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(73) Octrooihouder(s):
Protix Biosystems B.V. te 's-Hertogenbosch.

(72) Uitvinder(s):
Tarique Arsiwalla te 's-Hertogenbosch.
Kees Wilhelmus Petrus Aarts te 's-Hertogenbosch.

(74) Gemachtigde:
ir. C.M. Jansen c.s. te Den Haag.

(54) **Method to convert insects or worms into nutrient streams and compositions obtained thereby.**

(57) The present invention provides a method to convert insects or worms into nutrient streams, such as a fat-containing, an aqueous proteinaceous-containing and a solid-containing fraction. The method comprises the steps of (a) squashing insects or worms thereby obtaining a pulp, (b) subjecting the pulp to enzymatic hydrolysis obtaining thereby a hydrolysed mixture, (c) heating the hydrolysed mixture to a temperature of 70-100°C, and (d) subjecting the mixture to a physical separation step, preferably decanting and/or centrifuging. The fat-containing fraction comprises at least 80 wt.% insect or worm fat of which at least 30 wt.% are saturated fats. The aqueous proteinaceous fraction can be dried to obtain dried proteinaceous material, which contains at least 45 wt. % insect or worm protein-derived matter and at most 25 wt.% insect or worm fat based on dry weight, and has a pepsin digestibility of at least 50%. The resulting nutrient streams can be used in food, feed and pharmaceutical industry.

NL C 2009044

P97396NL00

Title: Method to convert insects or worms into nutrient streams and compositions obtained thereby

The invention relates to the field of obtaining nutrients, feed and foodstuffs from insects or worms. In particular, the invention presents a method to convert insects or worms into nutrient streams, encompassing a fat-containing fraction, an aqueous proteinaceous-containing fraction and/or a solid-containing fraction.

In the past decades, there has been a growing interest to use insects and worms as a food source, especially in view of the growth of global population and malnutrition in the developing world. Since insects and worms are rich in proteins and sometimes fats, they represent a relatively high caloric value. Although in some populations it is common to consume insects and worms, *e.g.* in Africa, Asia, Australia, these are usually eaten as such, be it as a whole or in parts, or used in the preparation of dishes.

However, it is desirable to be able to process insects and worms on an industrial scale to produce nutrients, which subsequently may be used in the preparation of food or feed products.

From several publications, it is known to obtain some particular nutrients from insects, such as proteins or fats.

JP2009254348 A concerns obtaining proteins from bee larvae. Dried larvae are suspended in water, whereto a lypolytic enzyme is added to decompose the lipids. After that, a proteolytic enzyme is added to hydrolyse proteins and the resulting mixture is filtered and the protein is collected. RU 2345139 C2 describes the recovery of chitin from cultivated larvae. WO 2008/091137 concerns an ethanol extract from house fly larvae, which is obtained by drying the larvae, dissolving these in an organic solvent to remove fats and mixing the residue with ethanol to obtain the extract. WO 2011/006276 describes obtaining fatty acids from insect larvae, wherein the fatty acids are extracted using organic solvent.

It is however not known to fully utilise insects or worms and to convert these into several nutrient streams, such as protein-derived matter, fats and chitin, from which streams the nutrients can optimally and easily be recovered.

An object of the present invention is therefore to provide a method that converts insects or worms into nutrient streams, and preferably into three nutrient streams, being a fat-containing stream, an aqueous stream containing protein-derived matter and yet another stream containing solids such as chitin.

Another object of the invention is to provide a processing method for insects or worms that results in nutrients that are not contaminated with toxic substances and are safe to be used in preparation of various food or feed products and pharmaceuticals.

Yet another object of the invention is to provide a method that is simple, does not require costly equipment or reagents and can easily be scaled up in a large production facility.

Accordingly, the invention provides, in a first aspect, a method to convert insects or worms into nutrient streams, comprising the steps of:

(a) squashing insects or worms thereby obtaining a pulp,

(b) subjecting the pulp to enzymatic hydrolysis obtaining thereby a hydrolysed mixture,

(c) heating the hydrolysed mixture to a temperature of 70-100°C, and

(d) subjecting the mixture to a physical separation step thereby obtaining a fat fraction, an aqueous proteinaceous fraction and a solid-containing fraction.

In another aspect, the present invention provides a fat-containing composition, comprising at least 80 wt.% insect or worm fat based on dry weight, of which at least 30 wt.% are saturated fats, the fat comprising at least

7 wt.% lauric acid C12:0, 10-30 wt.% palmitic acid C16:0, and 15-40 wt.% oleic acid C18:1.

In yet another aspect, the invention provides a composition comprising at least 45 wt.% insect or worm protein-derived matter and at most 25 wt.% insect or worm fat based on dry weight, which insect or worm protein-derived matter has a pepsin digestibility of at least 50%, as measured by the pepsin-HCl method, elaborated herein-below.

