acid, for example, may be used as starting material. Preferably, seeds of a plant chosen from the group formed by leguminous plants, cereals, oleaginous plants and crucifers may be used as starting materials. The use of lucerne, radish, fenugreek, lentil, pea and bean seeds, for example, may be particularly recommended.

In order to germinate the said seeds, they may be previously steeped for 0-30 h at 15-30°C, in pure water or water supplemented with 0-5% sodium chloride as stabilizing agent, for example.

The seeds, optionally previously steeped, may be placed in a suitable chamber containing an atmosphere whose hygroscopicity and temperature can be regulated and comprising means for sprinkling water over the germinating seeds, for example.

The said seeds may be germinated for 1-10 d at 15-30°C while intermittently spraying them with pure water or water supplemented with 0-5% sodium chloride as stabilizing agent, for example.

AP/P/96/00821

Preferably, the said seeds are germinated in a controlled atmosphere until the sprouts have a dry matter content of between 20 and 50%.

The sprouts can be matured, under the effect of their endogenous enzymes, at a temperature of between more than 30°C and 70°C, preferably at 45-65°C, for 12-72 h, for example. The whole sprouts can be matured in an atmosphere with a high relative humidity or with some water, and they may then be reduced to a purée, especially by homogenization, for example. They may also be matured in purée form, after homogenizing them, for example.

The enzymes may then be inactivated at 80-95°C for 2-30 min, for example.

The sprouts may then be dehydrated, especially by drying under reduced pressure, or a soluble extract of the said matured sprouts may be prepared and this extract may be dried, especially by spray-drying, for example.

In a first variant of the present process, a plant material rich in proteins, especially wheat gluten, is mixed with the said sprouts before they are matured.

In a second variant of the present process, exogenous enzymes are also added to the sprouts during their maturation, especially in order to reduce the viscosity of the said purée.

In a third variant of the present process, a fermentation of the seeds, during their germination, and/or of the whole sprouts and/or of the sprouts in subdivided form, especially in purée form, is in addition envisaged, with at least one microorganism of interest as regards its flavouring or acidifying power and/or its ability to degrade the reducing sugars and thereby to enhance the storage life of the present product, especially with at least one microorganism chosen from a group formed by *Lactobacillus plantarum, Lactobacillus sake, Bacillus*

AP/P/96/00821

natto, *Saccharomyces cerevisiae*, *Lactobacillus carnis*, *Staphylococcus xylosus*, *Debaryomyces hansenii*, *Pediococcus pentosaceus*, *Penicillium nalgiovensis* and mixtures thereof, for example.

The flavouring agent prepared by the process according to the present invention may be used as it is, in dehydrated form or otherwise, to flavour dishes, sauces or broths, for example.

Within the framework of the present invention, it is therefore also envisaged to use this flavouring agent as raw material for the preparation of products of the Maillard reaction, alone or mixed with other materials rich in flavour precursors and/or enhancers, such as a soyabean sauce or a yeast autolysate, or even with certain reducing sugars or certain substances rich in sulphur in the sulphide state, such as cystine, cysteine, methionine or thiamine, for example.

In order to carry out such a reaction, a mixture may be prepared having a water content of 35-55% and comprising, in % by weight of dry matter, 24-98% of the present flavouring agent, 2-40% of sodium chloride, 0-4% of added reducing sugar, 0-2% of a sulphur-containing substance, 0-15% of monosodium glutamate and 0-15% of sucrose, for example.

The mixture may be caused to react by heating at 80-150°C, preferably 120-150°C, for 1 min to 4 h, preferably for 1 to 40 min, relatively short durations corresponding to relatively high temperatures and conversely.

The reaction product may then be dried to a residual water content of less than or equal to 2%.

It is possible to perform the reaction and the drying in two separate apparatuses, especially in an autoclave or in a band cooker and in a vacuum dryer, and then to crush or grind, in a hammer mill, the compact mass

obtained, for example. It is also possible to carry out the reaction and the drying by extrusion cooking in a twin-screw extruder, and to carefully cut or grind the expanded pudding obtained, for example.

The examples below illustrate various embodiments of the process for the preparation of the flavouring agent, variants of the process and of the use of the flavouring agent according to the present invention.

In these examples, total nitrogen (Ntot) was determined by the Kjeldahl method. The protein content is defined and calculated as the product Ntot x 6.25. The alpha-amino nitrogen (Nalpha) was determined by the Slyke method. The degree of hydrolysate is determined as the quotient Nalpha/Ntot. The glutamic acid (Glu) content is determined enzymatically. The dry matter (DM) content is determined after drying for 4 h at 70°C, at 20 mbar. The percentages are given by weight, relative to the total weight (%) or relative to the weight of dry matter (% DM).

