

IMPROVED PROTEIN BIO-AVAILABILITY OF PALM KERNEL CAKE USING Milder EXPELLER CONDITIONS AND ENZYMATIC TREATMENT

Reference to sequence listing

5 This application contains a Sequence Listing in computer readable form. The computer readable form is incorporated herein by reference.

FIELD OF THE INVENTION

10 The present invention relates to an improved bio-availability of protein/amino acids in palm kernel cake by reduction in expeller processing temperature and/or time and/or force of palm kernels during palm kernel oil extraction. After expelling the palm kernel cake is upgraded by enzymatic hydrolysis and fermentation. The invention relates to methods for production of the upgraded PKC (termed half press PKC++) and to the half press PKC++ as such. The half press PKC++ can be used as an animal feed e.g. for feeding of mono-gastric animals such as poultry and pigs.

BACKGROUND OF THE INVENTION

15 The oil palm (*Elaeis* sp.) tree is grown for its fruits which each comprises an oily, fleshy outer layer termed the pericarp. Each fruit has a single seed termed the kernel. Oil can be extracted from both the pericarp and the kernel. Extraction of oil from the palm kernel has previously been described e.g. in MY131537 and MY141713.

20 Extraction of palm kernel oil from palm kernels can either be done by mechanical extraction or by solvent extraction. The residue obtained after mechanical extraction is called palm kernel cake/Expeller and the residue obtained after solvent extraction, is called palm kernel meal.

It has previously been disclosed that the palm kernel meal can be processed to an animal feed product with high nutritional value - cf. CN101248835.

25 Currently palm kernels are typically used for production of palm kernel oil and the residue obtained after the mechanical extraction (expelling) is mainly used for animal feed products for ruminants. The present invention relates to upgrading of palm kernel cake to animal feed for mono-gastric animals.

30 The Maillard reaction is a non-enzymatic browning reaction which takes place at elevated temperatures. The reaction occurs between the reducing end of a sugar molecule or a sugar polysaccharide, of structural or non-structural origin in a plant material, and an amino group in an amino acid. In food processing the reaction may be beneficial in order to produce favorable

color or flavors. The reaction diminishes the bio-available amino acids and is therefore not beneficial in animal feed since the reaction proceeds faster for amino acids having two amino groups, which is the case for lysine, which typically is the first limiting amino acid in the feeding of mono-gastric animals. Furthermore, heating may produce acrylamide (2-propenamide) and it has been shown that at neutral pH acrylamide may be eliminated by coupling of two molecules of acrylamide to one molecule of cysteine, glycine and lysine and thereby the concentration of these amino acids is reduced.

The Maillard reaction affects the nutritional value of the palm kernel cake. The Maillard reaction takes place between a reducing end of a carbohydrate molecule and the amino groups of free amino acids or amino acids in peptides and proteins. The reaction is favored by free ϵ -amino groups in the N-terminal part of the amino acid. Lysine which has a free ϵ -amino group is one of the most susceptible amino acids and reacts at rates of 5 to 15 times that of other amino acids, e.g. methionine, cysteine or tryptophan. Since lysine is one of the first limiting essential amino acids this may result in a notable loss of nutritive value since the Maillard compound is indigestible.

In feed components the acid detergent fiber (ADF) represents the cellulose and lignin fraction of the feed. The contents of nitrogen bound in this fraction serves as an indicator of insoluble protein (i.e. non bio-availability) damaged by heat treatment.

In food product processing the analysis of heat damaged carbohydrate and amino acid compounds requires painstaking analytical work but in animal feed this can be simplified by analysing the amount of nitrogen bound in the acid detergent fraction. The concentration of nitrogen found in this fraction has been shown to serve as an indicator of insoluble protein damaged by heat treatment (Goering, H.K., Gordon, C.H., Hemken, R.W., Waldo, D.R., VanSoest, P.J. and Smith, L.W. (1972). Analytical estimates of nitrogen digestibility in heat damaged forages. *Journal of Dairy Science* 55: 1275-1280).

The present invention demonstrates that a reduction of the expelling cycle (half press) reduces the formation of ADF-N and that this is beneficial in order to conserve the protein quality of the PKC. This will improve the digestibility of the protein but also leave more fat in the product and thereby its metabolisable energy content (energy that an animal may utilize for maintenance and growth) is increased. An improvement in the amount of digestible protein will also provide an energy contribution from the protein fraction.

The present invention demonstrates that alteration and/or elimination of processing steps that creates ADF bound nitrogen is important in order to preserve the protein bio-availability in palm kernel cake. The present invention relates to a new process comprising 1) alteration and/or elimination of processing steps that creates ADF bound nitrogen in PKC and 2) enzymatic hydroly-

sis/fermentation of the PKC in order to upgrade the PKC to be suitable for animal feed for mono-gastric animals.

SUMMARY OF THE INVENTION

The present invention relates to an improved processing step that minimizes the loss of protein bio-availability of palm kernel cake. A reduction of the expeller pressing cycles in temperature and/or time and/or force of palm kernels during palm kernel oil extraction increase the protein bio-availability in the protein rich press-residue termed the palm kernel cake. The palm kernel cake according to the present invention can be used as an animal feed e.g. for feeding of monogastric animals such as chickens.

The present invention relates to a new process comprising 1) alteration and/or elimination of processing steps that creates ADF bound nitrogen in palm kernel cake and 2) enzymatic hydrolysis/fermentation of the palm kernel cake. It is possible to convert mannans in the palm kernel cake to mannose by the use of mannanase and mannosidase in this enzymatic hydrolysis process. The mannose can be utilized by yeast in the fermentation process as energy source to produce ethanol. This typically depletes the kernels of mannan from an original level of approximately 44 % down to about 14%, and after removal of the ethanol, the protein concentration is increased from about 15% (Sauvant D, Perez J-M, Tran G, editors (2004); Tables of Composition and Nutritional Value of Feed Materials. INRA editions, Wageningen Publishers, ISBN 2-7380-1158-6) up to about 25 % to 29 % crude protein.

DEFINITIONS

Mannan-containing materials

The terms "mannan-containing material" used herein refers to a material comprising a significant amount of mannan. Any mannan-containing material is contemplated according to the present invention. In a preferred embodiment the mannan-containing material contains at least 1 wt-%, preferably at least 5 wt.-%, more preferably at least 10 wt-%, even more preferably at least 15 wt-% mannan, yet more preferably at least 20 wt-%, or most preferably at least 25 wt-% mannan. It is to be understood that the mannan-containing material may also comprise other constituents such as cellulosic material, including cellulose and/or hemicellulose, and may also comprise other constituents such as proteinaceous material, starch, sugars, such as fermentable sugars and/or un-fermentable sugars. Mannan can comprise or consist of galacto-mannan.

Mannan and galacto-mannan is found in plant, fungal and bacterial cell walls. Mannan-containing material is generally found, for example, in the stems, leaves, fruits, hulls, husks, and

cobs of plants or leaves, branches, and wood of trees. It is understood herein that mannan-containing material may be in the form of plant cell wall material containing lignin, cellulose, and hemicellulose in a mixed matrix.

The mannan-containing material may be selected from the list consisting of herbaceous
 5 and/or woody crops, agricultural food and feed crops, animal feed products, tubers, roots, stems, legumes, cassava peels, cocoa pods, copra meal, rice husks and/or hulls, rice bran, cobs, straw, hulls, husks, sugar beet pulp, locust bean pulp, vegetable pomaces, agricultural crop waste, straw, stalks, leaves, corn bran, husks, cobs, rind, shells, pods, wood waste, bark, shavings, sawdust, wood pulp, pulping liquor, waste paper, cardboard, wood waste, industrial or
 10 municipal waste water solids, manure, by-product from brewing and/or fermentation processes, wet distillers grain, dried distillers grain, spent grain, vinasse and bagasse.

In a preferred embodiment the mannan-containing material is derived from a plant from the tribe *Cocoseae* such as from the subtribe *Attaleinae*, *Bactridinae* or *Elaeidinae* such as from a genus selected from the group consisting of *Allagoptera*, *Attalea*, *Beccariophoenix*, *Butia*, *Cocos*,
 15 *Jubaea*, *Jubaeopsis*, *Lytocaryum*, *Orbignya*, *Parajubaea*, *Polyandrococos*, *Syagrus*, *Voanioala*, *Acrocomia*, *Aiphanes*, *Astrocaryum*, *Bactris*, *Desmoncus*, *Gastrococos*, *Hexopetion*, *Barcella* and *Elaeis*. In a more preferred embodiment the mannan-containing material is selected from the group consisting of *Cocos nucifera* (coconut palm), *Elaeis guineensis* (African oil palm) and *Elaeis oleifera* (American oil palm).

20 The mannan-containing material can be separated into oil and mannan-containing cake by one or more pressing steps. In a preferred embodiment the mannan-containing material is palm kernel material and the mannan-containing cake is palm kernel cake. The palm kernel material can be palm kernels that have been pretreated as described elsewhere herein.

The oil palm (*Elaeis* sp.) tree is grown for its fruits which each comprises an oily, fleshy outer layer (the pericarp), with a single seed (the kernel). Oil can be extracted from both the pericarp and the kernel. The mannan-containing material according to the invention can in one embodiment be the oil palm pericarp and/or the oil palm kernel.

Copra meal is the residue remaining after the coconut oil is pressed from copra, the albumen of *Cocos nucifera* (expelled copra meal) or the residue remaining after the coconut oil extraction using solvents (extracted copra meal). Copra meal contains 25-32% mannan.
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Both oil palm residues and coconut residues are highly suitable as substrates for the present invention.

Palm kernel cake

Extraction of palm kernel oil from palm kernels can either be done by mechanical extraction or by solvent extraction. The residue obtained after mechanical extraction is called palm kernel cake (PKC) and the residue obtained after solvent extraction, is called palm kernel meal. PKC is
5 herein used as an alternative term for palm kernel expeller (PKE).

Upgraded PKC (PKC++)

“Upgraded PKC” or “PKC++” are used interchangeably herein and is defined as half
10 press or full press PKC that has been subjected to an enzymatic hydrolysis step and a fermentation step for generation of half press PKC++ or full press PKC++, respectively. The hydrolysis step can comprise treatment of the PKC with one or more enzymes (such as all of the enzymes) selected from the group consisting of cellulase, mannanase and mannosidase. The fermentation step can comprise treatment of the PKC with yeast such as Baker yeast red, Thermosacc. Yeast; SS-2010-00452. PKC++ can e.g. be obtained as described in Example 1 or Example 2.

Upgraded mannan-containing cake

“Upgraded mannan-containing cake” or “mannan-containing cake with improved nutritional value” are used interchangeably herein and is defined as mannan-containing cake with an
20 oil content of from 8% to 16% (w/w%) that has been subjected to an enzymatic hydrolysis step and a fermentation step. The hydrolysis step can comprise treatment of the mannan-containing cake with one or more enzymes (such as all of the enzymes) selected from the group consisting of cellulase, mannanase and mannosidase. The fermentation step can comprise treatment of the mannan-containing cake with yeast such as Baker yeast red, Thermosacc. Yeast; SS-2010-00452. An example of upgraded mannan-containing cake is PKC++ such as half press PKC++.

Full press and half press PKC

“Half press PKC” is defined as PKC containing from 8% to 16% oil (w/w%). “Full press PKC” is defined as PKC containing 5.0 w/w% oil e.g. determined as described in Example 2.

Acid detergent fiber bound nitrogen (ADF-N)

ADF-N is determined as described in AOAC methods of analysis: Method 4.1 Determination of Acid Detergent Fiber by Refluxing, AOAC 973.18 Method 3.2 Nitrogen Determination by Kjeldahl (Block Digestion) AOAC 981.10.

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True metabolisable energy content (TME)

TME is determined as outlined by Sibbald (Sibbald, I.R. 1981; Metabolic plus endogenous energy and nitrogen losses of adult cockerels: The correction used in the bioassay for true metabolisable energy; Poultry Science 60: 805-811).

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Non-starch polysaccharide (NSP)

NSP contents of the PKC samples are determined by a gas chromatographic method (Theander O, Aaman P, Westerlund E, Andersson R, Pettersson D. (1995). Total dietary fibre determined as neutral sugar residue, uronic acid residue and Klason lignin (The Uppsala Method): Collaborative study. Journal of AOAC International 74 1030-1043).

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Sequence identity

The relatedness between two amino acid sequences or between two nucleotide sequences is described by the parameter "sequence identity".

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For purposes of the present invention, the sequence identity between two amino acid sequences is determined using the Needleman-Wunsch algorithm (Needleman and Wunsch, 1970, *J. Mol. Biol.* 48: 443-453) as implemented in the Needle program of the EMBOSS package (EMBOSS: The European Molecular Biology Open Software Suite, Rice *et al.*, 2000, *Trends Genet.* 16: 276-277), preferably version 5.0.0 or later. The parameters used are gap open penalty of 10, gap extension penalty of 0.5, and the EBLOSUM62 (EMBOSS version of BLOSUM62) substitution matrix. The output of Needle labeled "longest identity" (obtained using the -nobrief option) is used as the percent identity and is calculated as follows:

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$$(\text{Identical Residues} \times 100) / (\text{Length of Alignment} - \text{Total Number of Gaps in Alignment})$$

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In a preferred embodiment, the amino acid sequence of the mannanase has at least 70% sequence identity to the polypeptide of SEQ ID NO: 1 or SEQ ID NO: 3. In a more preferred embodiment, the amino acid sequence of the mannanase has at least 80% sequence identity to the polypeptide of SEQ ID NO: 1 or SEQ ID NO: 3. In an even more preferred embod-

iment, the amino acid sequence of the mannanase has at least 90% sequence identity to the polypeptide of SEQ ID NO: 1 or SEQ ID NO: 3. In an even more preferred embodiment, the amino acid sequence of the mannanase has at least 95%, such as at least 96%, at least 97%, at least 98%, or at least 99% sequence identity to the polypeptide of SEQ ID NO: 1 or SEQ ID NO: 3. In a further embodiment, the mannanase is the polypeptide of SEQ ID NO: 1 or SEQ ID NO: 3.

In a preferred embodiment, the amino acid sequence of the mannosidase has at least 70% sequence identity to the polypeptide of SEQ ID NO: 2. In a more preferred embodiment, the amino acid sequence of the mannosidase has at least 80% sequence identity to the polypeptide of SEQ ID NO: 2. In an even more preferred embodiment, the amino acid sequence of the mannosidase has at least 90% sequence identity to the polypeptide of SEQ ID NO: 2. In an even more preferred embodiment, the amino acid sequence of the mannosidase has at least 95%, such as at least 96%, at least 97%, at least 98%, or at least 99% sequence identity to the polypeptide of SEQ ID NO: 2. In a further embodiment, the mannosidase is the polypeptide of SEQ ID NO: 2.