The method according to the invention converts insects or worms into nutrient streams. The term "insects" refers to insects in any development stage, such as adult insects, insect larvae and insect pupae. Preferably, insect larvae or worms are used. A large variety of insects and worms can be used. Preferably, edible insects or edible worms are used. More preferably, the insects are flies, bugs, mosquitos, butterflies, moths, cicadas, termites, bees, ants, wasps, beetles, grasshoppers, or crickets. More preferably, the insects belong to the species: black soldier fly (Hermetia illucens), house fly (Musca domestica), morio worm (Zophobas Morio), mealworm (Tenebrio Molitor) or cricket (Gryllida). In a preferred embodiment, the insects belong to the species black soldier fly. The insects and worms are preferably cultivated, *e.g.* in an insect farm. The cultivation allows to control and reduces the risks associated with diseases of insects and with the toxicity of insect-derived foodstuffs, *e.g.* due to the presence insecticides, in contrast to insects harvested in the nature. The conversion of the insects or worms into nutrient streams can suitably be carried out in a reactor vessel.

In step (a) the insects or worms are squashed to obtain a pulp. Preferably, the insects or worms are thereby reduced in size. This results in a homogeneous starting material of viscous consistency. The squashing and reducing in size can conveniently be done in a micro-cutter mill, although other suitable techniques can also be used. During this step, the particle size of the insect or worm remains in the pulp is preferably less than 1 mm (the largest size to be determined using a microscope), more preferably less than

0.5 mm. The particle size can be controlled by selection of a specific knife and plate combination and rotating speed; for example one can use a single or double knife and rotating speed could vary between 1000 and 3000 rpm. A skilled person can find suitable conditions in order to reach a desired particle size. A small particle size is advantageous as it facilitates the enzymatic hydrolysis in the next step.

In step (b) the pulp is subjected to enzymatic hydrolysis. The hydrolysis is preferably carried out at a temperature of 35-65°C. Depending on the enzyme used, it may be necessary to adjust the pH of the pulp. Preferably, the enzymatic hydrolysis is carried out using a protease. The protease can be an acidic protease, a neutral protease or an alkaline protease. If an acidic protease is used, the pH of the pulp may be adjusted to acidic values, preferably to a pH of 3-6. This can be done with any suitable acid and, preferably, citric acid is used. A preferred acidic protease is for example pepsin. If a neutral protease is used, the pH is preferably 6-8. The enzymatic hydrolysis can also be done with an alkaline protease such as papain.

The enzymatic hydrolysis is preferably carried out by continuously stirring in the reactor vessel. It is desired to carry out a complete hydrolysis of the protein present in the insects or worms. Typically, the hydrolysis takes 1-6 hours, preferably 3-5 hours.

At the end of the hydrolysis step, the hydrolysed mixture is heated to a temperature in the range from 60 to 100°C, preferably in the range 80-95°C. This heating step can advantageously be used to stop the enzymatic reaction. The heating also assures that the majority of fats are liquefied in order to prepare the hydrolysed mixture for the following separation step.

In step (d), the hydrolysed mixture is subjected to a physical separation step to obtain nutrient streams. Preferably, the nutrient streams are a fat-containing fraction, an aqueous fraction containing proteinaceous matter and a solid-containing fraction. The physical separation preferably encompasses decanting, centrifuging, or a combination of the two methods.

In a preferred embodiment, first, a fat fraction is separated by decanting, and the remaining mixture is further separated into an aqueous proteinaceous-containing fraction and a solid-containing fraction. However, the fat, proteinaceous and solid-containing fractions can also be obtained in a different order, or simultaneously, *e.g.* by using a 3-phase decanter. In a preferred embodiment, the physical separation is carried out by using a 3-phase decanter.

The method according to the invention results in a fat fraction, an aqueous proteinaceous fraction and a solid-containing fraction. In this way, the method results in several nutrient streams. Under nutrients streams in the present description streams are understood that contain nutrients, such as fats, proteinaceous material, carbohydrates, minerals and/or chitin. For the purposes of the invention, chitin is also considered a nutrient.

The fat-containing fraction predominantly contains insect or worm fat. Under "predominantly containing", e.g. fat, it is understood that based on the dry weight, the stream contains more fat (on a weight basis) than any other component, or in other words, that fat constitutes the major part of all ingredients based on dry weight. Generally, "predominantly containing" means a content of at least 40 wt.% dry matter, more preferably at least 50 wt.% dry matter. The aqueous proteinaceous fraction predominantly contains protein