Example 1

1 kg of daikon radish (*Raphanus sativus*) is steeped in 4 l of water for 16 h at 25°C. They are drained and they are placed in a chamber containing an atmosphere whose hydroscopicity and temperatures can be regulated and comprising means for sprinkling water over the germinating seeds.

In this chamber, the seeds are allowed to germinate for 8 d at 20°C while spraying or sprinkling them with water every two hours.

The sprouts thus obtained are cut into small pieces and they are matured at 40°C for 48 h under the effect of their endogenous enzymes. The enzymes are inactivated by heating the mass of matured sprout pieces at

90°C for 12 min. The mass is dried at 60°C at a reduced pressure of 20 mbar for 8 h and it is reduced to a powder.

A powdered flavouring agent is obtained which can be used as it is, in other words which can be sprinkled on various dishes to enhance their flavour.

Example 2

Lucerne (*Medicago sativa*) seeds are steeped in water for 15 h at 25°C. They are germinated in a suitable chamber similar to that described in Example 1, for 2, 4 and 6 d at 25°C, while spraying them with water every 12 h. The sprouts are homogenized, they are matured at 55°C for 24 or 48 h, they are heated at 90°C for 3 min in order to inactivate the enzymes, and they are dried at 65°C under reduced pressure for 6-7 h.

The dry matter, total nitrogen, alpha-amino nitrogen and glutamic acid content is determined, and the alpha-amino nitrogen/total nitrogen and glutamic acid/protein quotients of the flavouring agent thus obtained are produced.

The results obtained are presented in Table 1 below where the values obtained for sprouts not matured or dried are also indicated for comparison:

Table 1

Germination (d)	Maturation (h)	DM (%)	Ntot (% DM)	Nalpha (% DM)	Glu (% DM)	Nalpha/Ntot (%)	Glu/protein (%)
2	0	20.2	6.43	0.99	0.09	15.39	0.22
2	24	98.5	5.98	1.00	0.31	16.72	0.83
2	48	98.1	6.22	1.19	0.33	19.13	0.85
4	0	8.8	6.59	1.59	0.08	24.13	0.19
4	24	97.8	6.33	1.90	0.36	30.02	0.91
4	48	97.1	6.59	2.04	0.50	30.96	1.21
6	0	7.6	5.92	1.57	0.12	26.52	0.32
6	24	98.1	6.93	2.14	0.43	30.88	0.99
6	48	98.3	6.91	2.23	0.60	32.27	1.39

It can be seen from this table that for germination periods of 2, 4 and 6 d and for maturation periods of 24 and 48 h, the content of glutamic acid, an important flavour enhancer, increases remarkably compared with the contents determined, for comparison, for a zero maturation time. A similar observation is made for the alpha-amino nitrogen content. Most particularly advantageous values of the alpha-amino nitrogen/total nitrogen and glutamic acid/protein quotients are noted from a germination period of 4 d and a maturation period of 24 h.

Example 3

The procedure is carried out in the manner described in Example 2, with daikon radish, fenugreek (*Trigonella phoenum-graceum*), yellow lentils (*Lens esculenta*), green pea (*Pisum sativum*) and mung bean (*Phaseolus radiatus*) seeds, for a germination period of 4 d and for a maturation period of 24 h.

The dry matter, total nitrogen, alpha-amino nitrogen and glutamic acid content is determined and the alpha-amino nitrogen/total nitrogen and glutamic

acid/protein quotients of the flavouring agent thus obtained are produced.

The results obtained are presented in Table 2 below where the values obtained for sprouts not matured or dried are also indicated for comparison:

Table 2

Seed	Germination (d)	Maturation (h)	DM (%)	Ntot (% DM)	Nalpha (% DM)	Glu (% DM)	Nalpha/Ntot (%)	Glu/protein (%)
Daikon radish	4	0	13.4	5.07	0.53	0.16	10.45	0.50
		24	98.1	4.79	0.83	0.54	17.32	1.80
Fenugreek	4	0	8.0	5.00	0.63	0.20	12.60	0.64
		24	97.4	5.44	1.62	0.47	29.78	1.38
Lentils	4	0	15.8	6.20	0.95	0.22	15.32	0.57
		24	96.9	4.95	1.45	0.66	29.29	2.13
Pea	4	0	22.5	4.62	0.57	0.25	12.34	0.86
		24	96.6	4.76	0.95	0.67	19.96	2.25
Mung bean	4	0	13.0	5.15	1.07	0.12	20.78	0.37
		24	97.9	4.59	1.07	0.46	23.31	1.60

It can be seen from this Table 2 that for each of these seeds, for a germination period of 4 d and for a maturation period of 24 h, the glutamic acid content increases remarkably compared with the content determined, for comparison, for a zero maturation time. A similar observation is made for the alpha-amino nitrogen content.