In a preferred embodiment, the amino acid sequence of the cellulase has at least 70% sequence identity to the polypeptide of SEQ ID NO: 4, SEQ ID NO: 5, SEQ ID NO: 6 or SEQ ID NO: 7. In a more preferred embodiment, the amino acid sequence of the cellulase has at least 80% sequence identity to the polypeptide of SEQ ID NO: 4, SEQ ID NO: 5, SEQ ID NO: 6 or SEQ ID NO: 7. In an even more preferred embodiment, the amino acid sequence of the cellulase has at least 90% sequence identity to the polypeptide of SEQ ID NO: 4, SEQ ID NO: 5, SEQ ID NO: 6 or SEQ ID NO: 7. In an even more preferred embodiment, the amino acid sequence of the cellulase has at least 95%, such as at least 96%, at least 97%, at least 98%, or at least 99% sequence identity to the polypeptide of SEQ ID NO: 4, SEQ ID NO: 5, SEQ ID NO: 6 or SEQ ID NO: 7. In a further embodiment, the cellulase is the polypeptide of SEQ ID NO: 4, SEQ ID NO: 5, SEQ ID NO: 6 or SEQ ID NO: 7.

In one embodiment the mannanase is obtained or obtainable from *Aspergillus niger* or *Talaromyces leycettanus*. The mannosidase can be obtained or obtainable from *Aspergillus niger*.

DETAILED DESCRIPTION OF THE INVENTION

The invention relates to one or more improved processing steps for oil extraction or a method for oil extraction that minimizes the loss of protein bio-availability of a mannan-containing cake such as palm kernel cake (PKC) compared to conventional oil extraction methods. More spe-

cifically, a reduction of the one or more expeller pressing cycles in temperature and/or time and/or force of palm kernels during palm kernel oil extraction is performed (termed half press).

This method results in an increased protein bio-availability in the palm kernel cake – i.e. characterized by improved amino acid availability. The improved protein bio-availability can be characterized by a reduced amount of acid detergent fiber bound nitrogen (ADF-N) in the palm kernel cake. In one embodiment the ADF-N is less than 20, such as less than 15, such as less than 10, such as less than 5.

More specifically the invention relates to a method of improving fiber digestibility in an animal feed comprising the steps of

- i) placing a mannan-containing material such as palm kernels in one or more press machines (expellers),
- ii) pressing the mannan-containing material one or more times to extract oil from the mannan-containing material to generate oil and mannan-containing cake such as palm kernel cake,
- iii) separating the oil from the mannan-containing cake such as the palm kernel cake, and
- iv) optionally hydrolyzing the mannan-containing cake such as the palm kernel cake with one or more enzymes to give an animal feed product,

wherein the pressing step uses less time and/or less pressure and/or lower temperature than in a conventional press.

The mannan-containing cake or PKC obtained in step iii) is typically used as an animal feed e.g. for feeding ruminants whereas the mannan-containing cake or PKC obtained in step iv) is typically used as an animal feed e.g. for feeding one or more monogastric animals such as chickens including layers and/or broilers, swine and aquatic animals (either vertebrate or invertebrate).

In a conventional press used in step ii) the residence time (the conventional press residence time) is typically less than 2 minutes such as less than 1 minute. In the following example the conventional press residence time is set to 1 minute and the present invention can in this embodiment relate to a press residence time selected from the group consisting of less than 90% of the conventional press residence time, such as less than 80% of the conventional press residence time, such as less than 70% of the conventional press residence time, such as less than 60% of the conventional press residence time, such as less than 50% of the conventional press residence time, such as less than 40% of the conventional press residence time, such as less than 30% of the conventional press residence time, such as less than 25% of the conventional press residence time, such as less than 20% of the conventional press residence time, and such as less than 10% of the conventional press residence time. In one embodiment the press residence time

can be selected from the group consisting of from 10% to 20% of the conventional press residence time, from 20% to 30% of the conventional press residence time, from 30% to 40% of the conventional press residence time, from 40% to 50% of the conventional press residence time, from 50% to 60% of the conventional press residence time, from 60% to 70% of the conventional press residence time, from 70% to 80% of the conventional press residence time, and from 80% to 90% of the conventional press residence time, or any combination of these intervals (wherein the conventional press residence time is set to 1 minute).

In a conventional press used in step ii) the pressure (the conventional pressure) is typically from 10 kPa to 100 kPa, such as from 10 kPa to 20 kPa, from 20 kPa to 30 kPa, from 30 kPa to 40 kPa, from 40 kPa to 50 kPa, from 50 kPa to 60 kPa, from 60 kPa to 70 kPa, from 70 kPa to 80 kPa, from 80 kPa to 90 kPa, or from 90 kPa to 100 kPa, or any combination of these intervals. In the following example the conventional pressure is set to 80 kPa and the present invention can in this embodiment relate to a method comprising a pressure for the oil extraction selected from the group consisting of less than 90% of the conventional pressure, such as less than 80% of the conventional pressure, such as less than 70% of the conventional pressure, such as less than 60% of the conventional pressure, such as less than 50% of the conventional pressure, such as less than 40% of the conventional pressure, such as less than 30% of the conventional pressure, such as less than 25% of the conventional pressure, such as less than 20% of the conventional pressure, and such as less than 10% of the conventional pressure. In one embodiment the pressure can be selected from the group consisting of from 10% to 20% of the conventional pressure, from 20% to 30% of the conventional pressure, from 30% to 40% of the conventional pressure, from 40% to 50% of the conventional pressure, from 50% to 60% of the conventional pressure, from 60% to 70% of the conventional pressure, from 70% to 80% of the conventional pressure, and from 80% to 90% of the conventional pressure, or any combination of these intervals (wherein the conventional pressure is set to 80 kPa).

In a conventional press used in step ii) the temperature (the conventional temperature) is typically from 95°C to 130°C. In the present invention the temperature can be selected from the group consisting of from 30°C to 35°C, from 35°C to 40°C, from 40°C to 45°C, from 45°C to 50°C, from 50°C to 55°C, from 55°C to 60°C, from 60°C to 65°C, from 65°C to 70°C, from 70°C to 75°C, from 75°C to 80°C, from 80°C to 85°C, from 85°C to 90°C, from 90°C to 95°C, or any combination of these intervals.

The oil content in palm kernels (prior to the one or more press cycles) is typically from 40 to 50% such as from 45 to 49%.

In a conventional press the oil content in the mannan-containing material such as in the palm kernel is typically reduced to an oil content of around 6% such as from 5 to 7% in the

mannan-containing cake such as the PKC. Typically the oil content is reduced in a first pressing cycle to around 8% to 16% such as from 12 to 14%. The first pressing cycle is typically followed by a second pressing cycle wherein the oil content is reduced to less than 7%, such as less than 6% or less than 5%.

5 In the present invention the one or more press cycles results in an oil content of from 8% to 30% in the mannan-containing cake such as the PKC (as defined in step iii) herein above). In one embodiment the mannan-containing cake such as the PKC after the one or more press cycles has an oil content of at least 8%, such as at least 9%, such as at least 10%, such as at least 11%, such as at least 12%, such as at least 13%, such as at least 14%, such as at least 15%, such as at least 16%, such as at least 18%, such as at least 20%, such as at least 22%, such as at least 24%, such as at least 26%, such as at least 28% and such as at least 29% . In another embodiment the mannan-containing cake such as the PKC after the one or more press cycles (as defined in step iii) has an oil content selected from the group consisting of from 8% to 10%, from 10% to 12%, from 12% to 14%, from 14% to 16%, from 16% to 18%, from 18% to 20%, from 20% to 22%, from 22% to 24%, from 24% to 26%, from 26% to 28% and from 28% to 30% or any combination of these intervals. When the above cited oil content of the mannan-containing cake such as of the PKC is obtained the mannan-containing cake such as the PKC is preferably not subjected to any further pressing steps. The oil content can however be reduced by other means than pressing.

Accordingly, the mannan-containing cake such as the PKC obtained after the one or more pressing steps has A) a higher oil content than conventional mannan-containing cake such as conventional PKC and B) a higher protein bioavailability and/or a higher amino acid availability and/or reduced nitrogen bound ADF and/or improved fiber digestibility compared to conventional mannan-containing cake such as conventional PKC.

15 In one embodiment the mannan-containing cake such as the PKC obtained after the one or more pressing steps has higher oil content than conventional mannan-containing cake such as conventional PKC.

In another embodiment the mannan-containing cake such as the PKC obtained after the one or more pressing steps has a higher protein bioavailability compared to conventional mannan-containing cake such as conventional PKC.

30 In another embodiment the mannan-containing cake such as the PKC obtained after the one or more pressing steps has a higher amino acid availability compared to conventional mannan-containing cake such as conventional PKC.

In another embodiment the mannan-containing cake such as the PKC obtained after the one or more pressing steps has reduced nitrogen bound ADF compared to conventional mannan-containing cake such as conventional PKC.

In another embodiment the mannan-containing cake such as the PKC obtained after the one or more pressing has improved fiber digestibility compared to conventional mannan-containing cake such as conventional PKC.

5 The mannan-containing cake such as the PKC obtained after the one or more pressing can have one or more of the properties listed herein above.

The oil content of the mannan-containing cake such as the PKC can subsequently be reduced by one or more further processing steps e.g. including one or more oil skimming steps e.g. during or after the hydrolysis step described elsewhere herein.

10 Further aspects of the method of the invention and the corresponding products are described in more detail herein below.

Preferred aspects of the invention

In a first preferred aspect (termed Aspect I) the present invention relates to a method for improving the nutritional value of mannan-containing cake comprising the steps of

15 i) providing mannan-containing cake (such as PKC) with an oil content from 8% to 16%,
ii) forming an aqueous slurry comprising the mannan-containing cake,
iii) contacting the slurry with one or more enzymes to produce a soluble hydrolysate,
iv) contacting the soluble hydrolyzate with a fermenting organism (such as yeast)
and thereby obtaining mannan-containing cake with improved nutritional value (such as half press
20 PKC++).

In one embodiment of the method according to Aspect I the mannan-containing cake is selected from the group consisting of palm kernel cake (PKC), palm kernel meal, coffee waste, guar meal, and copra cake.

The mannan-containing cake with improved nutritional value according to Aspect I has in

25 one embodiment a mannose content from 2% to 30%, such as from 2% to 20%, such as from 2% to 18%, such as from 2% to 16%, such as from 2% to 14%, such as from 2% to 12%, such as from 2% to 10%, such as from 2% to 8%, such as from 3% to 7%, such as from 4% to 7% (w/w% in a composition with 90 w/w% dry matter). In another embodiment of the method according to Aspect I the mannan-containing cake with improved nutritional value has a mannose content of less than

30 30%, such as less than 25%, such as less than 20%, such as less than 18%, such as less than 16%, such as less than 14%, such as less than 12%, such as less than 10%, such as less than 8% (w/w% in a composition with 90 w/w% dry matter).

The method according to Aspect I relates in one embodiment to mannan-containing cake with improved nutritional value with a content of non-starch polysaccharide from 2% to 40%, such

as from 2% to 30%, such as from 2% to 28%, such as from 2% to 24%, such as from 2% to 22%, such as from 2% to 20%, such as from 5% to 20%, such as from 10% to 20% and such as from 15% to 20% (w/w% in a 90 w/w% dry matter composition). In another embodiment of Aspect I the mannan-containing cake with improved nutritional value has a content of non-starch polysaccharide of less than 40%, such as less than 35%, such as less than 30%, such as less than 28%, such as less than 26%, such as less than 24%, such as less than 22%, and such as less than 20% (w/w% in a composition with 90 w/w% dry matter).

The method according to Aspect I relates in one embodiment to mannan-containing cake with improved nutritional value with lowered acid detergent fiber bound nitrogen (ADF-N) such as an ADF-N range selected from the group consisting of from 5% to 50%, such as from 5% to 35%, such as 5% to 30%, such as 5% to 25%. In another embodiment of Aspect I the mannan-containing cake with improved nutritional value has a lowered acid detergent fiber bound nitrogen (ADF-N) such as an ADF-N of less than 45%, such as less than 40%, such as less than 35%, such as less than 30% and such as less than 25%.

In an embodiment the method according to Aspect I relates to mannan-containing cake with improved nutritional value (such as half press PKC++) with lowered acid detergent fiber bound nitrogen (ADF-N) where the ADF-N value has been lowered by at least 20%, such as at least 30% such as at least 50%, such as at least 60% compared to full press mannan-containing cake after enzyme treatment/fermentation (e.g. half press PKC++ compared to full press PKC++).

The method according to Aspect I relates in one embodiment to mannan-containing cake with improved nutritional value with a crude fat content of from 8% to 16%, such as from 8% to 14%, such as from 10% to 14% and such as from 12% to 14%. In another embodiment of Aspect I the mannan-containing cake with improved nutritional value has a crude fat content of at least 8%, such as at least 9%, such as at least 10%, such as at least 11% such as at least 12%.

The method according to Aspect I relates to mannan-containing cake with improved nutritional value with a true metabolisable energy of 2400 kcal/kg to 4000 kcal/kg as determined in Example 2, such as a true metabolisable energy of 2400 kcal/kg to 3800 kcal/kg, such as a true metabolisable energy of 2400 kcal/kg to 3800 kcal/kg, such as a true metabolisable energy of 2400 kcal/kg to 3600 kcal/kg, such as a true metabolisable energy of 2400 kcal/kg to 3400 kcal/kg and such as a true metabolisable energy of 2400 kcal/kg to 3200 kcal/kg. In another embodiment of Aspect I the mannan-containing cake with improved nutritional value has a true metabolisable energy of at least 2400 kcal/kg, such as at least 2500 kcal/kg, such as at least 2600 kcal/kg, such as at least 2700 kcal/kg, such as at least 2800 kcal/kg and such as at least 2900 kcal/kg.

The method according to Aspect I relates in one embodiment to mannan-containing cake with improved nutritional value with an improvement in digestible amino acid content of from 4% to

12% such as from 6% to 10%, such as from 7% to 9% compared to full press PKC++ as defined in Example 2. In another embodiment of Aspect I the mannan-containing cake with improved nutritional value has an improvement in digestible amino acid content of at least 2%, such as at least 4%, such as at least 6%, such as at least 8% compared to full press PKC++ as defined in Example 2.

The method according to Aspect I relates in one embodiment to mannan-containing cake with improved nutritional value with an improvement in digestible cysteine content of at least 50%, such as at least 55%, such as at least 60%, such as at least 65%, such as at least 70% compared to full press PKC++ as defined in Example 2. In another embodiment Aspect I relates to mannan-containing cake with improved nutritional value with an improvement in digestible glycine content of at least 5%, such as at least 10%, such as at least 12%, such as at least 14%, such as at least 16% compared to full press PKC++ as defined in Example 2. In a further embodiment of Aspect I the mannan-containing cake with improved nutritional value has an improvement in digestible lysine content of at least 10%, such as at least 20%, such as at least 25%, such as at least 30%, such as at least 32% compared to full press PKC++ as defined in Example 2.

In yet another embodiment of Aspect I the one or more enzymes in step iii) have one or more of the enzyme activities selected from the group consisting of cellulase, mannanase and mannosidase. In a specific embodiment of Aspect I the one or more enzymes in step iii) comprises one or more enzyme activities selected from the group consisting of galactomannanase, beta-xylosidase, endo-1,4-beta xylanase, beta-glucosidase, alpha-galactosidase, beta-galactosidase, alpha-L-arabinofuranosidase, acetyl xylan esterase, ferulic acid esterase, protease and alpha-glucuronidase. In another specific embodiment of Aspect I the one or more enzymes in step iii) comprises one or more enzyme activities selected from the list consisting of alpha-amylase and amyloglycosidase.