The fat-containing fraction obtainable by the method according to the invention, preferably comprises at least 80 wt.%, more preferably 85-95 wt.%, of insect or worm fat based on the dry weight of the fat fraction. The insect or worm fat in the fat fraction comprises at least 30 wt.% and preferably 40-70 wt.% saturated fats, based on the total weight of the fat. The amount of unsaturated fats is 70 wt.% or less, preferably less than 60 wt.% and more preferably 25-55 wt.%, based on the total weight of the fat. The amount of mono unsaturated fatty acids (cis) is preferably from 20 to 45 wt.%, while the amount poly unsaturated fatty acids is preferably from 5 to 20 wt.%. In a preferred embodiment, the insect or worm fat contains at least 7 wt.%,

preferably 8-40 wt.%, of lauric acid C12:0. More preferably, the fat contains 20-40 wt.% of lauric acid. The insect or worm fat preferably contains 10-30 wt.% palmitic acid C16:0. Further, the insect or worm fat may further comprise omega-9 fatty acids, preferably in an amount 10-45 wt.%. Under omega-9 fatty acids, the sum of the following acids is understood: oleic acid C18:1, eicosenoic acid C20:1, mead acid C20:3, erucic acid C22:1, nervonic acid C24:1. In particular, the insect or worm fat preferably contains 15-40 wt.% oleic acid C18:1, more preferably, 15-35 wt.%. Omega-6 fatty acids are preferably present in an amount 2-20 wt.%. Under omega-6 fatty acids, the sum of the following acids is understood: linoleic acid C18:2, gamma-linolenic acid C18:3, eicosadienoic acid C20:2, dihomo-gamma-linolenic acid C20:3, arachidonic acid C20:4, docosadioenoic acid C22:2, adrenic acid C22:4, docosapentaenoic acid C22:5, tetracosatetraenoic acid C24:4, tetracosapentaenoic acid C24:5. For example, linoleic acid C18:2 is preferably present in an amount 5-15 wt.%. The amount of trans fatty acids is lower than 0.5 wt.%, preferably lower than 0.2 wt.%. Under trans fatty acids unsaturated fatty acids are meant with at least one carbon-carbon double bond with a trans configuration, e.g. elaidic acid C18:1. The amounts of fatty acids are based on the weight of the insect or worm fat, which is the fat component of the fat-containing fraction. The fatty acid composition is determined by a standard method NEN-EN-ISO 5508+5509, BF3.

Another fraction obtained in the separation step is an aqueous proteinaceous-containing fraction. Proteinaceous is understood to encompass protein, hydrolysed protein, peptides, amino acids and/or other protein-derived compounds obtainable by enzymatic hydrolysis of proteins. The aqueous proteinaceous fraction can further be dried to obtain dried proteinaceous material. This dried material can itself be used as a food or feed ingredient, or it can further be processed, *e.g.* to isolate amino acids. The aqueous fraction is preferably dried by spray-drying.

The dried proteinaceous material contains at least 45 wt.%, preferably at least 50 wt.%, more preferably 55-85 wt.%, insect or worm protein-derived matter and at most 25 wt.%, preferably 3-20 wt.%, insect or worm fat, based on dry weight. The material may further comprise residual moisture, minerals and/or carbohydrates. Under "insect or worm protein" and "insect or worm fat" respectively protein and fat derived from insects or worms are meant. The term "protein-derived matter" is considered synonymous with proteinaceous material. The insect or worm protein-derived matter may thus comprise proteins, hydrolysed proteins, peptides, amino acids. The insect or worm protein-derived matter in the composition above has preferably a pepsin digestibility of at least 50% as determined by a standard "pepsin-HCl" laboratory test such as following the guideline in the Third Commission Directive 72/199/EEC of 27 April 1972. In a preferred embodiment, the proteinaceous material contains at least 50 wt.% insect or worm protein-derived matter and has a protein digestibility of at least 85%, preferably 90-95%. Preferably, the proteinaceous material further contains amino acids, in particular one or more of arginine, lysine, isoleucine, methionine, tryptophan. In a preferred embodiment, the proteinaceous material contains 3-7 wt.% lysine, based on dry weight of the proteinaceous material. In another preferred embodiment, the proteinaceous material further contains minerals such as calcium or phosphorus. Preferably, the calcium content of the proteinaceous material is at least 4,500, more preferably 10,000-30,000 mg/kg, based on dry weight of the proteinaceous material. The phosphorus content of the proteinaceous material is preferably at least 5000 mg/kg, based on dry weight. The calcium and phosphorus content is determined by the OCP-OES method.

The fat fraction of the proteinaceous material preferably contains at least 40 wt.%, more preferably 45-70 wt.% saturated fats and at most 60 wt.%, preferably 25-55 wt.% unsaturated fats based on the weight of the fat fraction. Mono unsaturated fatty acids (cis) are preferably present in an amount 20-45 wt.%, and poly unsaturated fatty acids preferably in an amount 5-20 wt.%.

The fat fraction preferably comprises at least 7 wt.%, more preferably 20-45 wt.% of lauric acid C12:0. The fat fraction preferably also comprises 10-30 wt.% palmitic acid C16:0. Further, the fat fraction of the proteinaceous material preferably comprises one or more omega-9 fatty acids in an amount of 10-40 wt.%. In a preferred embodiment, it comprises 15-35 wt.% oleic acid C18:1. The fat fraction also preferably comprises one or more omega-6 fatty acids, in an amount of 5-15 wt.%. The amount of trans fatty acids is preferably lower than 0.5 wt.%. The amounts of fatty acids are based on the weight of the fat fraction of the proteinaceous material. The fat fraction of the proteinaceous material can be isolated for further use.

