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- as to the applicant's entitlement to claim the priority of the
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WO 2014/123466 A1

(54) **Title:** LIQUID OAT BASE

(57) **Abstract:** A process for preparing a liquid oat base or drink of improved soluble oat protein content from an oats material, in particular an oats material that has not been heat treated in a humid state, comprises solubilizing oat protein in an aqueous solvent by means of protein-deamidase. Also disclosed is a corresponding liquid oat base and uses thereof.

A liquid oat base and a process for preparing it using protein-deamidase.

FIELD OF THE INVENTION

5 The present invention relates to a liquid oat base, in particular a liquid oat base for use as a milk substitute or a food additive, and to a method for its manufacture.

BACKGROUND OF THE INVENTION

10

Oat drinks ("oat milk") for use as cow milk substitutes (EP 731646 B1; EP 1124441 B1; US 6451369 B1) and as a raw material for other non-dairy milk products (US 7160564 B2) are known in the art. They are preferred by many customers for
15 various reasons, such as for their content of soluble β -glucan fiber beneficial to health, their lack of potentially allergenic proteins and of lactose, which cannot be digested by the majority of the global population. The soluble protein content of oat milk is about 0.5 to about 1.0 % by weight. In the prior art processes
20 for preparing oat milk the starting material, such as oat flour or oat bran or the whole oats from which it is made or an aqueous suspending or mixture of it is heated to a temperature and for a time sufficient to substantially prevent the development of endogenous enzymatic activity, in particular lipase/ lipoxygenase
25 activity, but also β -glucanase activity, during the respective process. Known oat drinks may be termed "oat bases" since, in addition to be used as drinks, in particular milk drinks, they can be used as a base for food other products, such as oat yogurt or oat batter, or be used as a food additive.

30 Due to the low fat content of oat milk (typically 0.5 % by weight) fat in form of vegetable oil, such as rapeseed oil, is often added to the product.

In spite of the commercial success of oat drinks available on the market there is room for further improvement, in
35 particular in respect of increasing the protein content of the drinks. Processes for producing oat drinks known in the art do not adequately access the protein in oat raw material.

It is known to increase the content of water soluble protein in oat drinks by the use of proteinase in addition to amylase(s) in the enzymatic degradation of oat raw material. The use of proteinase however results in the formation of low-molecular
5 peptides, which may change the organoleptic properties of the drinks.

EP 976 829 A1 discloses a protein deamidating enzyme and a process for its production. EP 1 371 734 A1 discloses a method of denaturing milk protein by a deamidating enzyme to improve its
10 sensitivity to protease and its emulsifying, foaming and gelling characteristics. EP 1 839 491 discloses a dairy product and a method of its production by contacting milk with a deamidating enzyme to suppress acidic and bitter taste. WO 2008/138900 A2 discloses a method for producing an acidified milk drink by
15 contacting raw or processed milk with a deamidating enzyme.

In addition to/separate from deamidation by a deamidating enzyme glutamyl and asparagyl residues in peptides and proteins have been observed to undergo non-enzymatic deamidation *in vitro* and *in vivo* (Robinson N A, *Protein Deamidation*. Proc Nat Acad Sci,
20 99 (2002)5283-5288 = <http://www.pnas.org/content/99/8/5283.full> and literature cited therein).

OBJECTS OF THE INVENTION

25 It is an object of the invention to provide an oat drink or base of the aforementioned kind, which has improved protein content.

Another object of the invention is to provide said improvements while maintaining or even improving the organoleptic
30 properties of the drink.

A further object of the invention is to provide a process for producing the improved oat drink or base.

Additional objects of the invention will become evident from the following summary of the invention, a number of examples
35 describing preferred embodiments thereof, and the appended claims.

SUMMARY OF THE INVENTION

According to the present invention is provided an oat base of the aforementioned kind having an improved content of soluble oat protein. "Improved protein content" is a higher protein content than obtainable by methods known in the art from a given oat raw material with the proviso that the improved content is not due to the use of protease (peptidase/proteinase).

The oat base of the invention is provided by degrading an oats material with one or more amylases and protein-deamidase.