In a preferred embodiment of Aspect I the one or more enzymes in step iii) comprises a cellulase derived or derivable from *Trichoderma reesei*. In a preferred embodiment of Aspect I the one or more enzymes in step iii) comprises a mannosidase derived or derivable from *Aspergillus niger*. In a preferred embodiment of Aspect I the one or more enzymes in step iii) comprises a mannanase derived or derivable from *Aspergillus niger* or *Talaromyces leycettanus*.

In a preferred embodiment of Aspect I the one or more enzymes in step iii) comprises a mannosidase with at least 80% sequence identity to SEQ ID NO: 2 (such as at least 85%, such as at least 90%, such as at least 95%, such as 100%). In a preferred embodiment of Aspect I the one or more enzymes in step iii) comprises a mannanase with at least 80% sequence identity to SEQ ID NO:1 or SEQ ID NO:3 (such as at least 85%, such as at least 90%, such as

at least 95%, such as 100%). In a preferred embodiment of Aspect I the one or more enzymes in step iii) comprises a cellulase with at least 80% sequence identity to SEQ ID NO: 4, SEQ ID NO: 5, SEQ ID NO: 6 or SEQ ID NO:7 (such as at least 85%, such as at least 90%, such as at least 95%, such as 100%).

5 In yet another embodiment of Aspect I the method further comprises one or more oil skimming steps.

In an embodiment of Aspect I the mannan-containing cake provided in step i) has an oil content from 9% to 15%, such as 9% to 14%, such as from 10% to 15%, such as from 10% to 14%, such as from 11 to 14% and such as from 12 to 14% (w/w%). In an embodiment of Aspect I
10 the mannan-containing cake provided in step i) has an oil content selected from the group consisting of from 8% to 9%, from 9% to 10%, from 10% to 11%, from 11% to 12%, from 12% to 13%, from 13% to 14%, from 14% to 15%, from 15% to 16% (w/w%), or any combination of these intervals. In another embodiment of Aspect I the mannan-containing cake provided in step i) has an oil content of at least 8%, such as at least 10%, such as at least 12%, such as at least 14%, such as
15 at least 15%.

In a second preferred aspect (termed Aspect II) the invention relates to a mannan-containing cake with improved nutritional value e.g. obtained or obtainable by the method according to Aspect I (e.g. half press PKC++).

In one embodiment Aspect II relates to a mannan-containing cake with improved nutritional value such as half press PKC++ comprising one or more of the features selected from the group consisting of

- i) a content of less than 30% (such as less than 25%, such as less than 20%, such as less than 15%, such as less than 10%) mannose (w/w% in a composition with 90 w/w% dry matter),
- ii) a content of less than 40% (such as less than 35%, such as less than 30%, such as less than
25 25%, such as less than 20%) non-starch polysaccharide (w/w% in a composition with 90 w/w% dry matter),
- iii) a content of less than 40% (such as less than 35%, such as less than 30%, such as less than 25%, such as less than 20%) acid detergent fiber bound nitrogen,
- iv) a content of true metabolisable energy of at least 2400 kcal/kg (such as at least 2500 kcal/kg,
30 such as at least 2600 kcal/kg, such as at least 2800 kcal/kg),
- v) improvement in digestible amino acid content of at least 4% (such as at least 5%, such as at least 6%, such as at least 7%, such as at least 8%) compared to full press PKC++ as defined in Example 2,

vi) improvement in digestible cysteine content of at least 50% (such as at least 55%, such as at least 60%, such as at least 65%, such as at least 70%) compared to full press PKC++ as defined in Example 2,

vii) improvement in digestible glycine content of at least 5% (such as at least 10%, such as at least 15%) compared to full press PKC++ as defined in Example 2 and

viii) improvement in digestible lysine content of at least 10% (such as at least 15%, such as at least 20%, such as at least 25%, such as at least 30%) compared to full press PKC++ as defined in Example 2.

In one embodiment Aspect II relates to mannan-containing cake with improved nutritional value (such as half press PKC++) containing or comprising from 2% to 30% mannan, such as from 2% to 20%, such as from 2% to 18%, such as from 2% to 16%, such as from 2% to 14%, such as from 2% to 12%, such as from 2% to 10%, such as from 2% to 8%, such as from 3% to 7%, such as from 4% to 7% (w/w% in a composition with 90 w/w% dry matter). In another embodiment Aspect II relates to mannan-containing cake with improved nutritional value (such as half press PKC++) with a mannose content of less than 30%, such as less than 25%, such as less than 20%, such as less than 18%, such as less than 16%, such as less than 14%, such as less than 12%, such as less than 10%, such as less than 8% (w/w% in a composition with 90 w/w% dry matter).

In one embodiment Aspect II relates to mannan-containing cake with improved nutritional value (such as half press PKC++) with a content of non-starch polysaccharide from 2% to 40%, such as from 2% to 30%, such as from 2% to 28%, such as from 2% to 24%, such as from 2% to 22%, such as from 2% to 20%, such as from 5% to 20%, such as from 10% to 20% and such as from 15% to 20% (w/w% in a composition with 90 w/w% dry matter). In another embodiment Aspect II relates to mannan-containing cake with improved nutritional value (such as half press PKC++) with a content of non-starch polysaccharide of less than 40%, such as less than 35%, such as less than 30%, such as less than 28%, such as less than 26%, such as less than 24%, such as less than 22%, and such as less than 20% (w/w% in a composition with 90 w/w% dry matter).

In one embodiment Aspect II relates to mannan-containing cake with improved nutritional value (such as half press PKC++) with lowered acid detergent fiber bound nitrogen (ADF-N) such as an ADF-N range selected from the group consisting of from 5% to 50%, such as from 5% to 35, such as 5% to 30%, such as 5% to 25%. In another embodiment of Aspect II the mannan-containing cake with improved nutritional value (such as half press PKC++) has lowered acid detergent fiber bound nitrogen (ADF-N) such as an ADF-N value of less than 50%, such as less

than 45%, such as less than 40%, such as less than 35%, such as less than 30%, such as less than 25%.

In an embodiment Aspect II relates to mannan-containing cake with improved nutritional value (such as half press PKC++) with lowered acid detergent fiber bound nitrogen (ADF-N) where the ADF-N value has been lowered by at least 20%, such as at least 30% such as at least 50%, such as at least 60% compared to full press mannan-containing cake after enzyme treatment/fermentation (e.g. half press PKC++ compared to full press PKC++).

In one embodiment Aspect II relates to mannan-containing cake with improved nutritional value (such as half press PKC++) with a crude fat content of from 8% to 16%, such as from 8% to 14%, such as from 10% to 14% and such as from 12% to 14%. In another embodiment Aspect II relates to mannan-containing cake with improved nutritional value (such as half press PKC++) with a crude fat content of at least 8%, such as at least 9%, such as at least 10%, such as at least 11% such as at least 12%.

In a specific embodiment of Aspect II the mannan-containing cake with improved nutritional value (such as half press PKC++) has a true metabolisable energy of 2400 kcal/kg to 4000 kcal/kg as determined in Example 2, such as a true metabolisable energy of 2400 kcal/kg to 3800 kcal/kg, such as a true metabolisable energy of 2400 kcal/kg to 3800 kcal/kg, such as a true metabolisable energy of 2400 kcal/kg to 3600 kcal/kg, such as a true metabolisable energy of 2400 kcal/kg to 3400 kcal/kg, such as a true metabolisable energy of 2400 kcal/kg to 3200 kcal/kg, and such as a true metabolisable energy of 2400 kcal/kg to 3000 kcal/kg. In another preferred embodiment of Aspect II the mannan-containing cake with improved nutritional value (such as half press PKC++) has a true metabolisable energy of at least 2400 kcal/kg, such as at least 2500 kcal/kg, such as at least 2600 kcal/kg, such as at least 2700 kcal/kg, such as at least 2800 kcal/kg and such as at least 2900 kcal/kg.

In a specific embodiment of Aspect II the mannan-containing cake with improved nutritional value (such as half press PKC++) has an improvement in digestible amino acid content of from 4% to 12% such as from 6% to 10%, such as from 7% to 9% compared to full press PKC++ as defined in Example 2. In another specific embodiment of Aspect II the mannan-containing cake with improved nutritional value (such as half press PKC++) has improved nutritional value has an improvement in digestible amino acid content of at least 2%, such as at least 4%, such as at least 6%, such as at least 8% compared to full press PKC++ as defined in Example 2. In a further embodiment of Aspect II the mannan-containing cake with improved nutritional value (such as half press PKC++) has an improvement in digestible cysteine content of at least 50%, such as at least 55%, such as at least 60%, such as at least 65%, such as at least 70% compared to full press PKC++ as defined in Example 2. In yet another embodiment of Aspect II the mannan-containing

cake with improved nutritional value (such as half press PKC++) has an improvement in digestible glycine content of at least 5%, such as at least 10%, such as at least 12%, such as at least 14%, such as at least 16% compared to full press PKC++ as defined in Example 2. In yet another embodiment of Aspect II the mannan-containing cake with improved nutritional value (such as half
5 press PKC++) has an improvement in digestible lysine content of at least 10%, such as at least 20%, such as at least 25%, such as at least 30%, such as at least 32% compared to full press PKC++ as defined in Example 2.

In a third preferred Aspect (termed Aspect III) the invention relates to an animal feed or an animal feed additive comprising the mannan-containing cake with improved nutritional value (such
10 as half press PKC++) according to any of the embodiments of Aspect II. The animal feed or an animal feed additive according to Aspect III can in one embodiment further comprise one or more enzymes such as one or more enzymes selected from the group consisting of cellulase, mannanase and mannosidase. In a specific embodiment of Aspect III the animal feed or an animal feed additive can further comprise one or more enzyme activities selected from the group
15 consisting of galactomannanase, beta-xylosidase, endo-1,4-beta xylanase, beta-glucosidase, alpha-galactosidase, beta-galactosidase, alpha-L-arabinofuranosidase, acetyl xylan esterase, ferulic acid esterase, protease and alpha-glucuronidase. In another embodiment the animal feed or an animal feed additive according to Aspect III further comprise one or more enzyme activities selected from the list consisting of alpha-amylase and amyloglycosidase. In a preferred em-
20 bodiment the animal feed or animal feed additive according Aspect III is an animal feed or an animal feed additive for a mono-gastric animal.

In a fourth preferred aspect (termed Aspect IV) the invention relates to use of the mannan-containing cake with improved nutritional value (such as half press PKC++) according to Aspect II or the animal feed or the animal feed additive according Aspect III for feeding an animal
25 such as a mono-gastric animal.

Palm kernel oil extraction by expelling pressing cycles

As previously mentioned palm kernel oil can be extracted by mechanical and/or chemical/solvent extraction. The present invention relates to mechanical extraction or a combination
30 of mechanical and chemical extraction. The description herein below is exemplifying oil extraction by expelling pressing cycle(s). Alternative methods for oil extraction can be used.

Mechanical extraction

Mechanical extraction often comprises the steps of (a) kernel pre-treatment, (b) screw-pressing, and (c) oil clarification.

5 Kernel pre-treatment

Proper kernel pre-treatment is preferred to efficiently extract the oil from the kernels by mechanical extraction. The kernels are preferably first cleaned of foreign materials that may cause damage to the one or more presses such as one or more screw-presses. Magnetic separators are commonly installed to remove metal debris, while vibrating screens can be used to
10 sieve sand, stones or other undesirable materials.

A swinging hammer grinder, breaker rolls or a combination of both or a similar device can then be used to break the kernels into small fragments. This process increases the surface area of the kernels, thus facilitating flaking. The kernel fragments are subsequently typically subjected to flaking e.g. in a roller mill. A large roller mill can e.g. consist of up to five rollers
15 mounted vertically above one another, each revolving at e.g. 200-300 rpm. The thickness of the palm kernel material is progressively reduced as it travels from the top roller to the bottom. This progressive rolling initiates rupturing of cell walls. The flakes that leave the bottom nip are in one embodiment typically from 0.25 to 0.4 mm thick.

The kernel flakes are then typically conveyed to one or more cookers such as one or
20 more stack cookers for steam conditioning, the purpose of which is to:

- adjust the moisture content to an optimum level; and/or
- rupture cell walls (initiated e.g. by rolling); and/or
- reduce viscosity of oil; and/or
- coagulate the protein to facilitate separation of the oil from protein materials.

25 The palm kernel material can subsequently flow from the top compartment down to the last compartment in series. At each stage a mechanical stirrer can agitate the palm kernel material. Steam trays can be used to heat the cookers, and live steam may be injected into each compartment when necessary. The important variables are temperature, retention time and moisture content. Cooking can e.g. be performed at a moisture content of 1% to 5% such
30 as about 3%. The temperature for the cooking can be selected from the groups consisting of from 30°C to 35°C, from 35°C to 40°C, from 40°C to 45°C, from 45°C to 50°C, from 50°C to 55°C,

from 55°C to 60°C, from 60°C to 65°C, from 65°C to 70°C, from 70°C to 75°C, from 75°C to 80°C, from 80°C to 85°C, from 85°C to 90°C, from 90°C to 95°C, from 95°C to 100°C, from 100°C to 110°C, from 110°C to 120°C or any combination of these intervals.

5 Pressing

The palm kernel material such as the properly cooked palm kernel material is then fed to one or more presses such as one or more screw-presses, which can consist of an interrupted helical thread (worm) which revolves within a stationary perforated cylinder called the cage or barrel. The palm kernel material can be forced through the barrel by the action of the revolving worms. The volume axially displaced by the worm diminishes in one embodiment from the feed-
10 ing end to the discharge end, thus compressing the meal as it passes through the barrel.

The oil and the mannan-containing cake such as the PKC are separated. The expelled oil drains e.g. through the perforation of the lining bars of the barrel, while the de-oiled mannan-containing cake such as the PKC e.g. is discharged through an annular orifice. In order to pre-
15 vent extreme temperatures that could damage the oil and cake quality, the worm-shaft can be cooled with circulating water while the barrel is cooled externally by recycling some cooled oil.

Oil clarification

The expelled oil invariably contains a certain quantity of 'fines and foots' that need to be
20 removed. The oil from the presses can be drained to a reservoir. It can then e.g. be either pumped to a decanter or revolving coarse screen to remove a large part of the solid impurities. The oil can then be pumped to a filter press to remove the remaining solids and fines in order to produce clear oil prior to storage. The mannan-containing cake such as the PKC discharged from the presses can be conveyed for bagging or bulk storage.

25 Not all crushers use the same procedure for mechanical extraction of kernel oil. There are three main variations: direct pressing such as direct screw-pressing, partial pre-treatment, and complete pre-treatment. The present invention relates to any of these types of procedures or alternatives hereto.

Direct pressing such as direct screw-pressing

Some mills crush the kernels directly in the presses without any pre-treatment. Double pressing can be required to ensure efficient oil extraction. The present invention can relate to single pressing, double pressing or multiple pressing e.g. using screw-pressing.