The remaining solid-containing fraction obtained in the separation step (d), which step encompasses for example decanting or centrifuging, represents a wet pulp, or a suspension. This wet pulp can easily be distinguished from the aqueous proteinaceous fraction. The wet pulp contains solids such as chitin and chitin-derivatives. The wet pulp further preferably comprises proteinaceous matter. This proteinaceous matter has the composition as described herein-above for the aqueous proteinaceous fraction, and has a pepsin digestibility of the protein-derived matter in the range 50-80%, as can be determined by a standard "pepsin-HCl" laboratory test; and particularly by following the guideline in the Third Commission Directive 72/199/EEC of 27 April 1972.

The solid-containing fraction can further be dried to obtain solid material. Preferably, air drying is used. The solid-containing fraction can also be further processed to isolate chitin or proteinaceous material. Chitin is a polysaccharide that can be used in various applications. In food industry, chitin can be used as an additive to thicken and stabilise foods and pharmaceuticals. It can also be used in animal feed as a nutrient source.

The isolated nutrient streams can further be used in the preparation of food or feed, or of food or feed additives, or in pharmaceutical industry. For example, the proteinaceous material and the fat fraction can, respectively, be

used in animal feed as a crude protein and a crude fat source. The obtained streams can also be processed further, *e.g.* to isolate specific ingredients such as hydrolysed protein, amino acids, or specific fatty acids.

The invention is now illustrated in the following, non-limiting example.

Example

1000 kg fresh larvae of black soldier fly are squashed to obtain insect pulp with an average particle size less than 0.5 mm. The pulp is introduced in a reaction vessel and heated to a temperature of 50°C, whereto 1.1 l of proteolytic enzyme pepsin is added. The pH is lowered to 4 by adding citric acid. The mixture is maintained at 50°C for about 4 hours to allow the hydrolysis reaction. Subsequently, the reaction mixture is heated to 90°C for 1 hour and then brought into a 3-phase decanter. From the decanter three fractions are obtained, being a fat fraction, a "larvae water" and a solid fraction. The "larvae water" contains insect protein derivatives and is spray-dried to obtain insect proteinaceous material.

The composition of the fat fraction (before further separation steps) is given in Table 1. The fatty acids composition of the crude fat is given in Table 2, wherein the percentage is based on the weight of the crude fat. The fatty acids composition was determined by NEN-EN-ISO 5508+5509, BF3 method. The fatty acids are referred to as Cn:m, wherein n is the amount of carbon atoms, and n is the amount of unsaturated carbon-carbon bonds.

Table 1

Component	Content, %
Moisture	7.3
Crude protein (Dumas, N x 6.25)	0.5
Crude fat (petroleum-ether extraction)	79.3
Crude fiber (long method)	4.3
Crude ashes (550°C)	8.6
Energy value	2943 kJ/100 g

Table 2

Fatty acid	Content (wt.%)
C10:0	1.3
C12:0	38.2
C14:0	6.9
C14:1	0.3
C16:0	16.5
C16:1	3.3
C17:0	0.1
C18:0	2.8
C18:1	19.2
C18:1 cis	0.3
C18:2	8.0
C18:3n3	1.0
C20:5	0.2
trans fatty acids	0.1
saturated fatty acids	66.3
mono unsaturated fatty acids	23.2
poly unsaturated fatty acids	9.4
unsaturated fatty acids	32.5

omega-3 fatty acids	1.3
omega-6 fatty acids	8.0
omega-9 fatty acids	19.3

The composition of the spray-dried proteinaceous material is given in Table 3. The fat composition of the crude fat fraction of the proteinaceous material is given in Table 4, wherein the percentages refer to percentages by weight based on the total weight of the crude fat fraction. The amino acid composition is given in Table 5, wherein the percentages refer to percentages by weight based on the total weight of the dried proteinaceous material.

Table 3

Component	Content
Moisture (dry matter at 103°C), %	4.4
Crude protein (Dumas, N x 6.25)	66.2
Crude fat (after pre-extraction and hydrolysis)	6.4
Crude ashes (550°C)	7.0
Crude fiber (long method)	1.5
Energy value, kJ/100 g	1395
Phosphorus, mg/kg	5200
Calcium, mg/kg	4800

Table 4

Fatty acid	Content (wt.%)
C8:0	0.1
C10:0	0.9
C12:0	32.8
C14:0	6.8
C14:1	0.2

C15:0	0.2
C16:0	18.1
C16:1	3.4
C17:0	0.1
C18:0	3.5
C18:1	21.5
C18:1 cis	0.4
C18:2	8.9
C18:3n3	1.1
C20:1	0.1
C20:3n3	0.2
C20:5	0.2
trans fatty acids	0.1
saturated fatty acids	62.8
mono unsaturated fatty acids	25.7
poly unsaturated fatty acids	10.4
unsaturated fatty acids	36.1
omega-3 fatty acids	1.4
omega-6 fatty acids	8.9
omega-9 fatty acids	21.7