Example 4

The procedure is carried out in the manner described in Example 3 for mung beans. After the 24 h maturation and the inactivation of the enzymes, a portion of sprouts is homogenized with a portion of water. The homogenized mixture is centrifuged and a clear solution is recovered which is concentrated and which is spray-dried.

The flavouring agent thus obtained has a relatively neutral flavour and is particularly suitable for use as raw material for the preparation of products of the Maillard reaction.

Example 5

The procedure is carried out in the manner described in Example 2 for lucerne until sprouts are obtained after germinating for 4 d. Before maturation, a portion of the sprouts is homogenized with an equal portion by weight of wheat gluten dry matter. The mixture is then matured for 48 h at 40°C, it is heated for 3 min at 90°C in order to inactivate the enzymes and it is spray-dried.

The flavouring agent thus obtained comprises a mixture of peptides and amino acids obtained from using sprouts and from wheat gluten. It is particularly suitable for use as raw material for the preparation of products of the Maillard reaction.

Example 6

The process is carried out in the manner described in Example 3 for daikon radish until sprouts are obtained after germinating for 4 d. The sprouts are cut into small pieces and they are supplemented with 2% sodium chloride. They are inoculated with a commercial *Lactobacillus plantarum* culture, they are allowed to ferment and mature for 48 h at 32°C, and they are heated for 10 min at 90°C in order to inactivate the bacteria and the enzymes.

The flavouring agent thus obtained has a particularly strong flavour.

Example 7

Lentil seeds are steeped in water for 12 h at 20°C. They are germinated in a suitable chamber for 4 d at 23°C, while spraying them with water every 6 h. The sprouts are homogenized and they are inoculated with a commercial *Bacillus natto* culture. They are fermented for 24 h at 37°C. The temperature is raised to 55°C, they are allowed to mature for 24 h at this temperature, they are heated at 90°C for 10 min in order to inactivate the bacteria and the enzymes, and they are dried at 60°C at a reduced pressure of 20 mbar for 8 h.

The dry matter, total nitrogen, alpha-amino nitrogen, glutamic acid and reducing sugar (RS) content is determined and the alpha-amino nitrogen/total nitrogen quotient of the flavouring agent thus obtained is produced.

The results obtained are presented in Table 3 below where the corresponding values obtained in Example 3 for lentils are reproduced in order to facilitate comparison:

Table 3

Germination (d)	DM (%)	Ntot (% DM)	Nalpha (% DM)	Nalpha/Ntot (%)	Glu (% DM)	RS (% DM)
4	97.9	4.3	1.27	29.53	0.97	1.06
4 (Ex3)	96.9	4.95	1.45	29.29	0.66	4.79

Thus, if the results obtained in the present example are compared with those obtained in Example 3 for lentils, it can be seen that they are very similar, with the exception of the glutamic acid content and especially of the reducing sugar content. The latter is considerably lower in the flavouring agent obtained in the present example, which ensures a better storage life.

Example 8

Lentil seeds are steeped in water for 12 h at 20°C. They are germinated in a chamber under a controlled atmosphere containing 100% humidity and in the dark for 6 d at 30°C. A portion A of the sprouts is homogenized before allowing them to mature for 24 h at 55°C. A portion B of the sprouts is allowed to mature for 24 h at 55°C and they are then homogenized. Sprouts A and B are then heated at 90°C for 3 min in order to inactivate the enzymes and they are dried at 60°C at a reduced pressure of 20 mbar for 8 h.

The dry matter, total nitrogen, alpha-amino nitrogen and glutamic acid content is determined and the alpha-amino nitrogen/total nitrogen quotient of the flavouring agents thus obtained is produced.

The results obtained are presented in Table 4 below where the values obtained for a maturation time equal to zero have also been indicated for comparison.

Table 4

Maturation (h)	DM (%)	Ntot (% DM)	Nalpha (% DM)	Nalpha/Ntot (%)	Glu (% DM)
0	38.1	3.99	0.21	5.26	0.05
24 (A)	97.5	4.10	0.76	18.53	0.18
24 (B)	99.1	4.30	0.50	11.63	0.14

It can be seen from this Table 4 that the dry matter content of the sprouts obtained by germination in a controlled atmosphere and in the dark reaches remarkably high values. It can also be seen that the degree of hydrolysis of the sprouts homogenized before maturation is considerably greater than the degree of hydrolysis of the sprouts homogenized after maturation.

Example 9

Lentil seeds, germinated, fermented with a *Bacillus natto* culture, matured and inactivated are prepared in the manner described in Example 7.

This flavouring agent is mixed with water, xylose, sodium chloride, cysteine, monosodium glutamate and sucrose in proportions such that the mixture obtained has a water content of 49% and comprises, in % by weight of dry matter, 40% dry matter of the said flavouring agent including 0.44% reducing sugars, 1.5% xylose, 34% sodium chloride, 1.5% cysteine, 11.5% monosodium glutamate and 11.5% sucrose.