5

Partial pre-treatment

In the partial pre-treatment procedure the palm kernels are preferably first broken down to smaller fragments e.g. by grinding prior to pressing (such as screw-pressing). In some cases, cooking is also carried out in relation to the partial pre-treatment procedure.

10

Complete pre-treatment

The full pre-treatment processes described earlier can be carried out prior to pressing e.g. by screw-pressing. Plants with larger capacities (50-500 tons per day) often choose complete pre-treatment.

15

Animal feed

In the present invention it has been shown that a decrease in expeller pressing cycles and/or expeller pressing temperature and/or time and/or expeller pressing force of palm kernels during palm oil extraction decreases the content of ADF bound nitrogen in the protein rich press-residue that is used for animal feed.

20

Extending the time and/or force for the pressing condition removes more oil from the palm kernel and increases the crude protein content in the PKC in conventional methods. In conventional methods e.g. when the final second press is applied the protein in the PKC becomes heat damaged as indicated by a notable increase in ADF bound nitrogen

25

The present invention relates to the mannan-containing cake such as the PKC obtained according to the method of present invention – i.e. the method of improving fiber digestibility in an animal feed comprising the steps of

- i) placing a mannan-containing material such as palm kernels in one or more press machines (expellers),

- ii) pressing the mannan-containing material one or more times to extract oil from the mannan-containing material to generate oil and mannan-containing cake such as palm kernel cake,
 - iii) separating the oil from the mannan-containing cake such as the palm kernel cake, and
 - iv) optionally hydrolyzing the mannan-containing cake such as the palm kernel cake with one
- 5 or more enzymes to give an animal feed product,

wherein the compression uses less time and/or less pressure and/or lower temperature than in a conventional press.

The mannan-containing cake such as the PKC obtained in step iii) is typically used as an animal feed e.g. for feeding ruminants whereas the mannan-containing cake such as the PKC
10 obtained in step iv) is typically used as an animal feed e.g. for feeding one or more monogastric animals such as chickens including layers and/or broilers, swine and aquatic animals (either vertebrate or invertebrate).

The increased bio-availability of protein/amino acids of the mannan-containing cake such as the PKC obtained by the present invention is e.g. advantageous when the mannan-containing
15 cake such as the PKC is used as an animal feed product.

The mannan-containing cake such as the PKC according to the present invention can be used as an animal feed product or an animal feed additive.

The solids remaining after enzymatic hydrolysis and fermentation described herein below have an improved nutritional value as an animal feed compared to the un-treated manna-
20 containing material, e.g., un-treated PKC after the one or more pressing cycles. During hydrolysis and fermentation the protein content is increased through the removal of polysaccharides just as the digestibility of the remaining polysaccharides is increased. Furthermore, the protein composition is improved by the yeast residues.

Accordingly, the solids remaining after the process of the first aspect are very suitable as
25 an animal feed or for inclusion in an animal feed composition.

The term animal includes all animals. In one embodiment human beings are however excluded. Examples of animals are non-ruminants, and ruminants. Ruminant animals include, for example, animals such as sheep, goats, and cattle, e.g. beef cattle, cows, and young calves. In a particular embodiment, the animal is a non-ruminant animal. Non-ruminant animals include
30 mono-gastric animals, e.g. pigs or swine (including, but not limited to, piglets, growing pigs, and sows); poultry such as turkeys, ducks and chicken (including but not limited to broiler chicks, layers); horses (including but not limited to hotbloods, coldbloods and warm bloods), young calves; and fish (including but not limited to salmon, trout, tilapia, catfish and carps; and crustaceans (including but not limited to shrimps and prawns).

The term feed or feed composition means any compound, preparation, mixture, or composition suitable for, or intended for intake by an animal.

Hydrolysis Pretreatment

5 The mannan-containing material and/or the mannan-containing cake such as the PKC derived from the one or more pressing steps may optionally be pretreated before one or more hydrolysis steps and/or optionally one or more fermentation steps. The goal of pretreatment is to separate and/or release non-mannan constituents such as cellulose, hemicellulose and/or lignin and this way improve the rate of hydrolysis of the cellulose and hemicellulose compounds. Pretreat-
10 ment methods such as wet-oxidation and alkaline pretreatment targets lignin, while dilute acid and auto-hydrolysis targets hemicellulose.

 The mannan-containing material and/or the mannan-containing cake such as the PKC derived from the one or more pressing steps may be chemically, mechanically and/or biologically pre-
15 treated before hydrolysis and/or fermentation. Mechanical treatment (often referred to as physical treatment) may be used alone or in combination with subsequent or simultaneous hydrolysis, especially enzymatic hydrolysis.

 Preferably, the chemical, mechanical and/or biological pretreatment is carried out prior to the hydrolysis and/or fermentation. Alternatively, the chemical, mechanical and/or biological pre-
20 treatment may be carried out simultaneously with hydrolysis, such as simultaneously with addition of one or more of the enzyme activities mentioned below, to release, e.g., fermentable sugars, such as mannose, glucose and/or maltose.

 The term "chemical treatment" refers to any chemical pretreatment which promotes the separation and/or release of cellulose, hemicellulose and/or lignin. Examples of suitable chemical pretreatments include treatment with; for example, dilute acid, lime, alkali, organic solvent, ammo-
25 nia, sulfur dioxide, carbon dioxide. Further, wet oxidation and pH-controlled hydrothermolysis are also considered chemical pretreatment.

 The chemical pretreatment may be an acid treatment, more preferably, a continuous dilute and/or mild acid treatment, such as, treatment with sulfuric acid, or another organic acid, such as acetic acid, citric acid, tartaric acid, succinic acid, hydrogen chloride or mixtures thereof.
30 Other acids may also be used. Mild acid treatment means that the treatment pH lies in the range from 1-5, preferably pH 1-3.

Alkaline chemical pretreatment with base, e.g., NaOH, Na₂CO₃ and/or ammonia or the like, is also contemplated according to the invention. Pretreatment methods using ammonia are described in, e.g., WO 2006/110891, WO 2006/11899, WO 2006/11900, WO 2006/110901 (which are hereby incorporated by reference)

5 Wet oxidation techniques involve use of oxidizing agents, such as: sulphite based oxidizing agents or the like. Examples of solvent pretreatments include treatment with DMSO (Dimethyl Sulfoxide) or the like. Chemical pretreatment is generally carried out for 1 to 60 minutes, such as from 5 to 30 minutes, but may be carried out for shorter or longer periods of time dependent on the material to be pretreated.

10 Other examples of suitable pretreatment methods are described by Schell et al. (2003) Appl. Biochem and Biotechn. Vol. 105-108, p. 69-85, Mosier et al. Bioresource Technology 96 (2005) 673-686, Ahning et al. in WO2006032282 and WO200160752, Foody et al. in WO2006034590, and Ballesteros et al. in US publication no. 2002/0164730, which references are hereby all incorporated by reference.

15 The term "mechanical pretreatment" refers to any mechanical (or physical) treatment which promotes the separation and/or release of cellulose, hemicellulose and/or lignin from the mannan-containing material. For example, mechanical pretreatment includes various types of milling, irradiation, steaming/steam explosion, wet oxidation, and other hydrothermal treatments.

 Mechanical pretreatment includes comminution (mechanical reduction of the size). Comminution includes dry milling, wet milling and vibratory ball milling. Mechanical pretreatment may involve high pressure and/or high temperature (steam explosion). In an embodiment of the invention high pressure means pressure in the range from 300 to 600 psi, preferably 400 to 500 psi, such as around 450 psi. In an embodiment of the invention high temperature means temperatures in the range from about 100 to 300°C, preferably from about 140 to 235°C. In a preferred embodiment
20 mechanical pretreatment is a batch-process, steam gun hydrolyzer system which uses high pressure and high temperature as defined above. A Sunds Hydrolyzer (available from Sunds Defibrator AB (Sweden) may be used for this.

 As used in the present invention the term "biological pretreatment" refers to any biological pretreatment which promotes the degradation of the mannan-containing material. Biological pretreatment techniques can involve applying lignin-solubilizing microorganisms (see, for example,
30 Hsu, T.-A., 1996, Pretreatment of biomass, in *Handbook on Bioethanol: Production and Utilization*, Wyman, C. E., ed., Taylor & Francis, Washington, DC, 179-212; Ghosh, P., and Singh, A., 1993, Physicochemical and biological treatments for enzymatic/microbial conversion of lignocellulosic biomass, *Adv. Appl. Microbiol.* 39: 295-333; McMillan, J. D., 1994, Pretreating lignocellulosic bio-

mass: a review, in *Enzymatic Conversion of Biomass for Fuels Production*, Himmel, M. E., Baker, J. O., and Overend, R. P., eds., ACS Symposium Series 566, American Chemical Society, Washington, DC, chapter 15; Gong, C. S., Cao, N. J., Du, J., and Tsao, G. T., 1999, Ethanol production from renewable resources, in *Advances in Biochemical Engineering/Biotechnology*, Scheper, T., ed., Springer-Verlag Berlin Heidelberg, Germany, 65: 207-241; Olsson, L., and Hahn-Hagerdal, B., 1996, Fermentation of lignocellulosic hydrolysates for ethanol production, *Enz. Microb. Tech.* 18: 312-331; and Vallander, L., and Eriksson, K.-E. L., 1990, Production of ethanol from lignocellulosic materials: State of the art, *Adv. Biochem. Eng./Biotechnol.* 42: 63-95).

In a preferred embodiment wherein the mannan-containing material is palm kernel meal or palm kernel cake the mannan-containing material is not subjected to a hydrolysis pretreatment.

Hydrolysis

The mannan-containing material and/or the mannan-containing cake such as the PKC derived from the one or more pressing steps can be hydrolyzed to break down mannan, cellulose and hemicellulose into sugars and/or oligosaccharides. The hydrolysis may be performed prior to and/or simultaneously with a fermentation step.

According to the invention the mannan-containing material and/or the mannan-containing cake such as the PKC derived from the one or more pressing steps to be hydrolyzed is contacted by a cellulase and/or a mannanase and/or a mannosidase. The mannan-containing material and/or the mannan-containing cake such as the PKC derived from the one or more pressing steps can be converted into a soluble hydrolyzate, in a process comprising a) forming an aqueous slurry comprising the biomass substrate, b) contacting the slurry with an enzyme composition comprising the enzyme activities cellulose and/or mannanase and/or mannosidase and producing a soluble hydrolyzate.

In an embodiment one or more enzymes of protease, galactomannanase, beta-xylosidase, endo-1,4-beta xylanase, beta-glucosidase, alpha-galactosidase, alpha-L-arabinofuranosidase, acetyl xylan esterase, ferulic acid esterase and alpha-glucuronidase are present. Also alpha-amylase, glucoamylase and/or the like may be present during hydrolysis and/or fermentation as the mannan-containing material may include some starch. Galactomannanase is preferably applied if the substrate comprises galactomannan.

The enzyme(s) used for hydrolysis is(are) capable of directly or indirectly converting mannan and other carbohydrate polymers into fermentable sugars which can be fermented into a de-

sired fermentation product, such as ethanol. Suitable enzymes are described in the "Enzymes"-section below.

The dry solids content during hydrolysis may be in the range from 5-50 wt-%, preferably 10-40 wt-%, preferably 20-30 wt-%. Hydrolysis may in a preferred embodiment be carried out as a fed batch process where the mannan-containing material (substrate) is fed gradually to an enzyme containing hydrolysis solution. The mannan-containing material may be supplied to the enzyme containing hydrolysis solution either in one or more distinct batches, as one or more distinct continuous flows or as a combination of one or more distinct batches and one or more distinct continuous flows.

Suitable hydrolysis time, temperature and pH conditions can readily be determined by one skilled in the art. The temperature should be decided with regard to the optimum temperatures of the applied enzymes. Generally a temperature between 25°C and 70°C, preferably between 40°C and 60°C or 65°C, more preferably between 45°C and 55°C, especially around 50°C will be suitable. In another embodiment the temperature is preferably around 60°C or 65°C. The process is preferably carried out at a pH in the range from 3-8, preferably pH 4-6, especially around pH 5, e.g., in the range of pH 4.5 to 5.5.. Preferably, hydrolysis is carried out for between 12 and 144 hours, preferable 16 to 120 hours, more preferably between 24 and 96 hours, such as between 32 and 48 hours.

Preferably the hydrolysis is allowed to proceed for a time and under conditions allowing that at least 60%, at least 70%, at least 75%, at least 80%, at least 85%, or even at least 90% of the mannan content of the mannan-containing material is degraded to mannose and/or for a time and under conditions allowing that at least 60%, at least 70%, at least 75%, at least 80%, at least 85%, at least 90%, or even at least 95% of the cellulose content of the mannan-containing material is degraded to glucose.

During the hydrolysis of substrates such as oil palm or palm kernel cake, palm kernel meal and/ or copra cake residual oil is released and can easily be isolated from the soluble hydrolyzate. Thus in a preferred embodiment the process comprises isolating or skimming of an oil fraction from the soluble hydrolyzate, e.g., where the mannan-containing material is derived from a plant comprising plant oil, such as a residue produced in a vegetable oil extraction process.

Fermentation

According to the invention the hydrolyzed mannan-containing material and/or the mannan-containing cake such as the PKC derived from the one or more pressing steps can optionally be

fermented by at least one fermenting organism capable of fermenting fermentable sugars, such as mannose, glucose, and galactose directly or indirectly into a desired fermentation product. Accordingly, in an embodiment a mannan-containing material is converted into a soluble hydrolyzate, in a process comprising a) forming an aqueous slurry comprising the biomass substrate, b) contacting the slurry with an enzyme composition comprising the enzyme activities cellulase, mannanase, and mannosidase and producing a soluble hydrolyzate, and c) contacting the soluble hydrolyzate with a fermenting organism to produce a fermentation product.

Preferred for ethanol fermentation is yeast of the species *Saccharomyces cerevisiae*, preferably strains which are resistant towards high levels of ethanol, i.e., up to, e.g., about 10, 12 or 15 vol. % ethanol or more, such as 20 vol. % ethanol. The fermentation is preferably ongoing for between 8 to 96 hours, preferably 12 to 72, more preferable from 24 to 48 hours.

In an embodiment the fermentation is carried out at a temperature between 20 to 40°C, preferably 26 to 34°C, in particular around 32°C. In an embodiment the pH is from pH 3 to 6, preferably around pH 4 to 5.

According to the invention hydrolysis and fermentation may be carried out simultaneously or sequentially. In an embodiment there is no separate holding stage for the hydrolysis, meaning that the hydrolysing enzyme(s) and the fermenting organism are added together. When the fermentation (e.g., ethanol fermentation using *Saccharomyces* yeast) is performed simultaneous with hydrolysis the temperature is preferably between 26°C and 35°C, more preferably between 30°C and 34°C, such as around 32°C. A temperature program comprising at least two holding stages at different temperatures may be applied according to the invention.

The process of the invention as well as the individual steps may be performed as a batch, fed-batch or as a continuous process/process step. Preferably the fermentation step is performed as a continuous fermentation.