Table 5

Amino acid	Content (wt.%)
Alanine	5.11
Arginine	3.29
Asparagic acid	6.67
Cystine	0.43
Glutamic acid	9.08
Glycine	3.43

Histidine	2.27
Isoleucine	3.03
Leucine	4.60
Lysine	4.33
Methionine	1.09
Phenyl-alanine	2.64
Proline	4.48
Serine	2.83
Threonine	2.99
Tryptophan	0.87
Tyrosine	3.87
Valine	4.38

The composition of the air-dried solid fraction is given in Table 6. The fat composition of the crude fat fraction is given in Table 7, wherein the percentages refer to percentages by weight based on the total weight of the crude fat fraction. The amino acid composition is given in Table 8, wherein the percentages refer to percentages by weight based on the total weight of the dried solid fraction. Chitin and chitin-derivatives are comprised in the crude fiber in Table 6.

Table 6

Component	Content
Moisture (dry matter at 103°C), %	10.9
Crude protein (Dumas, N x 6.25)	53.2
Crude fat (after pre-extraction and hydrolysis)	11.4
Crude ashes (550°C)	5.8
Crude fiber (long method)	20.5
Energy value, kJ/100 g	1331

Phosphorus, mg/kg	5100
Calcium, mg/kg	11000

Table 7

Fatty acid	Content (wt.%)
C8:0	0.1
C10:0	1.3
C12:0	33.3
C14:0	6.0
C14:1	0.3
C15:0	0.2
C16:0	16.4
C16:1	3.5
C17:0	0.1
C18:0	3.2
C18:1	21.4
C18:1 cis	0.4
C18:2	9.1
C18:3n3	1.1
C20:1	0.1
C20:3n3	0.2
C20:5	0.2
trans fatty acids	0.2
saturated fatty acids	61.2
mono unsaturated fatty acids	25.7
poly unsaturated fatty acids	10.6
unsaturated fatty acids	36.3
omega-3 fatty acids	1.5
omega-6 fatty acids	9.1

omega-9 fatty acids	21.5

Table 8

Amino acid	Content (wt.%)
Alanine	3.71
Arginine	2.33
Asparagic acid	4.38
Cystine	0.34
Glutamic acid	5.01
Glycine	3.01
Histidine	1.53
Isoleucine	2.49
Leucine	4.02
Lysine	3.01
Methionine	0.91
Phenyl-alanine	2.08
Proline	3.15
Serine	2.24
Threonine	2.09
Tryptophan	0.78
Tyrosine	3.12
Valine	3.29

Conclusies

1. Werkwijze om insecten of wormen in voedingsstromen om te zetten, omvattende de stappen van:

(a) pletten van insecten of wormen waarbij een pulp wordt verkregen,

(b) onderwerpen van de pulp aan enzymatische hydrolyse waarbij een gehydrolyseerd mengsel wordt verkregen,

(c) verwarmen van het gehydrolyseerde mengsel tot een temperatuur van 70-100°C, en

(d) onderwerpen van het mengsel aan een fysische scheidingsstap waarbij een vetfractie, een waterige eiwitachtige fractie en een vaste stof omvattende fractie worden verkregen.

2. De werkwijze volgens conclusie 1, waarbij de insecten volwassen insecten, larven of poppen zijn.

3. De werkwijze volgens conclusie 1 of 2, waarbij de insecten tot de soort huisvlieg, zwarte soldaat vlieg, morio worm, meelworm of krekel behoren.

4. De werkwijze volgens één van de voorgaande conclusies, waarbij de pulp wordt gehydrolyseerd met gebruik van een protease bij een temperatuur 35-65°C.

5. De werkwijze volgens conclusie 4, waarbij de protease een zuur protease is en de pulp wordt aangezuurd tot een pH 3-6.

6. De werkwijze volgens conclusie 4, waarbij de protease bij een pH 6-8 wordt gebruikt.

7. De werkwijze volgens één van de voorgaande conclusies, waarbij de fysische scheiding decanteren en/of centrifugeren omvat.

8. De werkwijze volgens één van de voorgaande conclusies, waarbij de waterige eiwitachtige fractie en/of de vaste stof omvattende fractie verder worden gedroogd.

9. De werkwijze volgens conclusie 8, waarbij de waterige eiwitachtige fractie wordt gesproeidroogd.

10. De werkwijze volgens één van de voorgaande conclusies, waarbij de deeltjesgrootte van insect- of wormresten in de pulp kleiner dan 1 mm is.

11. Een vet omvattende samenstelling omvattende ten minste 80 gew.% insecten- of wormenvet gebaseerd op droog gewicht, waarvan ten minste 30 gew.% verzadigde vetten zijn, waarbij het vet ten minste 7 gew.% laurierzuur C12:0, 10-30 gew.% palmitinezuur C16:0, en 15-40 gew.% oliezuur C18:1 omvat.