The mixture is caused to react by heating a jacketed tank at 100°C for 3 h. It is dried at a reduced pressure of 15 mbar at 95°C to a dry matter content of 1.5%. It is crushed and it is reduced to a powder.

In order to taste this product of the Maillard reaction, 5 g of it, supplemented with 5 g of sodium chloride, are dissolved in 1 l of boiling water. The water thus flavoured has a pleasant taste, free of any bitterness, similar to that of a meat stock, and it does not have the characteristic and penetrating odour of traditional natto.

Example 10

Powdered flavouring agent obtained in Example 4 is mixed with water, xylose, sodium chloride, cysteine, monosodium glutamate and sucrose in proportions such that the mixture obtained has a water content of 40% and comprises, in % by weight of dry matter, 40% dry matter of the said flavouring agent including 2.4% reducing sugars,

1.5% xylose, 34% sodium chloride, 1.5% cysteine, 11.5% monosodium glutamate and 11.5% sucrose.

The mixture is caused to react by heating in a jacketed tank at 120°C for 40 min. It is dried, it is crushed and it is reduced to a powder.

A product of the Maillard reaction is obtained which, when tasted under the same conditions as that of Example 9, has a pleasant taste, lacking any bitterness, similar to that of a meat stock.

Example 11

Powdered flavouring agent obtained in Example 5 is mixed with water, xylose, sodium chloride, cysteine, monosodium glutamate and sucrose in proportions such that the mixture obtained has a water content of 43% and comprises, in % by weight of dry matter, 40% dry matter of the said flavouring agent including 1.8% reducing sugars, 1.5% xylose, 34% sodium chloride, 1.5% cysteine, 11.5% monosodium glutamate and 11.5% sucrose.

The mixture is caused to react by heating in a jacketed tank at 120°C for 40 min. It is dried, it is crushed and it is reduced to a powder.

A product of the Maillard reaction is obtained which, when tasted under the same conditions as that of Example 9, has a pleasant taste, lacking any bitterness, similar to that of a meat stock.

Claims

1. A process for the preparation of a flavouring agent, in which seeds of an edible plant are germinated, the sprouts obtained are matured under the effect of their endogenous enzymes, the said enzymes are inactivated and all or part of the matured sprouts is recovered, wherein said seeds are germinated for 1-10 d at 15-30° C while spraying them intermittently and the said sprouts are matured for 12-72 h at a temperature of between more than 30° C and 70° C, preferably at 45-65° C, and the said enzymes are inactivated for 2-30 min at 80-95° C.

2. A process according to Claim 1, in which seeds of a plant chosen from the group formed by leguminous plants, cereals, oleaginous plants and crucifers are germinated.

3. A process according to claim 1, in which the said seeds are germinated in a controlled atmosphere until the sprouts have a dry matter content of between 20 and 50%.

4. A process according to Claim 1, in which the matured sprouts are dehydrated by drying under reduced pressure.

5. A process according to Claim 1, in which a soluble extract of the matured sprouts is prepared and this extract is spray-dried.

6. A process according to Claim 1, in which a plant material rich in proteins, especially wheat gluten, is mixed with the sprouts before they are matured.

7. A process according to Claim 1, in which exogenous enzymes are also added to the sprouts during their maturation.

8. A process according to Claim 1, in which fermentation of the seeds, during their germination, and/or of the whole sprouts and/or of the sprouts in subdivided form, is in addition envisaged with at least one microorganism having a flavouring or acidifying power and/or an ability to degrade the reducing sugars.

9. A process according to Claim 8, in which the said microoroganism is chosen from the group formed by Lactobacillus plantarum, Lactobacillus sake, Bacillus natto, Saccharomyces cerevisiae, Lactobacillus carnis, Staphylococcus xylosus, Debaryomyces hansenii, Pediococcus pentosaceus, Penicillium nalgiovensis and mixtures thereof.

10. A flavouring agent obtained by the process according to one of Claims 1-9.

11. A use of the flavouring agent according to one of Claims 1-9, as raw material for the preparation of products of the Maillard reaction, alone or mixed with other materials rich in flavour precursors and/or enhancers.

12. A use according to Claim 11, in which a mixture having a water content of 35-55% and comprising, in % by weight of dry matter, 24-98% of the said flavouring agent, 2-40% of sodium chloride, 0-4% of added reducing sugar, 0-2% of a sulphur-containing substance, 0-15% of monosodium glutamate and 0-15% of sucrose, is prepared.

13. A use according to Claim 12, in which the said mixture is caused to react by heating at 80-150° C, preferably 120-150° C, for 1 min to 4 h, preferably for 1 to 40 min.

AP/P/96/00821