According to one preferred aspect of the invention the protein-deamidase is one capable of deamidating high-molecular oat protein, such as oat globulin.

According to a preferred aspect of the invention the protein-deamidase does not comprise substantial protease (peptidase) activity. The protein-deamidase of the invention is preferably free from protease activity. Examples for protein-deamidases useful in the invention are disclosed in EP 976829 B1. A preferred amount of protein-deamidase is from 0.5 - 20 U/g oat protein.

According to another preferred aspect of the invention, deamidation is carried out in parallel amidolysis, that is, with starch degradation by amylase(s). "In parallel with amidolysis" is understood as simultaneous with the enzymatic degradation of starch by amylase(s). In the process of the invention deamidation of oat protein may however be continued even after amidolysis has ceased or substantially ceased.

The process of the invention can be stopped at a desired viscosity, such as at a viscosity of from 100 cP to 200 cP or from 50 cP to 100 cP or from 25 cP to 50 cP or from 10 cP to 25 cP (sp2/60 rpm/25±2 °C). The process of the invention is preferably stopped by heating to a temperature at which any enzymatic activity is destroyed within a short time, such as within ten seconds or one minute or five minutes, said temperature being > 80 °C, preferably greater than 90 °C, in particular greater than 100 °C, such as about 105 °C, at which

temperature heating for about 10 seconds is sufficient to destroy any enzymatic activity.

The improved oat base of the invention differs from prior art oat bases (oat drinks) by its increased content of soluble oat protein. In this application "soluble" signifies "water soluble". The improvement in soluble protein content obtainable by the method of the invention is 10 per cent by weight and up to 20 per cent by weight or more.

Thus, according to the present invention, the content of soluble protein in the oat base is not one increased by addition of soluble protein to the base or to the raw material from which it is made or during the process by which it is manufactured but by use of an appropriate oat raw material and an appropriate protein solubilization process. It is preferable to use a raw material with a high content of protein preserved in its natural state. "Preserved in its (a) natural state" signifies that the protein in the raw material has not been denatured or has only been denatured to a minor extent, such as by 10 % by weight or 20 % by weight.

Oats used for producing oat drinks is dry- or wet-heated prior to use as starting material for producing oat bases or drinks. The purpose with the heat treatment is twofold. On the one hand, the purpose is to destroy beta-glucanase present and/or to prevent it from being formed during starch hydrolysis so as to preserve water-soluble beta-glucans in their native state. Beta-glucans in their native state are high-molecular beta-glucans, such as of a molecular weight of 50,000 D or more. High molecular beta-glucans are considered to constitute a valuable health-promoting component of oat drinks. Inactivation of beta-glucanase by heat treatment is however only indicated if the oat drink to be manufactured is desired to contain substantial amounts of beta-glucans.

On the other hand and, in a more general manner, the purpose with the traditional heat treatment is to inactivate lipase and lipoxigenase. Inactivation of lipase and lipoxigenase is indicated to prevent the product from turning rancid. According to a preferred aspect of the invention the need of inactivating

lipase and lipoxigenase can be avoided by removing the lipids of the raw material, such as by extraction with ethanol or supercritical carbon dioxide. Preferably at least 90 % and even at least 95 % of the lipids are removed.

5 While the content of water-soluble protein in untreated oats is about 60 % to about 70 % weight of total protein, it is only about 30 % weight in microwave-treated oats (Skånemöllan, Sweden) and in steam-treated (102 °C for 50 min, then air-dried (110 °C - 120 °C min for 50 min) oats.

10 In the method of the invention this kind of heat treatment, in particular steaming, should be avoided or at least be kept as short as possible and/or carried out at a temperature as low as possible to keep oat protein denaturation low. If avoided, the lipids should be removed from the oats. If heating is the
15 preferred method of preventing the product from turning rancid and from preventing substantial degradation of β -glucan, a compromise between heating temperature and/or length of heating, at the one hand, and completeness of inactivation of β -glucanase and lipase/lipoxxygenase, at the other hand is attempted.