12. De samenstelling volgens conclusie 11, omvattende 85-95 gew.% insecten- of wormenvet gebaseerd op droog gewicht.

13. De samenstelling volgens conclusie 11 of 12, waarbij het insecten- of wormenvet 40-70 gew.% verzadigde vetten omvat.

14. De samenstelling volgens één van conclusies 11 tot en met 13, waarbij het vet 8-40 gew.% laurierzuur C12:0 omvat.

15. De samenstelling volgens één van conclusies 11 tot en met 14, waarbij het vet 5-15 gew.% linolzuur C18:2 omvat.

16. Een samenstelling omvattende ten minste 45 gew.% van insecten- of wormeneiwit afgeleid materiaal en ten hoogste 25 gew.% insecten- of wormenvet gebaseerd op droog gewicht, welk van insecten- of wormeneiwit afgeleid materiaal een pepsineverteerbaarheid van ten minste 50% heeft, gemeten met de pepsine-HCl methode.

17. De samenstelling volgens conclusie 16, omvattende ten minste 50 gew.% van insecten- of wormeneiwit afgeleid materiaal welk een pepsineverteerbaarheid van ten minste 85%, bij voorkeur 90-95% heeft, gemeten met de pepsine-HCl methode.

18. De samenstelling volgens conclusie 16, waarbij de samenstelling voorts chitine omvat en waarbij het van eiwit afgeleide materiaal een pepsineverteerbaarheid van 50-80% heeft, gemeten met de pepsine-HCl methode.

19. De samenstelling volgens één van conclusies 16-18, waarbij het insecten- of wormenvet ten minste 40 gew.%, bij voorkeur 45-70 gew.% verzadigde vetten omvat gebaseerd op het gewicht van de vetfractie.

20. De samenstelling volgens één van conclusies 16-19, voorts omvattende ten minste 4.500 mg/kg Ca gebaseerd op droog gewicht.

21. De samenstelling volgens één van conclusies 16-20, omvattende 55-85 gew.% van insecten- of wormeneiwit afgeleid materiaal.

22. De samenstelling volgens een van conclusies 16-21, voorts omvattende één of meer van aminozuren gekozen uit de groep bestaande uit arginine, lysine, isoleucine, methionine, en tryptofaan.

23. De samenstelling volgens conclusie 22, omvattende 3-7 gew.% lysine gebaseerd op het droge gewicht van de samenstelling.

SAMENWERKINGSVERDRAG (PCT)

RAPPORT BETREFFENDE NIEUWHEIDSONDERZOEK VAN INTERNATIONAAL TYPE

IDENTIFICATIE VAN DE NATIONALE AANVRAGE	KENMERK VAN DE AANVRAGER OF VAN DE GEMACHTIGDE
	P97396NL00
Nederlands aanvraag nr. **2009044**	Indieningsdatum **21-06-2012**
	Ingeroepen voorrangsdatum
Aanvrager (Naam) **Protix Biosystems B.V.**	
Datum van het verzoek voor een onderzoek van internationaal type **04-08-2012**	Door de Instantie voor Internationaal Onderzoek aan het verzoek voor een onderzoek van internationaal type toegekend nr. **SN 58637**

I. CLASSIFICATIE VAN HET ONDERWERP (bij toepassing van verschillende classificaties, alle classificatiesymbolen opgeven)

Volgens de internationale classificatie (IPC)

A23L1/48 **A23J3/00** **A23L1/29** **A61K35/64**

C11B1/02 **C11B1/10** **A23J3/34**

II. ONDERZOCHTE GEBIEDEN VAN DE TECHNIEK

Onderzochte minimumdocumentatie

Classificatiesysteem	Classificatiesymbolen
IPC8	**A23L A23J A61K C11B**

Onderzochte andere documentatie dan de minimum documentatie, voor zover dergelijke documenten in de onderzochte gebieden zijn opgenomen

III.	☐ **GEEN ONDERZOEK MOGELIJK VOOR BEPAALDE CONCLUSIES**	(opmerkingen op aanvullingsblad)
IV.	☐ **GEBREK AAN EENHEID**	(opmerkingen op aanvullingsblad)

ONDERZOEKSRAPPORT BETREFFENDE HET RESULTAAT VAN HET ONDERZOEK NAAR DE STAND VAN DE TECHNIEK VAN HET INTERNATIONALE TYPE

Nummer van het verzoek om een onderzoek naar de stand van de techniek
NL 2009044

A. CLASSIFICATIE VAN HET ONDERWERP
INV. A23L1/48 A23J3/00 A23L1/29 A61K35/64 C11B1/02
C11B1/10 A23J3/34
ADD.
Volgens de Internationale Classificatie van octrooien (IPC) of zowel volgens de nationale classificatie als volgens de IPC.