Recovery

Subsequent to fermentation the fermentation product may be separated from the fermentation broth. The broth may be distilled to extract the fermentation product or the fermentation product may be extracted from the fermentation broth by micro or membrane filtration techniques. Alternatively the fermentation product may be recovered by stripping. Recovery methods are well known in the art. Oil recovery can also be included e.g. by use of one or more oil skimming steps.

Fermentation Products

The process of the invention may be used for producing any fermentation product. Especially contemplated fermentation products include alcohols (e.g., ethanol, methanol, butanol); organic acids (e.g., acrylic acid, citric acid, acetic acid, itaconic acid, lactic acid, gluconic acid); ketones (e.g., acetone); amino acids (e.g., glutamic acid); gases (e.g., H₂ and CO₂); antibiotics (e.g., penicillin and tetracycline); enzymes; vitamins (e.g., riboflavin, B12, beta-carotene); and hormones.

In a preferred embodiment the fermentation product is an alcohol, especially ethanol. The fermentation product, such as ethanol, obtained according to the invention, may preferably be fuel alcohol/ethanol. However, in the case of ethanol it may also be used as potable ethanol.

Fermenting Organism

The term "fermenting organism" refers to any organism, including bacterial and fungal organisms, suitable for producing a desired fermentation product. Especially suitable fermenting organisms according to the invention are able to ferment, i.e., convert, C6 sugars, such as mannose and glucose, directly or indirectly into the desired fermentation product. Also suitable are fermenting organisms capable of converting C5 sugars such as xylose into a desired fermentation product. Examples of fermenting organisms include fungal organisms, especially yeast. Preferred yeast includes strains of *Saccharomyces* spp., in particular a strain of *Saccharomyces cerevisiae* or *Saccharomyces uvarum*; a strain of *Pichia*, preferably *Pichia stipitis*, such as *Pichia stipitis* CBS 5773; a strain of *Candida*, in particular a strain of *Candida utilis*, *Candida diddensii*, or *Candida boidinii*. Other contemplated yeast includes strains of *Zymomonas*; *Hansenula*, in particular *H. anomala*; *Kluyveromyces*, in particular *K. fragilis*; and *Schizosaccharomyces*, in particular *S. pombe*.

Commercially available yeast includes, e.g., ETHANOL RED™ yeast (available from Fermentis/Lesaffre, USA), FALI (available from Fleischmann's Yeast, USA), SUPERSTART and THERMOSACC™ fresh or dry yeast (available from Ethanol Technology, WI, USA), BIOFERM AFT and XR (available from NABC - North American Bioproducts Corporation, GA, USA), GERT STRAND (available from Gert Strand AB, Sweden), and FERMIOL (available from DSM Specialties). ANQI YEAST (available from Anqi yeast (CHIFENG) CO., LTD, China).

Compositions

The present invention also relates to compositions suitable for use in the processes of the invention, said compositions comprising one or more cellulases, one or more mannanases,

and one or more mannosidases. The composition may comprise additional enzymatic activities, such as one or more selected from the group consisting of acetylxy lan esterase, alpha-glucuronidase, arabinofuranosidase, aminopeptidase, amylase, carbohydase, carboxypeptidase, catalase, cellulase, chitinase, cutinase, cyclodextrin glycosyltransferase, deoxyribonuclease, esterase, ferulic acid esterase, galactomannanase alpha-galactosidase, beta-galactosidase, beta-xylosidase, glucoamylase, alpha-glucosidase, beta-glucosidase, haloperoxidase, invertase, laccase, lipase, mannosidase, oxidase, pectinolytic enzyme, peptidoglutaminase, peroxidase, phytase, polyphenoloxidase, proteolytic enzyme, protease, ribonuclease, transglutaminase, xylanase or xylosidase.

Enzymes

Even though not specifically mentioned in context of a process of the invention, it is to be understood that the enzymes (as well as other compounds) are used in an “effective amount”.

Cellulases

The term “cellulases” as used herein are understood as comprising the cellobiohydrolases (EC 3.2.1.91), e.g., cellobiohydrolase I and cellobiohydrolase II, as well as the endo-glucanases (EC 3.2.1.4) and beta-glucosidases (EC 3.2.1.21).

In order to be efficient, the digestion of cellulose and hemicellulose requires several types of enzymes acting cooperatively. At least three categories of enzymes are necessary to convert cellulose into fermentable sugars: endo-glucanases (EC 3.2.1.4) that cut the cellulose chains at random; cellobiohydrolases (EC 3.2.1.91) which cleave cellobiosyl units from the cellulose chain ends and beta-glucosidases (EC 3.2.1.21) that convert cellobiose and soluble cellodextrins into glucose. Among these three categories of enzymes involved in the biodegradation of cellulose, cellobiohydrolases are the key enzymes for the degradation of native crystalline cellulose. The term “cellobiohydrolase I” is defined herein as a cellulose 1,4-beta-cellobiosidase (also referred to as exo-glucanase, exo-cellobiohydrolase or 1,4-beta-cellobiohydrolase) activity, as defined in the enzyme class EC 3.2.1.91, which catalyzes the hydrolysis of 1,4-beta-D-glucosidic linkages in cellulose and cellotetraose, by the release of cellobiose from the non-reducing ends of the chains. The definition of the term “cellobiohydrolase II activity” is identical, except that cellobiohydrolase II attacks from the reducing ends of the chains.

Endoglucanases (EC No. 3.2.1.4) catalyses endo hydrolysis of 1,4- beta -D-glycosidic link-
ages in cellulose, cellulose derivatives (such as carboxy methyl cellulose and hydroxy ethyl cellu-
lose), lichenin, beta-1,4 bonds in mixed beta-1,3 glucans such as cereal beta-D-glucans or xyloglu-
cans and other plant material containing cellulosic parts. The authorized name is endo-1,4- beta -
5 D-glucan 4-glucano hydrolase, but the abbreviated term endoglucanase is used in the present
specification.

The cellulases may comprise a carbohydrate-binding module (CBM) which enhances the
binding of the enzyme to a cellulose-containing fiber and increases the efficacy of the catalytic ac-
tive part of the enzyme. A CBM is defined as contiguous amino acid sequence within a carbohy-
10 drate-active enzyme with a discreet fold having carbohydrate-binding activity. For further infor-
mation of CBMs see the CAZy internet server (Supra) or Tomme et al., (1995) in *Enzymatic Deg-
radation of Insoluble Polysaccharides* (Saddler, J.N. & Penner, M., eds.), Cellulose-binding do-
mains: classification and properties. pp. 142-163, American Chemical Society, Washington.

The cellulase activity may, in a preferred embodiment, be derived from a fungal source,
15 such as a strain of the genus *Trichoderma*, preferably a strain of *Trichoderma reesei*; a strain of the
genus *Humicola*, such as a strain of *Humicola insolens*; or a strain of *Chrysosporium*, preferably a
strain of *Chrysosporium lucknowense*.

In a preferred embodiment the cellulases may a preparation as defined in co-pending ap-
plication US application US 60/941,251, which is hereby incorporated by reference. In a pre-
20 ferred embodiment the cellulase preparation comprising a polypeptide having cellulolytic en-
hancing activity (GH61A), preferably the one disclosed as SEQ ID NO:2 in WO 2005/074656.
The cellulase preparation may further comprise a beta-glucosidase, such as the fusion protein
disclosed in US 60/832,511. In an embodiment the cellulase preparation also comprises a CBH
II, preferably *Thielavia terrestris* cellobiohydrolase II CEL6A. In an embodiment the cellulase
25 preparation also comprises a cellulase enzymes preparation, preferably the one derived from
Trichoderma reesei. In a preferred embodiment the cellulase preparation is Cellulase prepara-
tion A used in Example 1 and disclosed in co-pending US application US 60/941,251.

In an embodiment the cellulase preparation is the commercially available product CELLU-
CLAST® 1.5L or CELLUZYME™ (Novozymes A/S, Denmark).

30 The cellulase may be dosed in the range from 0.1-100 FPU per gram dry solids (DS), pref-
erably 0.5-50 FPU per gram DS, especially 1-20 FPU per gram DS.

Mannanases

In the context of the present invention a mannanase is a beta-mannanase and defined as an enzyme belonging to EC 3.2.1.78.

Mannanases have been identified in several *Bacillus*, *Aspergillus* and *Talaromyces* organisms. For example, Talbot et al., Appl. Environ. Microbiol., Vol.56, No. 11, pp. 3505-3510 (1990) describes a beta-mannanase derived from *Bacillus stearothermophilus* having an optimum pH of 5.5-7.5. Mendoza et al., World J. Microbiol. Biotech., Vol. 10, No. 5, pp. 551-555 (1994) describes a beta-mannanase derived from *Bacillus subtilis* having an optimum activity at pH 5.0 and 55°C. JP-03047076 discloses a beta-mannanase derived from *Bacillus* sp., having an optimum pH of 8-10. JP-63056289 describes the production of an alkaline, thermostable beta-mannanase. JP-08051975 discloses alkaline beta-mannanases from alkalophilic *Bacillus* sp. AM-001. A purified mannanase from *Bacillus amyloliquefaciens* is disclosed in WO 97/11164. WO 94/25576 discloses an enzyme from *Aspergillus aculeatus*, CBS 101.43, exhibiting mannanase activity and WO 93/24622 discloses a mannanase isolated from *Trichoderma reesei*.

The mannanase may be derived from a strain of the genus *Bacillus*, such as the amino acid sequence having the sequence deposited as GENESEQP accession number AAY54122 and shown herein as SEQ ID NO:1 or an amino acid sequence which is homologous to this amino acid sequence.

A suitable commercial mannanase preparation is Mannaway® produced by Novozymes A/S.

Mannosidases

In the context of the present invention a mannosidase, such as a beta-mannosidase, is defined as an enzyme belonging to EC 3.2.1.25.

A suitable mannosidase preparation can be obtained from *Aspergillus niger*, such as e.g. a mannosidase preparation comprising the mannosidase having the amino acid sequence deposited as Swiss-Prot accession number A2QWU and shown herein as SEQ ID NO:2., or an amino acid sequence which is homologous to this amino acid sequence.

Hemicellulases

Any hemicellulase suitable for use in hydrolyzing hemicellulose may be used. Preferred hemicellulases include xylanases, arabinofuranosidases, acetyl xylan esterase, feruloyl esterase, glucuronidases, endo-galactanase, endo or exo arabinases, exo-galactanases, and mixtures of two or more thereof. Preferably, the hemicellulase for use in the present invention is an exo-acting hemicellulase, and more preferably, the hemicellulase is an exo-acting hemicellulase which has the ability to hydrolyze hemicellulose under acidic conditions of below pH 7, preferably pH 3-7. An example of hemicellulase suitable for use in the present invention includes ULTRAFLO L (available from Novozymes A/S, Denmark).

Arabinofuranosidase (EC 3.2.1.55) catalyses the hydrolysis of terminal non-reducing alpha-L-arabinofuranoside residues in alpha -L-arabinosides.

Galactanase (EC 3.2.1.89), arabinogalactan endo-1,4-beta-galactosidase, catalyses the endohydrolysis of 1,4-D-galactosidic linkages in arabinogalactans.

Pectinase (EC 3.2.1.15) catalyses the hydrolysis of 1,4-alpha-D-galactosiduronic linkages in pectate and other galacturonans. Xyloglucanase catalyses the hydrolysis of xyloglucan.

The hemicellulase may be added in an amount effective to hydrolyze hemicellulose, such as, in amounts from about 0.001 to 0.5 wt.-% of dry solids (DS), more preferably from about 0.05 to 0.5 wt.-% of DS.

Alpha-Amylase

According to the invention an alpha-amylase may be used. In a preferred embodiment the alpha-amylase is an acid alpha-amylase, e.g., fungal acid alpha-amylase or bacterial acid alpha-amylase. The term "acid alpha-amylase" means an alpha-amylase (E.C. 3.2.1.1) which added in an effective amount has activity optimum at a pH in the range of 3 to 7, preferably from 3.5 to 6, or more preferably from 4-5.

Bacterial Alpha-Amylase

According to the invention the bacterial alpha-amylase is preferably derived from the genus *Bacillus*.

In a preferred embodiment the *Bacillus* alpha-amylase is derived from a strain of *B. licheniformis*, *B. amyloliquefaciens*, *B. subtilis* or *B. stearothermophilus*, but may also be derived from other *Bacillus* sp. Specific examples of contemplated alpha-amylases include the *Bacillus licheni-*

formis alpha-amylase shown in SEQ ID NO:4 in WO 99/19467, the *Bacillus amyloliquefaciens* alpha-amylase SEQ ID NO:5 in WO 99/19467 and the *Bacillus stearothermophilus* alpha-amylase shown in SEQ ID NO:3 in WO 99/19467 (all sequences hereby incorporated by reference). In an embodiment of the invention the alpha-amylase may be an enzyme having a degree of identity of at least 60%, preferably at least 70%, more preferred at least 80%, even more preferred at least 90%, such as at least 95%, at least 96%, at least 97%, at least 98% or at least 99% to any of the sequences shown in SEQ ID NOS: 1, 2 or 3, respectively, in WO 99/19467.

The *Bacillus* alpha-amylase may also be a variant and/or hybrid, especially one described in any of WO 96/23873, WO 96/23874, WO 97/41213, WO 99/19467, WO 00/60059, and WO 02/10355 (all documents hereby incorporated by reference). Specifically contemplated alpha-amylase variants are disclosed in US patent nos. 6,093,562, 6,297,038 or US patent no. 6,187,576 (hereby incorporated by reference) and include *Bacillus stearothermophilus* alpha-amylase (BSG alpha-amylase) variants having a deletion of one or two amino acid in positions R179 to G182, preferably a double deletion disclosed in WO 1996/023873 – see e.g., page 20, lines 1-10 (hereby incorporated by reference), preferably corresponding to delta(181-182) compared to the wild-type BSG alpha-amylase amino acid sequence set forth in SEQ ID NO:3 disclosed in WO 99/19467 or deletion of amino acids R179 and G180 using SEQ ID NO:3 in WO 99/19467 for numbering (which reference is hereby incorporated by reference). Even more preferred are *Bacillus* alpha-amylases, especially *Bacillus stearothermophilus* alpha-amylase, which have a double deletion corresponding to delta(181-182) and further comprise a N193F substitution (also denoted I181* + G182* + N193F) compared to the wild-type BSG alpha-amylase amino acid sequence set forth in SEQ ID NO:3 disclosed in WO 99/19467.

Fungal Alpha-Amylase

Fungal alpha-amylases include alpha-amylases derived from a strain of the genus *Aspergillus*, such as, *Aspergillus oryzae*, *Aspergillus niger* and *Aspergillus kawachii* alpha-amylases.

A preferred acidic fungal alpha-amylase is a Fungamyl-like alpha-amylase which is derived from a strain of *Aspergillus oryzae*. According to the present invention, the term "Fungamyl-like alpha-amylase" indicates an alpha-amylase which exhibits a high identity, i.e. at least 70%, at least 75%, at least 80%, at least 85% at least 90%, at least 95%, at least 96%, at least 97%, at least 98%, at least 99% or even 100% identity to the mature part of the amino acid sequence shown in SEQ ID NO:10 in WO 96/23874.