20 A preferred raw material for use in the invention is dehulled or hullless/naked, dry milled oat flour that has not been heat treated, in particular steamed. However, wet milled oat flour that has not been heat treated or dry milled flour of any oats fraction can also be used. Particularly preferred is the use
25 of dry milled non-heat treated oats, non-heat treated oat bran, and non-steamed oats.

According to the invention it has been found that heating of oats in any form at a temperature of up to about 50 °C or even up to about 65 °C for a few hours, such as for one or two or even
30 five hours, does not result in substantial denaturation. On the other hand, heating such oats material for a corresponding time period at a temperature of 80 °C or more does result in a substantial reduction of soluble protein, in particular if the material is in a humid state. Steaming of oats in any form
35 results in substantial denaturation, such as denaturation of 30 % or more and even of 50 % of more. Consequently, steamed oats materials, such as, for instance, those disclosed in US 6165365 A

and US 7494683 B2, are not preferred for use in the present invention.

According to one preferred aspect of the invention the oat base of the invention is prepared by milling groats (dehulled
5 oats) with water to obtain a mash containing from 8 % by weight to 13 % by weight dry substance, then adding amylase(s) and degrading the oat starch at a temperature of from 50 °C to 75 °C. The amylase may be beta- and alpha amylase or a mixture thereof, the amylases being added as a mixture or their mixture in the
10 mash being formed by their simultaneous or sequential addition.

The amylases are added in amount(s) sufficient for significant hydrolysis of starch over a time period of from 0.5 h to 4 hrs, in particular from about 1 h to about 2 hrs, hydrolysis of more than 50 % by weight of the starch, in particular of more
15 than 80 % by weight or even more than 90 % weight being considered significant.

Typically the amylase(s) are added in an amount to provide amylase activity of from 140 to 250 Betamyl-3 units and from 0.5 to 4 Ceralpha units per g of starch, in particular of about 180
20 Betamyl-3 units and about 1 Ceralpha unit per g of starch.

Also disclosed according to the invention is a liquid oat base prepared by the process of the invention and a liquid oat base comprising oat protein deamidated by protein deamidase. It is preferred for the oat base protein to comprise 10 % by weight
25 or 20 % by weight or more of protein deamidated by protein deamidase.

According to the invention is furthermore disclosed the use of the liquid oat base of the invention as a food, a food additive or a starting material for production of a food, all
30 intended for human consumption.

DESCRIPTION OF PREFERRED EMBODIMENTS

Material and Methods

35

Oat kernels: Dehulled, steam treated, wet ground or dry ground.

Oat bran (Frebaco Kvarn AB, Lidköping, Sweden): Prepared from steam treated Swedish oat grain by grinding in a rolling mill. Composition (% by weight): Protein 18, fat 7, carbohydrate 45, fiber 16 %, water 9.5.

5

Enzymes: Protein-glutaminase "Amano 50", 50 U/g (Amano Inc., Japan). Commercial alpha-amylase and beta-amylase are available from various commercial sources.

10 *Alpha-amylase activity:* One Ceralpha unit is defined as the amount of enzyme required to release one micromole of p-nitrophenol from BPNPG7 (non-reducing end blocked p-nitrophenyl maltoheptaoside) in one minute under defined assay conditions: http://secure.megazyme.com/files/BOOKLET/K-BETA3_1010_DATA.pdf

15

Beta-amylase activity: One BNP β -G3 (p-nitrophenyl- β -D-maltotrioside) unit is defined as the amount of enzyme required to release one micromole of p-nitrophenol from PNP β -G3 in one minute under defined assay conditions:

20 http://secure.megazyme.com/files/BOOKLET/K-BETA3_1010_DATA.pdf

Protein-glutaminase activity: One activity unit (U) is defined as the quantity of enzyme producing one μ mol of ammonia per min in the reaction with 10 mM aqueous benzylocarbonyl-L-glutaminyglycine (Cbz-Gln-Gly).

25

Viscosity: Measured with a Brookfield Visco DV-II+ instrument (<http://www.brookfieldengineering.com/products/viscosimeters/laboratory-dv-ii.asp>).