B. ONDERZOCHTE GEBIEDEN VAN DE TECHNIEK

Onderzochte miminum documentatie (classificatie gevolgd door classificatiesymbolen)
A23L A23J A61K C11B

Onderzochte andere documentatie dan de mimimum documentatie, voor dergelijke documenten, voor zover dergelijke documenten in de onderzochte gebieden zijn opgenomen

Tijdens het onderzoek geraadpleegde elektronische gegevensbestanden (naam van de gegevensbestanden en, waar uitvoerbaar, gebruikte trefwoorden)
EPO-Internal, WPI Data

C. VAN BELANG GEACHTE DOCUMENTEN

Categorie °	Geciteerde documenten, eventueel met aanduiding van speciaal van belang zijnde passages	Van belang voor conclusie nr.
X	CN 1 297 691 A (INST OF ZOOLOGY ACADEMIA SINIC [CN] INST ZOOLOGY CAS [CN]) 6 juni 2001 (2001-06-06) * samenvatting * * conclusie 1 * -----	1-23
X,D	JP 2009 254348 A (SHINGY KK) 5 november 2009 (2009-11-05) in de aanvraag genoemd * samenvatting * -----	1-23
A	CN 102 422 974 A (INST OF AGRO FOOD SCIENCE AND TECHNOLOGY SAAS) 25 april 2012 (2012-04-25) * samenvatting * -----	1-23
A	CN 1 415 757 A (UNIV JIANGNAN [CN]) 7 mei 2003 (2003-05-07) * samenvatting * -----	1-23

☐ Verdere documenten worden vermeld in het vervolg van vak C.

☒ Leden van dezelfde octrooifamilie zijn vermeld in een bijlage

° Speciale categorieën van aangehaalde documenten

"A" niet tot de categorie X of Y behorende literatuur die de stand van de techniek beschrijft

"D" in de octrooiaanvrage vermeld

"E" eerdere octrooi(aanvrage), gepubliceerd op of na de indieningsdatum, waarin dezelfde uitvinding wordt beschreven

"L" om andere redenen vermelde literatuur

"O" niet-schriftelijke stand van de techniek

"P" tussen de voorrangsdatum en de indieningsdatum gepubliceerde literatuur

"T" na de indieningsdatum of de voorrangsdatum gepubliceerde literatuur die niet bezwarend is voor de octrooiaanvrage, maar wordt vermeld ter verheldering van de theorie of het principe dat ten grondslag ligt aan de uitvinding

"X" de conclusie wordt als niet nieuw of niet inventief beschouwd ten opzichte van deze literatuur

"Y" de conclusie wordt als niet inventief beschouwd ten opzichte van de combinatie van deze literatuur met andere geciteerde literatuur van dezelfde categorie, waarbij de combinatie voor de vakman voor de hand liggend wordt geacht

"&" lid van dezelfde octrooifamilie of overeenkomstige octrooipublicatie

Datum waarop het onderzoek naar de stand van de techniek van internationaal type werd voltooid	Verzenddatum van het rapport van het onderzoek naar de stand van de techniek van internationaal type
21 maart 2013	

Naam en adres van de instantie	De bevoegde ambtenaar
European Patent Office, P.B. 5818 Patentlaan 2 NL - 2280 HV Rijswijk Tel. (+31-70) 340-2040, Fax: (+31-70) 340-3016	Picout, David

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ONDERZOEKSRAPPORT BETREFFENDE HET RESULTAAT VAN HET ONDERZOEK NAAR DE STAND VAN DE TECHNIEK VAN HET INTERNATIONALE TYPE

Informatie over leden van dezelfde octrooifamilie

Nummer van het verzoek om een onderzoek naar de stand van de techniek
NL 2009044

In het rapport genoemd octrooigeschrift	Datum van publicatie	Overeenkomend(e) geschrift(en)	Datum van publicatie
CN 1297691 A	06-06-2001	GEEN	
JP 2009254348 A	05-11-2009	JP 4384249 B2	16-12-2009
		JP 2009254348 A	05-11-2009
CN 102422974 A	25-04-2012	GEEN	
CN 1415757 A	07-05-2003	GEEN	

WRITTEN OPINION

File No.	Filing date *(day/month/year)*	Priority date *(day/month/year)*	Application No.
SN58637	21.06.2012		NL2009044

International Patent Classification (IPC)
INV. A23L1/48 A23J3/00 A23L1/29 A61K35/64 C11B1/02 C11B1/10 A23J3/34

Applicant
Protix Biosystems B.V.

This opinion contains indications relating to the following items:

- ☒ Box No. I Basis of the opinion
- ☐ Box No. II Priority
- ☐ Box No. III Non-establishment of opinion with regard to novelty, inventive step and industrial applicability
- ☐ Box No. IV Lack of unity of invention
- ☒ Box No. V Reasoned statement with regard to novelty, inventive step or industrial applicability; citations and explanations supporting such statement
- ☐ Box No. VI Certain documents cited
- ☐ Box No. VII Certain defects in the application
- ☐ Box No. VIII Certain observations on the application

	Examiner
	Picout, David

Application number

WRITTEN OPINION

NL2009044

Box No. I Basis of this opinion

1. This opinion has been established on the basis of the latest set of claims filed before the start of the search.

2. With regard to any **nucleotide and/or amino acid sequence** disclosed in the application and necessary to the claimed invention, this opinion has been established on the basis of:

   a. type of material:

   ☐ a sequence listing

   ☐ table(s) related to the sequence listing

   b. format of material:

   ☐ on paper

   ☐ in electronic form

   c. time of filing/furnishing:

   ☐ contained in the application as filed.