Another preferred acidic alpha-amylase is derived from a strain *Aspergillus niger*. In a preferred embodiment the acid fungal alpha-amylase is the one from *A. niger* disclosed as

"AMYA_ASPNG" in the Swiss-prot/TrEMBL database under the primary accession no. P56271 and described in WO 89/01969 (Example 3).

Other contemplated wild-type alpha-amylases include those derived from a strain of the genera *Rhizomucor* and *Meripilus*, preferably a strain of *Rhizomucor pusillus* (WO 2004/055178 incorporated by reference) or *Meripilus giganteus*. In a preferred embodiment the alpha-amylase is derived from *Aspergillus kawachii* and disclosed by Kaneko et al. J. Ferment. Bioeng. 81:292-298(1996) "Molecular-cloning and determination of the nucleotide-sequence of a gene encoding an acid-stable alpha-amylase from *Aspergillus kawachii*."; and further as EMBL:#AB008370.

Glucoamylases

A glucoamylase used according to the invention may be derived from any suitable source, e.g., derived from a microorganism or a plant. Preferred glucoamylases are of fungal or bacterial origin, selected from the group consisting of *Aspergillus* glucoamylases, in particular *A. niger* G1 or G2 glucoamylase (Boel et al. (1984), EMBO J. 3 (5), p. 1097-1102), or variants thereof, such as those disclosed in WO 92/00381, WO 00/04136 and WO 01/04273 (from Novozymes, Denmark); the *A. awamori* glucoamylase disclosed in WO 84/02921, *A. oryzae* glucoamylase (Agric. Biol. Chem. (1991), 55 (4), p. 941-949), or variants or fragments thereof. Other *Aspergillus* glucoamylase variants include variants with enhanced thermal stability: G137A and G139A (Chen et al. (1996), Prot. Eng. 9, 499-505); D257E and D293E/Q (Chen et al. (1995), Prot. Eng. 8, 575-582); N182 (Chen et al. (1994), Biochem. J. 301, 275-281); disulphide bonds, A246C (Fierobe et al. (1996), Biochemistry, 35, 8698-8704; and introduction of Pro residues in position A435 and S436 (Li et al. (1997), Protein Eng. 10, 1199-1204).

Other glucoamylases include *Athelia rolfsii* (previously denoted *Corticium rolfsii*) glucoamylase (see US patent no. 4,727,026 and (Nagasaka,Y. et al. (1998) "Purification and properties of the raw-starch-degrading glucoamylases from *Corticium rolfsii*, Appl Microbiol Biotechnol 50:323-330), *Talaromyces* glucoamylases, in particular derived from *Talaromyces emersonii* (WO 99/28448), *Talaromyces leycettanus* (US patent no. Re. 32,153), *Talaromyces duponti*, *Talaromyces thermophilus* (US patent no. 4,587,215).

Bacterial glucoamylases contemplated include glucoamylases from the genus *Clostridium*, in particular *C. thermoamylolyticum* (EP 135,138), and *C. thermohydrosulfuricum* (WO 86/01831) and *Trametes cingulata* disclosed in WO 2006/069289 (which is hereby incorporated by reference).

Also hybrid glucoamylase are contemplated according to the invention. Examples the hybrid glucoamylases disclosed in WO 2005/045018. Specific examples include the hybrid glucoamylase disclosed in Table 1 and 4 of Example 1 (which hybrids are hereby incorporated by reference.).

5 Contemplated are also glucoamylases which exhibit a high identity to any of above mention glucoamylases, i.e., at least 70%, at least 75%, at least 80%, at least 85% at least 90%, at least 95%, at least 96%, at least 97%, at least 98%, at least 99% or even 100% identity to the mature enzymes sequences.

Commercially available compositions comprising glucoamylase include AMG 200L; AMG
10 300 L; SANTM SUPER, SANTM EXTRA L, SPIRIZYMETM PLUS, SPIRIZYMETM FUEL, SPIRIZYMETM B4U and AMGTM E (from Novozymes A/S); OPTIDEXTM 300 (from Genencor Int.); AMIGASETM and AMIGASETM PLUS (from DSM); G-ZYMETM G900, G-ZYMETM and G990 ZR (from Genencor Int.). Glucoamylases may in an embodiment be added in an amount of 0.02-20 AGU/g DS, preferably 0.1-10 AGU/g DS, especially between 1-5 AGU/g DS, such as 0.5 AGU/g
15 DS.

Protease

Suitable proteases include those of bacterial, fungal, plant, viral or animal origin e.g. vegetable or microbial origin. Microbial origin is preferred. Chemically modified or protein engi-
20 neered mutants are included. It may be an alkaline protease, such as a serine protease or a metalloprotease. A serine protease may for example be of the S1 family, such as trypsin, or the S8 family such as subtilisin. A metalloproteases protease may for example be a thermolysin from e.g. family M4 or other metalloprotease such as those from M5, M7 or M8 families.

The term "subtilases" refers to a sub-group of serine protease according to Siezen et
25 al., Protein Engng. 4 (1991) 719-737 and Siezen et al. Protein Science 6 (1997) 501-523. Serine proteases are a subgroup of proteases characterized by having a serine in the active site, which forms a covalent adduct with the substrate. The subtilases may be divided into 6 subdivisions, i.e. the Subtilisin family, the Thermitase family, the Proteinase K family, the Lantibiotic peptidase family, the Kexin family and the Pyrolysin family.

30 Examples of commercially available proteases include Ronozyme® ProAct (DSM Nutritional Products).

PREFERRED EMBODIMENTS

Preferred embodiments are disclosed in the sets of items listed herein below.

ITEM SET I:

1. A method of improving bio-availability of protein in an animal feed comprising the steps of

- 5 i) placing a mannan-containing material from the tribe *Cocoseae* in one or more press machines,
- ii) pressing the mannan-containing material one or more times to extract oil from the mannan-containing material to generate oil and mannan-containing cake,
- iii) separating the oil from the mannan-containing cake, and
- 10 iv) optionally subsequently hydrolyzing the mannan-containing cake with one or more enzymes

wherein

- a) the oil content of the mannan-containing cake obtained from step iii) is from 8% to 16%;
- and
- 15 b) no further pressing is performed to decrease the oil content of the mannan-containing cake;
- and
- c) the mannan-containing cake obtained in step iii) or step iv) is used as an animal feed.

20 2. The method according to item 1, wherein the mannan-containing material is palm kernel material and wherein the mannan-containing cake is palm kernel cake.

3. The method according to any of items 1 and 2, wherein the animal feed has an improved amino acid availability.

25 4. The method according to any of items 1 to 3, wherein the animal feed has reduced acid detergent fiber bound nitrogen (ADF-N).

5. The method according to item 4, wherein the ADF-N is less than 30, such as less than 25, such as less than 20, such as less than 15, such as less than 10, such as less than 5.

30 6. The method according to any of items 1 to 5, wherein the one or more enzymes in step iv) in claim 1 have one or more of the enzyme activities selected from the group consisting of cellulase, mannanase and mannosidase.

7. The method according to any of items 1 to 6, wherein the one or more enzymes in step iv) in claim 1 comprises one or more enzyme activities selected from the list consisting of galactomannanase, beta-xylosidase, endo-1,4-beta xylanase, beta-glucosidase, alpha-galactosidase, beta-galactosidase, alpha-L-arabinofuranosidase, acetyl xylan esterase, ferulic acid esterase, protease and alpha-glucuronidase.
8. The method according to any of items 1 to 7, wherein the one or more enzymes in step iv) in claim 1 comprises one or more enzyme activities selected from the list consisting of alpha-amylase and amyloglycosidase.
9. The method according to any of items 1 to 8, wherein the cellulase is derived from *Trichoderma reesei*.
10. The method according to any of items 1 to 9, wherein the mannosidase is derived from *Aspergillus niger*.
11. The method according to any of claims 1 to 9, wherein the mannanase is derived from *Bacillus sp.*
12. The method according to any of items 1 to 11, wherein the mannosidase is at least 80% identical to SEQ ID NO:2.
13. The method according to any of items 1 to 12, wherein the mannanase is at least 80% identical to SEQ ID NO:1.
14. The method according to any of items 1 to 13, wherein the method further comprises one or more oil skimming steps.
15. An animal feed obtained or obtainable by the method according to any of items 1 to 14.
16. An animal feed comprising palm kernel cake wherein the palm kernel cake has an ADF-N of less than 30, such as less than 25, such as less than 20, such as less than 15, such as less than 10, such as less than 5.

ITEM SET II:

1. A method for improving the nutritional value of mannan-containing cake comprising the steps of
 - i) providing mannan-containing cake with an oil content from 8% to 16% (w/w%),
 - 5 ii) forming an aqueous slurry comprising the mannan-containing cake,
 - iii) contacting the slurry with one or more enzymes to produce a soluble hydrolysate,
 - iv) contacting the soluble hydrolysate with a fermenting organismand thereby obtaining mannan-containing cake with improved nutritional value (such as half press PKC++).
- 10 2. The method according to item 1, wherein the mannan-containing cake is selected from the group consisting of palm kernel cake, palm kernel meal, coffee waste, guar meal, and copra cake.
- 15 3. The method according to any of items 1 and 2, wherein the mannan-containing cake with improved nutritional value has a mannose content from 2% to 30%, such as from 2% to 20%, such as from 2% to 18%, such as from 2% to 16%, such as from 2% to 14%, such as from 2% to 12%, such as from 2% to 10%, such as from 2% to 8%, such as from 3% to 7%, such as from 4% to 7% (w/w% in a composition with 90 w/w% dry matter).
- 20 4. The method according to any of items 1 to 3, wherein the mannan-containing cake with improved nutritional value has a mannose content of less than 30%, such as less than 25%, such as less than 20%, such as less than 18%, such as less than 16%, such as less than 14%, such as less than 12%, such as less than 10%, such as less than 8% (w/w% in a composition with 90 w/w% dry matter).
- 25 5. The method according to any of items 1 to 4, wherein the mannan-containing cake with improved nutritional value has a content of non-starch polysaccharide from 2% to 40%, such as from 2% to 30%, such as from 2% to 28%, such as from 2% to 24%, such as from 2% to 22%,
30 such as from 2% to 20%, such as from 5% to 20%, such as from 10% to 20% and such as from 15% to 20% (w/w% in a composition with 90 w/w% dry matter).
6. The method according to any of items 1 to 5, wherein the mannan-containing cake with improved nutritional value has a content of non-starch polysaccharide of less than 40%, such as
35 less than 35%, such as less than 30%, such as less than 28%, such as less than 26%, such as

less than 24%, such as less than 22%, and such as less than 20% (w/w% in a composition with 90 w/w% dry matter).

- 5 7. The method according to any of items 1 to 6, wherein the mannan-containing cake with improved nutritional value has reduced acid detergent fiber bound nitrogen (ADF-N) such as an ADF-N selected from the group consisting of from 5% to 50%, such as from 5% to 35%, such as 5% to 30%, such as 5% to 25% .
- 10 8. The method according to any of items 1 to 7, wherein the mannan-containing cake with improved nutritional value has reduced acid detergent fiber bound nitrogen (ADF-N) such as an ADF-N of less than 45%, such as less than 40%, such as less than 35%, such as less than 30% and such as less than 25%.
- 15 9. The method according to any of items 1 to 8, wherein the mannan-containing cake with improved nutritional value has a crude fat content of from 8% to 16%, such as from 8% to 14%, such as from 10% to 14% and such as from 12% to 14%.
- 20 10. The method according to any of items 1 to 9, wherein the mannan-containing cake with improved nutritional value has a crude fat content of at least 8%, such as at least 9%, such as at least 10%, such as at least 11% such as at least 12%.
- 25 11. The method according to any of items 1 to 10, wherein the mannan-containing cake with improved nutritional value has a true metabolisable energy of 2400 kcal/kg to 4000 kcal/kg as determined in Example 2, such as a true metabolisable energy of 2400 kcal/kg to 3800 kcal/kg, such as a true metabolisable energy of 2400 kcal/kg to 3800 kcal/kg, such as a true metabolisable energy of 2400 kcal/kg to 3600 kcal/kg, such as a true metabolisable energy of 2400 kcal/kg to 3400 kcal/kg and such as a true metabolisable energy of 2400 kcal/kg to 3200 kcal/kg.
- 30 12. The method according to any of items 1 to 11, wherein the mannan-containing cake with improved nutritional value has a true metabolisable energy of at least 2400 kcal/kg, such as at least 2500 kcal/kg, such as at least 2600 kcal/kg, such as at least 2700 kcal/kg, such as at least 2800 kcal/kg and such as at least 2900 kcal/kg.
- 35 13. The method according to any of items 1 to 12, wherein the mannan-containing cake with improved nutritional value has an improvement in digestible amino acid content of from 4% to 12%

such as from 6% to 10%, such as from 7% to 9% compared to full press PKC++ as defined in Example 2.

14. The method according to any of items 1 to 13, wherein the mannan-containing cake with improved nutritional value has an improvement in digestible amino acid content of at least 2%, such as at least 4%, such as at least 6%, such as at least 8% compared to full press PKC++ as defined in Example 2.

15. The method according to any of items 1 to 14, wherein the mannan-containing cake with improved nutritional value has an improvement in digestible cysteine content of at least 50%, such as at least 55%, such as at least 60%, such as at least 65%, such as at least 70% compared to full press PKC++ as defined in Example 2.

16. The method according to any of items 1 to 15, wherein the mannan-containing cake with improved nutritional value has an improvement in digestible glycine content of at least 5%, such as at least 10%, such as at least 12%, such as at least 14%, such as at least 16% compared to full press PKC++ as defined in Example 2.

17. The method according to any of items 1 to 15, wherein the mannan-containing cake with improved nutritional value has an improvement in digestible lysine content of at least 10%, such as at least 20%, such as at least 25%, such as at least 30%, such as at least 32% compared to full press PKC++ as defined in Example 2.

18. The method according to any of items 1 to 17, wherein the one or more enzymes in step iii) in item 1 have one or more (such as all) of the enzyme activities selected from the group consisting of cellulase, mannanase and mannosidase.

19. The method according to any of items 1 to 18, wherein the one or more enzymes in step iii) in item 1 comprises one or more enzyme activities selected from the list consisting of galactomannanase, beta-xylosidase, endo-1,4-beta xylanase, beta-glucosidase, alpha-galactosidase, beta-galactosidase, alpha-L-arabinofuranosidase, acetyl xylan esterase, ferulic acid esterase, protease and alpha-glucuronidase.

20. The method according to any of items 1 to 19, wherein the one or more enzymes in step iii) in item 1 comprises one or more enzyme activities selected from the list consisting of alpha-amylase and amyloglycosidase.
- 5 21. The method according to item 18, wherein the cellulase is derived or derivable from *Trichoderma reesei*.
22. The method according to items 18, wherein the mannosidase is derived or derivable from *Aspergillus niger*.
- 10 23. The method according to item 18, wherein the mannanase is derived or derivable from *Aspergillus niger* or *Talaromyces Leycettanus*.
24. The method according to item 18, wherein the mannosidase is at least 80% identical to SEQ
15 ID NO:2.
25. The method according to item 18, wherein the mannanase is at least 80% identical to SEQ ID NO:1 or SEQ ID NO: 3.
- 20 26. The method according to item 18, wherein the cellulase is at least 80% identical to SEQ ID NO:4, SEQ ID NO: 5, SEQ ID NO: 6, or SEQ ID NO: 7.
27. The method according to any of items 1 to 26, wherein the method further comprises one or more oil skimming steps.
- 25 28. The method according to any of items 1 to 27, wherein the mannan-containing cake provided in step i) has an oil content from 9% to 15%, such as 9% to 14%, such as from 10% to 15%, such as from 10% to 14%, such as from 11 to 14% and such as from 12 to 14%.
- 30 29. A mannan-containing cake with improved nutritional value (such as half press PKC++) obtained or obtainable by the method according to any of items 1 to 28.