30

EXAMPLE 1. *Pilot scale process for producing the improved oat base of the invention*

35 Dehulled, steam treated oat kernels (675 kg) were wet ground in a colloidal mill at a temperature of 54 °C and directly fed into a stainless steel enzyme treatment tank over a period of about 20 min. Stirring was started at a mash volume of about 100

L. About 7.5 L of an aqueous solution of alpha-and beta-amylase (1 Ceralpha unit per 180 Betamyl-3 units per g of starch) was used. Enzyme activity may vary depending on the commercial source of the enzymes; in this experiment the total weight of amylases
5 was 432 g. The enzyme solution was fed into the tank in parallel with the mash over a period of about 12 min at the end of which about 3000 L of the mash had been fed into the tank. The rest of the mash was fed into the tank over a period of about 8 min to bring the total contents of the tank to about 5600 L. The
10 temperature of the mash was kept constant at 56 °C.

Protein-glutaminase (PG) dosing. PG (687.5 g) was dissolved in 1.5 L water at room temperature. The PG solution was added to the mash at a viscosity of 160.5 (sp2/60 rpm/25±2 °C). Stirring
15 was continued for about 120 min at a temperature of about 56 °C to reach a mash viscosity of 35 (sp2/60 rpm/25±2 °C) and a pH of 6.6. Any enzyme activity was then destroyed by heating the product to 95 °C. The mash was cooled to room temperature and decanted. Decantation can be omitted if a whole grain product is
20 to be produced.

The thus produced oat base of the invention can be transferred into a formulation tank in which rapeseed oil, vitamins, sodium chloride, di- and tricalcium phosphate, and calcium carbonate is added. The thus obtained enriched oat drink
25 has a viscosity (sp2/60 rpm/25±2 °C) of 17.5 cP and a pH of 6.8. The formulated oat drink or oat milk is transferred to a storage tank from which it is dispensed for UHT treatment and packaging.

Product analysis. Deamidation of product: 7.3 % of total
30 releaseable ammonia (by treatment with 2 N sulphuric acid at 100 °C for 4 h). Deamidation of control (non-enzymatic deamidation): 1.6 % of total releaseable ammonia (same process in absence of PG). Soluble protein: 78 % of total protein (product of the invention) v. 64 % of total protein (control).

35

Instead of dehulled steam treated oat kernels also corresponding naked kernels may be used, for instance, as a starting material.

5 EXAMPLE 2. *Modified and down-scaled (1:10⁵) process of Example 1*

Wet-milled oat slurry is heated to 60 °C under stirring. Alpha-and beta-amylase as well as protein glutaminase (1 U/g of oat protein) are added and reacted with the slurry under stirring
10 at 60 °C for two hours. The slurry is then heated to 95 °C for 5 min. Insoluble matter is removed by pulse centrifugation (pulses of 1100 g) and analyzed.

Product analysis. Deamidation of product: 6.9 % of total
15 releaseable ammonia. Deamidation of control (non-enzymatic deamidation): 1.9% of total releaseable ammonia (same process in absence of PG). Soluble protein: 84 % of total protein (product of the invention) v. 56 % of total protein (control).

20 EXAMPLE 3. *Modified and down-scaled process of Example 1*

As Example 2 but with heat treated dry milled and sieved oat kernels, fraction size <0.5 mm mixed with water to a dry weight of 11 %.

25

Product analysis. Deamidation of product: 6.1 % of total releaseable ammonia. Deamidation of control (non-enzymatic deamidation): 1.5 % of total releaseable ammonia (same process in absence of PG). Soluble protein: 59 % of total protein (product
30 of the invention) v. 48 % of total protein (control).

EXAMPLE 4. *Modified and down-scaled process of Example 1*

As Example 2 but with non-heat treated dry milled and sieved
35 oat kernels, fraction size <0.5 mm mixed with water to a dry weight of 11 %.

Product analysis. Deamidation of product: 8.9 % of total releaseable ammonia. Deamidation of control (non-enzymatic deamidation): 1.5 % of total releaseable ammonia (same process in absence of PG). Soluble protein: 81 % of total protein (product of the invention) v. 62 % of total protein (control).