   ☐ filed together with the application in electronic form.

   ☐ furnished subsequently for the purposes of search.

3. ☐ In addition, in the case that more than one version or copy of a sequence listing and/or table relating thereto has been filed or furnished, the required statements that the information in the subsequent or additional copies is identical to that in the application as filed or does not go beyond the application as filed, as appropriate, were furnished.

4. Additional comments:

Box No. V Reasoned statement with regard to novelty, inventive step or industrial applicability; citations and explanations supporting such statement

1. Statement

Novelty	Yes:	Claims	1-23
	No:	Claims	
Inventive step	Yes:	Claims	
	No:	Claims	1-23
Industrial applicability	Yes:	Claims	1-23
	No:	Claims	

2. Citations and explanations

see separate sheet

Re Item V

Reasoned statement with regard to novelty, inventive step or industrial applicability; citations and explanations supporting such statement

1. Reference is made to the following documents:

D1 CN 1 297 691 A (INST OF ZOOLOGY ACADEMIA SINIC [CN] INST ZOOLOGY CAS [CN]) 6 juni 2001 (2001-06-06)

D2 JP 2009 254348 A (SHINGY KK) 5 november 2009 (2009-11-05)

2. Novelty

2.1 The documents D1 and D2 can both be regarded as being the closest prior arts to the subject-matter of independent claims 1, 11 and 16.

D1: It discloses the separation and extraction process of effective components in housefly maggot (insects). Through shell/liquid separation, enzymolysis, oil/water separation, chemical extraction and other processes, protein hydrolysate, maggot oil and chitin are produced separately from housefly maggot **(abstract)**.

In D1 the maggot is washed and ground and the slurry is subjected to enzymatic hydrolysis in order to obtain after a physical separation step a fat fraction, a protein fraction and a chitin fraction **(claim 1)**.

D2: It discloses a process wherein dried larvae of bees are powdered and suspended in water, and a lipolytic enzyme is added to break down lipids in the larvae of bees. A proteolytic enzyme is then added to hydrolyze the proteins in the larvae of bees.

In the process, a lipase is added to break down the lipids. Any of at least an acidic protease, an alkaline protease, a neutral protease, a complex protease, trypsin, pepsin, papain, bromelain, peptidase, a flavorzyme, a vegetable protease, an animal protease, cathepsin A, cathepsin B, cathepsin C, cathepsin D, cathepsin E, or chymotrypsin is used as the proteolytic enzyme **(abstract)**.

The subject-matter of independent claim 1 of the present invention differs from these known documents D1 and D2 in that the method involves squashing insects obtaining a pulp and after subjecting the pulp to hydrolysis heating the hydrolysed mixture to a temperature of 70-100 °C.

The subject-matter of independent claims 11 and 16 of the present invention differs from these known documents D1 and D2 in that the fat-containing composition comprises at least 80 wt.% insect or worm fat (claim 11) and at least 45 wt.% insect or worm protein-derived matter and at most 25 wt.% insect or worm fat (claim 16)

The subject-matter of independent claims 1, 11 and 16 appears therefore to be novel.

2.2 Dependent claims 2-10, 12-15 and 17-23 are therefore also novel.

3. Inventive step

3.1 The problem to be solved by the present invention may be regarded as to provide a method that converts insects or worms into nutrient streams, and preferably into three nutrient streams, being a fat-containing stream, an aqueous stream containing protein-derived matter and another stream containing solids such as chitin.

3.2 The solution to this problem proposed in the present application is the method of claim 1 which consists of separating fat, protein and chitin from insects or worms and to use the obtained compositions in the preparation of food or feed products.

D1 and D2 appear to solve the same problem and the method of the invention would easily be foreseen by a person skilled in the art with the teaching of D1 and/or D2.

Indeed, for example, starting from D1, a person skilled in the art would arrive to the present invention without exercising any inventive skills since slight changes in the method of extraction of fat, protein and chitin from an insect slurry (temperature range, separation steps etc.) would come within the scope of the customary practice followed by persons skilled in the art, especially as the advantages thus achieved can readily be foreseen.

The same reasoning can apply starting from D2. Moreover the compositions obtained would therefore also be foreseen by a person skilled in the art.

The subject-matter of independent claims 1, 11 & 16 does therefore not appear to involve an inventive step.

3.3 In view of D1 and D2, dependent claims 2-10, 12-15 and 17-23 do not appear to contain any additional features, which in combination with the features of the claim to which they refer, meet the requirement of involving an inventive step.