30. A mannan-containing cake with improved nutritional value (such as half press PKC++) comprising one or more of the features selected from the group consisting of

- i) a content of less than 30% mannose (w/w% in a composition with 90 w/w% dry matter),
- ii) a content of less than 40% non-starch polysaccharide (w/w% in a composition with 90 w/w% dry matter),
- iii) a content of less than 40% acid detergent fiber bound nitrogen,
- iv) a content of true metabolisable energy of at least 2400 kcal/kg,
- v) improvement in digestible amino acid content of at least 4% compared to full press PKC++ as defined in Example 2,
- vi) improvement in digestible cysteine content of at least 50% compared to full press PKC++ as defined in Example 2,
- vii) improvement in digestible glycine content of at least 5% compared to full press PKC++ as defined in Example 2 and
- viii) improvement in digestible lysine content of at least 10% compared to full press PKC++ as defined in Example 2.

31. The mannan-containing cake with improved nutritional value (such as half press PKC++) according to any of items 29 to 30 comprising or containing from 2% to 30% mannan, such as from 2% to 20%, such as from 2% to 18%, such as from 2% to 16%, such as from 2% to 14%, such as from 2% to 12%, such as from 2% to 10%, such as from 2% to 8%, such as from 3% to 7%, such as from 4% to 7% (w/w% in a composition with 90 w/w% dry matter).

32. The mannan-containing cake with improved nutritional value (such as half press PKC++) according to any of items 29 to 31, wherein the mannan-containing cake has a mannose content of less than 30%, such as less than 25%, such as less than 20%, such as less than 18%, such as less than 16%, such as less than 14%, such as less than 12%, such as less than 10%, such as less than 8% (w/w% in a composition with 90 w/w% dry matter).

33. The mannan-containing cake with improved nutritional value (such as half press PKC++) according to any of items 29 to 32, wherein the mannan-containing cake has a content of non-starch polysaccharide from 2% to 40%, such as from 2% to 30%, such as from 2% to 28%, such as from 2% to 24%, such as from 2% to 22%, such as from 2% to 20%, such as from 5% to 20%,

such as from 10% to 20% and such as from 15% to 20% (w/w% in a composition with 90 w/w% dry matter).

- 5 34. The mannan-containing cake with improved nutritional value (such as half press PKC++) according to any of items 29 to 33, wherein the mannan-containing cake has a content of non-starch polysaccharide of less than 40%, such as less than 35%, such as less than 30%, such as less than 28%, such as less than 26%, such as less than 24%, such as less than 22%, and such as less than 20% (w/w% in a composition with 90 w/w% dry matter).
- 10 35. The mannan-containing cake with improved nutritional value (such as half press PKC++) according to any of items 29 to 34, wherein the mannan-containing cake has reduced acid detergent fiber bound nitrogen (ADF-N) such as an ADF-N selected from the group consisting of from 5% to 50%, such as from 5% to 35, such as 5% to 30%, such as 5% to 25%.
- 15 36. The mannan-containing cake such as PKC The mannan-containing cake with improved nutritional value (such as half press PKC++) according to any of items 29 to 35, wherein the mannan-containing cake has reduced acid detergent fiber bound nitrogen (ADF-N) such as an ADF-N of less than 50%, such as less than 45%, such as less than 40%, such as less than 35%, such as less than 30%, such as less than 25%.
- 20 37. The mannan-containing cake with improved nutritional value (such as half press PKC++) according to any of items 29 or 36, wherein the mannan-containing cake with improved nutritional value has a crude fat content of from 8% to 16%, such as from 8% to 14%, such as from 10% to 14% and such as from 12% to 14%.
- 25 38. The mannan-containing cake with improved nutritional value (such as half press PKC++) according to any of items 29 to 37, wherein the mannan-containing cake has a crude fat content of at least 8%, such as at least 9%, such as at least 10%, such as at least 11% such as at least 12%.
- 30 39. The mannan-containing cake with improved nutritional value (such as half press PKC++) according to any of items 29 to 38, wherein the mannan-containing cake has a true metabolisable energy of 2400 kcal/kg to 4000 kcal/kg as determined in Example 2, such as a true metabolisable energy of 2400 kcal/kg to 3800 kcal/kg, such as a true metabolisable energy of 2400 kcal/kg to 3800 kcal/kg, such as a true metabolisable energy of 2400 kcal/kg to 3600 kcal/kg, such as a true metabolisable energy of 2400 kcal/kg to 3400 kcal/kg, such as a true metabolisable energy of 2400 kcal/kg to 3200 kcal/kg, and such as a true metabolisable energy of 2400 kcal/kg to 3000 kcal/kg.
- 35

40. The mannan-containing cake with improved nutritional value (such as half press PKC++) according to any of items 29 to 39, wherein the mannan-containing cake has a true metabolisable energy of at least 2400 kcal/kg, such as at least 2500 kcal/kg, such as at least 2600 kcal/kg, such as at least 2700 kcal/kg, such as at least 2800 kcal/kg and such as at least 2900 kcal/kg.

5

41. The mannan-containing cake with improved nutritional value (such as half press PKC++) according to any of items 29 to 40, wherein the mannan-containing cake has an improvement in digestible amino acid content of from 4% to 12% such as from 6% to 10%, such as from 7% to 9% compared to full press PKC++ as defined in Example 2.

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42. The mannan-containing cake with improved nutritional value (such as half press PKC++) according to any of items 29 to 41, wherein the mannan-containing cake with improved nutritional value has an improvement in digestible amino acid content of at least 2%, such as at least 4%, such as at least 6%, such as at least 8% compared to full press PKC++ as defined in Example 2.

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43. The mannan-containing cake with improved nutritional value (such as half press PKC++) according to any of items 29 to 42, wherein the mannan-containing cake with improved nutritional value has an improvement in digestible cysteine content of at least 50%, such as at least 55%, such as at least 60%, such as at least 65%, such as at least 70% compared to full press PKC++ as defined in Example 2.

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44. The mannan-containing cake with improved nutritional value (such as half press PKC++) according to any of items 29 to 43, wherein the mannan-containing cake with improved nutritional value has an improvement in digestible glycine content of at least 5%, such as at least 10%, such as at least 12%, such as at least 14%, such as at least 16% compared to full press PKC++ as defined in Example 2.

25

45. The mannan-containing cake with improved nutritional value (such as half press PKC++) according to any of items 29 to 44, wherein the mannan-containing cake with improved nutritional value has an improvement in digestible lysine content of at least 10%, such as at least 20%, such as at least 25%, such as at least 30%, such as at least 32% compared to full press PKC++ as defined in Example 2.

30

46. An animal feed or an animal feed additive comprising the mannan-containing cake with improved nutritional value (such as half press PKC++) according to any of items 29 to 45.

35

47. The animal feed or an animal feed additive according to item 46 further comprising one or more enzymes such as one or more enzymes selected from the group consisting of cellulase, mannanase and mannosidase.
- 5 48. The animal feed or an animal feed additive according to item 46 further comprising one or more enzyme activities selected from the group consisting of galactomannanase, beta-xylosidase, endo-1,4-beta xylanase, beta-glucosidase, alpha-galactosidase, beta-galactosidase, alpha-L-arabinofuranosidase, acetyl xylan esterase, ferulic acid esterase, protease and alpha-glucuronidase.
- 10 49. The animal feed or an animal feed additive according to item 46 further comprising one or more enzyme activities selected from the group consisting of alpha-amylase and amyloglycosidase.
- 15 50. The animal feed or an animal feed additive according to any of items 46 to 49, wherein the animal feed or an animal feed additive is an animal feed or an animal feed additive for a monogastric animal.
- 20 51. Use of mannan-containing cake with improved nutritional value (such as half press PKC++) according to any of items 29 or 45 or the animal feed or the animal feed additive according to any of items 46 to 50 for feeding a mono-gastric animal.

EXAMPLES

EXAMPLE 1

Chemicals

5 Arabinose, galactose, glucose, xylose, mannose, and succinic acid were purchased from Merck (Darmstadt, Germany). Sulphuric acid was from Bie & Berntsen A/S (Rødovre, Denmark).

Substrate

10 Palm kernel cake with 25%, 50% and 100% of normal expelling was obtained from Malaysia. The palm kernel cake with 25%, 50% and 100% of normal expelling had a dry matter content of 93wt%, 95wt%, and 97wt%, respectively.

Enzymes

15 Cellulase composition A is Novozymes Cellic® CTec3.

 Mannanase composition B contains an *Aspergillus niger* mannanase having the sequence of SEQ ID NO:3.

 Mannosidase composition C contains a mannosidase derived from *Aspergillus niger* and having the sequence deposited as SWISSPROT accession number A2QWU9 and shown
20 herein as SEQ ID NO:2.

High Performance Anion Exchange Chromatography (HPAEC)

Hydrolysates (10 µl) were applied onto a Dionex BioLC system fitted with a Dionex CarboPac™ PA1 analytical column (4 x 250 mm)(Dionex Corporation, Sunnyvale, CA, USA) combined with
25 a CarboPac™ PA1 precolumn (4 x 50 mm). The monosaccharides were separated isocratically with 0.001 M KOH for 40 min, flow: 1 mL·min⁻¹. Monosaccharides were detected by a pulsed electrochemical detector in the pulsed amperimetric detection mode. The potential of the electrode was programmed for +0.1 V (t = 0-0.4 s) to -2.0 V (t = 0.41-0.42 s) to 0.6 V (t = 0.43 s) and finally -0.1 V (t = 0.44-0.50 s), while integrating the resulting signal from t = 0.2-0.4 s. A mixture

of arabinose, galactose, glucose, xylose, and mannose (concentration of each component: 0.0025-0.075 g·L⁻¹) was used as standard.

The effect of milder expelling conditions during oil extraction from palm kernels was investigated. The bio-availability of the resulting PKC/PKC++ was analysed – cf. Table 1.1 herein below.

Table 1.1. The content (g/100 g) of crude protein (Nx6.25) and acid detergent bound nitrogen (ADF-N) in palm kernel cake and hydrolysed/fermented palm kernel cake

ID	*Crude protein (g/100 g)	ADF-N (g/100 g)	Expeller process
	(EC) No 152/2009	LW04T / AOAC 973.18 (1995)	Press time
PKC-130°C	27.5	24	
PKC Freeze Dried	26.6	17	
PKC_without hydrolysis and fermentation	14.3	8	25% of normal expelling
PKC_without hydrolysis and fermentation	16.3	8	50% of normal expelling
PKC_without hydrolysis and fermentation	17.5	37	Normal expelling
PKC_with hydrolysis and fermentation (PKC++)	24.4	6	25% of normal expelling
PKC_with hydrolysis and fermentation (PKC++)	24.6	8	50% of normal expelling
PKC_with hydrolysis and fermentation (PKC++)	27.1	22	Normal expelling

*Standard crude protein factor in animal feed 6.25xN.

Palm kernel cake was made by conventional methods using two press cycles (termed normal expelling). In addition, palm kernel cake was made by reduced pressing – i.e. by 50% of normal expelling and by 25% of the normal expelling.

Palm kernel cake was oven dried at 130°C over night (16 h) to produce a sample with a high level of damaged protein and this was compared with the original material that was subjected to freeze drying. Three PKC samples and three PKC samples that had been hydrolysed and fermented for ethanol production and previously subjected to normal expelling, 50% of normal expelling and 25% of normal expelling were included in the study.

Palm kernel cake hydrolysis and fermentation

PKC obtained by 25%, 50% and 100% of normal expelling as described above was subsequently subjected to an enzymatic hydrolysis step and a fermentation step. The hydrolyzed and fermented PKC is termed PKC++.

The enzymatic hydrolysis of the PKC was done in Infors HT 2 L reactors. The substrate was put in the reactor and water and lactrol were added and stirring was started. Temperature was 55°C, pH=5.0, and stirring 250 rpm. Enzyme mixture (52% *Aspergillus niger* mannanase, 36% *Aspergillus niger* beta-mannosidase and 12% Novozymes Cellic® CTec3) was added. Samples were taken regularly and after 94 hours the temperature were lowered to 33°C and the yeast (Baker yeast red, Thermosacc. Yeast; SS-2010-00452) was added and the fermentation was initiated in the reactor for another 96 hours with regularly sampling. Thereafter the stirring was stopped and the resulting PKC++ recovered from the reactor.

The experimental products were processed in Infors HT 2 L reactors as follows:

- Enzyme dosage: 0.75 mg of enzyme/g dry matter (i.e. total amount of mannanase, beta-mannosidase and cellulase/g dry matter).
- Scale: 1000 g (total amount in reactor)
- Dry matter: 25 %
- Biomass addition: all at beginning
- Lactrol – was added at the start of the hydrolysis, prior to addition of the enzymes: 15 ppm
- Temperature: 55°C.
- Buffer: none – pH was adjusted frequently as required with a target pH of 5.0.
- Stirring: First 24 h 250 rpm and decreased to 200 rpm for the remaining time
- After 4 days (94 h) yeast was added
- Temperature during the yeast fermentation was 33°C and yeast dosage was 0.4 g/kg dry matter.

After hydrolysis and fermentation the PKC++ samples were dried and sent for external analyses of crude protein (Kjeldahl-Nx6.25) and acid detergent bound nitrogen as described in Official Journal of the European Union L54/1, No 152/2009 of 27 January 2009. Acid detergent fibre was analysed according to AOAC 973.18/1995.

In feed components the acid detergent fibre represents the cellulose and lignin fraction of the feed. The contents of nitrogen bound in this fraction serves as an indicator of insoluble

protein damaged by heat treatment (Goering, H.K., Gordon, C.H., Hemken, R.W., Waldo, D.R., VanSoest, P.J. and Smith, L.W. 1972. Analytical estimates of nitrogen digestibility in heat damaged forages. Journal of Dairy Science 55: 1275-1280).

Eliminations or alterations of processing steps that creates ADF bound nitrogen is important in order to preserve the protein bio-availability. In the current case it has been shown that a decrease in expeller pressing of palm kernels during palm oil extraction notably decreases the content of ADF bound nitrogen in the protein rich press-residue that is used for animal feed. Extending the time for the pressing condition removes more oil and increases the crude protein content in the residue (from 14.3 % up to 17.5 %) but when applying the final pressing the protein becomes heat damaged as indicated by a notable increase in ADF bound nitrogen (Table 1.1). Based on the above we conclude that milder expelling conditions can improve the bio-availability of PKC and PKC++.

EXAMPLE 2

Substrate

Half and full press palm kernel cake were obtained from Malaysia.