EXAMPLE 5. *Deamidation of oat drink at laboratory and pilot plant scale by protein-glutaminase*

The oat base or drink used in the example was prepared according to the method disclosed in European patent no. 731 646. This oat drink is a commercial product manufactured by Oatly AB, Landskrona, Sweden. In Table 1 important features of a number of products according to the invention are shown. Also shown are corresponding features of deamidation products obtained from dry-milled heat-treated oats. The products were obtained in absence of deamidase (0 U) and in presence of deamidase at two deamidase addition regimes (1 U; 2x0.5 U/g oat protein). From Table 1 it is evident that the content of total protein is substantially increased in the presence of deamidase. It is also evident that, at otherwise identical conditions, a non-heat treated starting material yields a product with higher protein content than a corresponding heat-treated starting material.

It is furthermore evident that that, at otherwise identical conditions, sequential addition of deamidase (2x0.5 U) yields a product of higher protein content than obtained by a single addition of the same amount of amylase (1 U). A higher protein content of the product is paralleled by increased emulsion stability (reduced sedimentation rate) of the product.

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Table 1. Deamidation of oat drink at laboratory and pilot plant scale

Oat raw material	Protein-Glutaminase, U/g of Oat Protein	Deamidation (%)	Soluble protein, g/100g (% of total)	Total protein g/100g	Droplet size (μm), 1.5 % fat	Sedimentation
<i>Laboratory scale</i>						
Wet-milled	0 U	1.9	0.71 (57 %)	0.84	3.2	14% UPH**
	1 U	6.7	0.90 (72 %)	0.92	1.7	2 white PH*
	2x0.5 U***	6.9	1.06 (87 %)	0.95	0.8	2 white PH
Dry-milled, heat treated	0 U	1.5	0.59 (46 %)	0.64	4.5	17 % UPH
	1 U	6.1	0.75 (59 %)	0.81	3.6	No sediment
	2x0.5 U***	8.6	0.80 (63 %)	0.80	4.0	No sediment
Dry milled, non-heat treated	0 U	1.5	0.88 (64 %)	0.80	2.6	38 % UPH
	1 U	8.9	1.16 (81 %)	0.92	1.2	30 % UPH
	2x0.5 U***	9.5	1.26 (93 %)	0.94	1.2	28 % UPH
Oat bran	0 U	1.8	0.41 (17 %)	1.25	7.1	13 % UPH
	1 U	5.9	1.05 (42 %)	1.62	6.3	7 % UPH
	2x0.5 U***	6.1	1.09 (44 %)	1.60	5.6	4 % UPH
<i>Small pilot scale</i>						
Wet-milled	0 U	2.3	0.65 (56 %)	0.82	15.8	10 % UPH
	1 U	7.9	0.70 (67 %)	0.80	15.8	2 white PH
	2x0.5 U***	13.0	0.83 (72 %)	1.01	17.8	No sediment
<i>Large pilot scale</i>						
Wet-milled	0 U	1.6	0.70 (64 %)	0.75	7.9	63 % UPH
	2x0.5 U***	7.3	1.00 (78 %)	0.91	10.0	2 white PH

*PH = Phase; ** UPH = Upper phase; *** 0.5 U added to each of two amylase enzymation steps

Claims

1. A process for preparing a liquid oat base or drink of improved soluble oat protein content from an oats material comprising starch and oat protein, characterized by solubilizing oat protein in an aqueous solvent, in particular water, by means of protein-deamidase; optionally decanting the product.
2. The process of claim 1, wherein the oats material is a material of which the protein has not been denaturated or has only been denaturated to an extent of up to 10 % by weight or up to 20 % by weight.
3. The process of claim 2, wherein the oats material is one that has not been steamed.
4. The process of any of claims 1 to 3, wherein the protein-deamidase is glutaminase.
5. The process of any of claims 1 to 4, wherein the amount of protein glutaminase used in the process is from 0.5 U/g of oat protein to 2 U/g of oat protein, in particular about 1 U/g of oat protein.
6. The process of claim 1 to 5, wherein the oats material is selected from non-steamed wet milled oats, non-steamed dry milled oats and non-steamed oat bran.
7. The process of the invention of claim 1 to 5, wherein the oats material is selected from non-steamed dehulled or hullless/naked dry milled oat flour.
8. The process of any of claims 1 to 7, wherein the improvement in content of soluble protein is 10 per cent by

weight or more, in particular up to 20 per cent by weight or more of protein solubilized in absence of protease.

9. The process of any of claims 1 to 8, wherein the oats
5 material is suspended in an aqueous medium, in particular water, and the starch thereof is degraded by amylase.

10. The process of claim 9, wherein the amylase is β -amylase or a mixture of α -amylase and β -amylase.

10

11. The process of claim 9 or 10, wherein oat protein is solubilized by protein-deamidase concurrently with starch degradation.

15 12. The process of claim 11, wherein protein-deamidase is added in two or more portions during the process.

13. The process of claim 12, wherein said second portion is added during a period extending from 30 min to 90 min after
20 addition of the first portion.

14. The process of claim 12 or 13, wherein the first portion is added during a first step of starch hydrolysis by amylase and the second portion is added during a second step
25 of starch hydrolysis by amylase.

15. The method of any of claims 11 to 14, wherein oat protein solubilization and starch degradation is carried out at a temperature of from 40 °C to 65 °C, in particular at a
30 temperature of from 50 °C to 60 °C, most preferred at a temperature of about 55 °C.

16. The method of any of claims 11 to 15, wherein the oat protein solubilization period and/or the starch degradation
35 period is from 30 min to 120 min.

17. The method of any of claims 1 to 16, wherein the process of oat protein solubilization and starch degradation is stopped at a desired viscosity.
- 5 18. The method of any of the preceding claims, wherein the product is UHT treated.
19. The method of any of the preceding claims, wherein the oat base is enriched with one or more of vegetable oil,
10 sodium chloride, dicalcium phosphate, tricalcium phosphate, calcium carbonate, vitamin.
20. Liquid oat base prepared by the method of any of claims 1 to 18.
- 15 21. Liquid oat base comprising oat protein dissolved therein deamidated by protein deamidase.
22. The oat base of claim 21, wherein 10 % by weight or
20 more of said oat protein is protein deamidated by protein deamidase.
23. Use of the liquid oat base of any of claims 20 to 22 as
25 production of food, all of them intended for human consumption.

INTERNATIONAL SEARCH REPORT

International application No.
PCT/SE2014/000010

A. CLASSIFICATION OF SUBJECT MATTER

IPC: see extra sheet

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)

IPC: A23L

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

SE, DK, FI, NO classes as above

Electronic data base consulted during the international search (name of data base and, where practicable, search terms used)

EPO-Internal, PAJ, WPI data, CHEM ABS Data, fsta

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	US 6036983 A (NIELSEN PER MUNK), 14 March 2000 (2000-03-14); claims	1-7, 17-23
Y	--	9-16
Y	US 20120034341 A1 (CHEN KWAN-HAN ET AL), 9 February 2012 (2012-02-09); claims	9-16
A	WO 0030457 A1 (KELLOG CO), 2 June 2000 (2000-06-02); claims	1-23

Further documents are listed in the continuation of Box C. See patent family annex.

* Special categories of cited documents:	“T” later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention
“A” document defining the general state of the art which is not considered to be of particular relevance	“X” document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone
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Name and mailing address of the ISA/SE Patent- och registreringsverket Box 5055 S-102 42 STOCKHOLM Facsimile No. + 46 8 666 02 86	Authorized officer Carolina Gomez Lagerlöf Telephone No. + 46 8 782 25 00
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A23L 1/105 (2006.01)

A23L 1/29 (2006.01)

INTERNATIONAL SEARCH REPORT

Information on patent family members

International application No.

PCT/SE2014/000010

US	6036983 A	14/03/2000	NONE			
US	20120034341 A1	09/02/2012	TW	201105251 A	16/02/2011	
			US	8337880 B2	25/12/2012	
WO	0030457 A1	02/06/2000	AU	1736200 A	13/06/2000	