Enzymes

Cellulase composition A is a *Trichoderma reesei* cellulase preparation containing a CBHI of SEQ ID NO: 4, a CBHII of SEQ ID NO: 5, a beta-glucosidase variant of SEQ ID NO: 6, and a AA9 (GH61) of SEQ ID NO: 7. Optionally, the preparation could further comprise one or more endoglucanases.

Mannanase composition B contains a *Talaromyces leycettanus* mannanase having the sequence deposited as GENESEQP accession number AAY54122 and shown herein as SEQ ID NO:1.

Mannosidase composition C contains a mannosidase derived from *Aspergillus niger* and having the sequence deposited as SWISSPROT accession number A2QWU9 and shown herein as SEQ ID NO:2.

Palm kernel cake production

Palm kernel cake (PKC) is the byproduct from the mechanical extraction of oils from the palm kernels. It is produced by feeding the kernels to screw presses. In the first stage – half press – the oil content is reduced from approximately 45-49 % to approximately 11.1% (cf. Table 2.0). The PKC is then feed into another screw press (full press) to extrude the remaining oil

until the oil content in the PKC is approximately 5.0% (cf. Table 2.0). The last stage – full press - is done at elevated temperatures and more harsh conditions.

Table 2.0: moisture and oil content in half and full press PKC

	PKC half press	PKC full press
Moisture (w/w%)	4.51%	1.84%
Crude fat (w/w%)*	11.1%	5.0%

5 * Crude fat determined according to Official Journal L257, 19/09/1998 P. 0014-0028.

The expelling time for each press is immediate within a few minutes. The mechanical pressing on the palm kernel leads to generation of heat. The expelling temperature is approximately 70°C after the half press and approximately 90°C after full press. Although the expelling time is immediate the heat generated will remain in the PKC after each press and it takes time to cool the PKC down to ambient temperature.

Palm kernel cake hydrolysis and fermentation

PKC obtained by full and half pressing as described above was subsequently subjected to an enzymatic hydrolysis step and a fermentation step. The hydrolyzed and fermented PKC is termed PKC++.

The enzymatic hydrolysis of the PKC was done in Infors HT 2 L reactors. The substrate was put in the reactor and water and lactol were added and stirring was started. Temperature was 60°C, pH=5.0, and stirring 250 rpm. Enzyme mixture (10% Cellulase composition A, 45% Mannanase composition B and 45% Mannosidase composition C) was added. Samples were taken regularly and after 96 hours the temperature was lowered to 33°C and the yeast (Baker yeast red, Thermosacc. Yeast; SS-2010-00452) was added and the fermentation was initiated in the reactor for another 96 hours with regularly sampling. Thereafter the stirring was stopped and the resulting PKC++ recovered from the reactor.

25 The experimental products were processed in Infors HT 2 L reactors as follows:

- Enzyme dosage: 0.75 mg of enzyme/g dry matter (i.e. total amount of mannanase, mannosidase and cellulase/g dry matter).
- Scale: 1000 g (total amount in reactor)
- Dry matter: 25 % PKC
- Biomass addition: all at beginning

- Lactrol – was added at the start of the hydrolysis, prior to addition of the enzymes: 15 ppm
 - Temperature: 60°C.
 - Buffer: none – pH was adjusted frequently as required with a target pH of 5.0.
 - Stirring: First 24 h 250 rpm and decreased to 200 rpm for the remaining time
- 5
- After 4 days (96 h) yeast was added
 - Temperature during the yeast fermentation was 33°C and yeast dosage was 0.4 g/kg dry matter.

After removal of ethanol the PKC/PKC++ samples were freeze dried.

10

In summary the following PKC/PKC++ samples were prepared as described above:

PKC samples	Pressing conditions	Enzymatic Hydrolysis	Fermentation
Sample 1 (Full press PKC)	Full press	No	No
Sample 2 (Half press PKC)	Half press	No	No
Sample 3 (Full press PKC++)	Full press	Yes	Yes
Sample 4 (Half press PKC++)	Half press	Yes	Yes

15 ***Palm kernel cake analysis***

The PKC samples obtained after full and half pressing with and without hydrolysis and fermentation as described above were analysed with respect to gross chemical analyses and determination of non-starch polysaccharide contents. In addition, the PKC++ samples were used in an *in vivo* trial for the determination of digestible amino acids and true metabolisable energy content (TME) as outlined by Sibbald (Sibbald, I.R. 1981; Metabolic plus endogenous energy and nitrogen losses of adult cockerels: The correction used in the bioassay for true metabolisable energy; Poultry Science 60: 805-811).

20

Gross chemical compositions of the PKC/PKC++ samples were analysed by Eurofins according to internal and international standard procedures [List of official EU methods of analysis: Crude fat according Crude fat according to procedure Official Journal L257, 19/09/1998 P. 0014-0028, Crude ash 1971L0250-EN-17.06.1971-000.000 (8-9). AOAC methods of analysis: Method 4.1 Determination of Acid Detergent Fiber by Refluxing, AOAC 973.18. Method 3.2 Nitrogen Determination by Kjeldahl (Block Digestion) AOAC 981.10].

25

The non-starch polysaccharide (NSP) contents of the PKC++ samples were determined by a gas chromatographic method (Theander O, Aaman P, Westerlund E, Andersson R, Pettersson D. (1995). Total dietary fibre determined as neutral sugar residue, uronic acid residue and Klason lignin (The Uppsala Method): Collaborative study. Journal of AOAC International 74 1030-1043).

In vivo trial

An *in vivo* trial was conducted with adult Bovans White roosters at the Department of Poultry Science at the University of Georgia under supervision of Dr. Adam Davis. Adult roosters were utilized for the TME and amino acid digestibility determinations – i.e. roosters in the range from 22 to 80 weeks of age. The body weight of Bovans White roosters increases rapidly up to about 20 weeks of age and after that they only grow slowly reaching an end point of about 2450 g live weight. For the TME it is important that birds are not in a rapid growth phase.

Roosters (10 per feed ingredient tested) were fasted for 24 hours. Fasted roosters were then moved to a room equipped with 40 individual wire cages. Each cage was equipped with a nipple drinker to provide each rooster free access to water. Each cage was also equipped with a stainless steel faeces-collection pan. Each rooster was tube fed 35 grams of the test feed ingredient/diet (PKC++/diet) and then placed back in to a cage. The excreta from each individual bird was collected over the next 48 hours. The roosters had free access to water but were fasted during this 48 hour period. The collected faeces from conventional birds (2x10) was used to determine the true metabolizable energy of the tested feed ingredient, while cecectomized birds (2x10) were used for the determination of digestible amino acids in order to minimize the effects of hind gut fermentation. A set of roosters (6 conventional and 6 cecectomized) deprived of feed for 24 hours were used for collection of excreta during 48 hours to obtain corrections for endogenous energy and amino acid excretion. At the end of the 48 hour period all roosters were returned to free access of both feed and water.

TME calculations

Calculations for TME: {Gross energy of the feed on a g basis multiplied by the g feed – [(Gross energy of the excreta on a g basis multiplied by the excreta weight) – 8.73 multiplied by the (grams of excreta multiplied by the nitrogen content of the excreta) – (grams of feed multiplied by the nitrogen content in the feed)] – [(grams of endogenous control excreta multiplied by the endogenous control excreta gross energy on a g basis – (grams of endogenous control excreta multiplied by the endogenous control nitrogen multiplied by 8.73)} this is all divided by the g

of feed. This result is multiplied by 1000 to go from energy per g of diet to kg of diet. The factor of 8.73 reflects the correction factor for voided nitrogen that is mostly uric acid.

Calculations for amino acid digestibility: [the gram amount of the feed ingredient fed*(% AA content in the feed/100)] – [grams of excreta*(% AA content in the excreta/100) – endogenous] this result is divided by the gram amount of the feed ingredient fed (% AA content in the feed/100). This number would then be multiplied by 100 for the percent value.

Results

Non-starch polysaccharide determinations of the PKC samples (Table 2.1) indicated a notable reduction in mannose polysaccharides after hydrolysis and fermentation (i.e. PKC++ full and PKC++ half press). Glucose containing polysaccharides including cellulose were not enriched in the PKC++ material due to the loss of mannose during fermentation, as was the case for all other polysaccharide residues. This indicates that the enzymatic treatment also broke down glucose polymers to a degree of polymerisation below DP 10. The dietary fibre definition and the method used for analysis does not quantify short chain oligomers below DP 10 and, hence, such chains that are not by definition polysaccharide chains are excluded from the analysis. After hydrolysis and fermentation the major remaining polysaccharides were still of mannose and glucose origin although mannose concentration displayed a 6 fold reduction. There was no fucose observed in the samples before or after hydrolysis/fermentation.

Table 2.1. Content of non-starch polysaccharide (NSP) residues (% of fresh weight) in PKC (full and half press) and hydrolysed/fermented PKC++ (full and half press)

	NSP (% of fresh weight, air dry basis)							
	Rham-nose	Fuc-ose	Ara-binose	Xyl-ose	Man-nose	Gal-actose	Glu-cose	Sum of NSP
Full press PKC	0.17	-	0.99	2.14	35.12	1.60	6.94	46.97
<i>SD</i>	0.008	-	0.056	0.294	1.186	0.095	0.627	1.295
Half press PKC	0.18	-	1.07	1.96	36.66	1.71	6.48	48.08
<i>SD</i>	0.013	-	0.035	0.358	1.167	0.047	0.144	1.430
Full press PKC++	0.22	-	1.32	2.60	5.98	2.42	5.72	18.25
<i>SD</i>	0.019	-	0.041	0.136	0.154	0.022	0.022	0.247
Half press PKC++	0.23	-	1.32	2.45	5.84	2.46	4.69	16.98
<i>SD</i>	0.013	-	0.064	0.666	0.555	0.211	0.103	0.597

SD: Standard deviation.

Gross chemical analyses of PKC++ (full and half press) revealed a lower crude fat content in full press PKC++ compared to half press PKC++. Full press PKC++ also had a higher content of nitrogen bound ADF (ADF-N) which indicates a higher level of Maillard formation (Table 2.2) compared to half press PKC++.

Table 2.2. Gross chemical composition* of PKC++ (full and half press)

	Crude fat (3 M HCl) (%)	Crude protein (%)	ADF-N (%)	Ash (%)
Full press PKC++	6.7	25.9	>50	8.0
Half press PKC++	13.8	23.5	21	7.7

*Analysed by Eurofins according to internal and international standard procedures (List of official EU methods of analysis: Crude fat according to procedure Official Journal L257, 19/09/1998 P. 0014-0028, Crude ash 1971L0250-EN-17.06.1971-000.000 (8-9). AOAC methods of analysis: Method 4.1 Determination of Acid Detergent Fiber by Refluxing, AOAC 973.18 Method 3.2 Nitrogen Determination by Kjeldahl (Block Digestion) AOAC 981.10). Results are expressed on fresh weight (air dry basis).

A true metabolisable energy (TME) trial was conducted with the half and full press PKC++ as described in material and methods. The half press PKC++ displayed a higher content of metabolisable energy (Table 2.3) due to a higher fat content but also an improved amino acid digestibility (Table 2.4).

Table 2.3. The content of true metabolisable energy measured in conventional roosters with results expressed on fresh weight (air dry basis)

Half press PKC++		Full press PKC++	
kcal/kg	MJ/kg	kcal/kg	MJ/kg
3016	12.6	2203	9.2
2947	12.3	2173	9.1
2908	12.2	2193	9.2
2957	12.4	2284	9.6
3025	12.7	2345	9.8
3045	12.7	2314	9.7
2967	12.4	2254	9.4
3172	13.3	2213	9.3
2918	12.2	na*	na*
3016	12.6	na*	na*
Average 2997	Average 12.5	Average 2247	Average 9.4

*Two roosters were removed since the results were considered to be outliers.

The content of digestible amino acids in the half and full press PKC++ (Table 2.4) was calculated based on the digestibility coefficients determined in the trial (digestibility coefficients not shown). Overall the average digestibility improvement of amino acids was about 8 % for the half press PKC++.

Table 2.4. The content (%) of amino acids (total and digestible) in PKC++ (half and full press)

Amino acid	Half press PKC++		Full press PKC++		Improvement (%)
	Total amino acid content (%)	Digestible amino acid content (%)*	Total amino acid content (%)	Digestible amino acid content (%)*	Increase in digestible amino acid content in half press PKC++
Alanine	0.95	0.563	1.04	0.577	-2.43
Arginine	2.69	2.075	2.58	1.924	7.85
Aspartic acid	1.91	0.788	1.99	0.792	-0.51
<i>Cysteine</i>	<i>0.31</i>	<i>0.074</i>	<i>0.3</i>	<i>0.043</i>	<i>72.09</i>
Glutamic acid	3.88	2.252	4.33	2.233	0.85
<i>Glycine</i>	<i>1.05</i>	<i>0.443</i>	<i>1.14</i>	<i>0.376</i>	<i>17.82</i>
Histidine	0.49	0.313	0.52	0.279	12.19
Isoleucine	0.93	0.622	0.99	0.627	-0.80
Leucine	1.57	1.048	1.73	1.084	-3.32
<i>Lysine</i>	<i>0.58</i>	<i>0.24</i>	<i>0.51</i>	<i>0.179</i>	<i>34.08</i>
Methionine	0.39	0.266	0.42	0.266	0.00
Phenylalanine	1.06	0.755	1.16	0.768	-1.69
Proline	0.81	0.444	0.86	0.413	7.51
Serine	1	0.557	1	0.515	8.16
Threonine	0.79	0.389	0.8	0.371	4.85
Tryptophan	0.15	0.101	0.17	0.112	-9.82
Tyrosine	0.69	0.492	0.74	0.495	-0.61
Valine	1.24	0.793	1.33	0.795	-0.25
					Average improvement 8.1 %

*Digestible amino acid content was determined from the measured digestibility coefficients.

Conclusions:

Currently the residual product from palm kernel oil expelling – i.e. PKC - is mainly used for ruminant feed due to the low protein content and high dietary fibre content. The purpose of the processing described above is to utilise the high mannan content in the palm kernels for the production of ethanol, and while doing so the protein fraction is concentrated and the dietary fibre content is notably reduced. This makes it possible to use this novel PKC++ product also for feeding of other animals than ruminants (i.e. mono-gastric animals such as poultry and pigs). An important property of feed components for mono-gastric animals is the protein quality and the metabolisable energy content. The current study demonstrates that a mild processing during the oil extraction procedure (i.e. half pressing) will lead to improved protein availability in the hydrolysed/fermented PKC++ product. This is indicated by a marked reduction in nitrogen bound in the ADF fraction. The TME trial conducted also corroborates these novel findings. Amino acid digestibility was on average improved by 8 % in the half time pressed material.

There are energetic benefits of a higher fat content in the half press material as well as a better protein digestibility. The metabolisable energy in the 71 g of fat extra per kg product corresponds to about 634 kcal/kg (the crude fat digestibility is not taken into account), while the 80 g/kg product improvement in digestible protein represents about 324 kcal/kg; a total of 954 kcal of additional metabolisable energy per kg half press PKC++ product. The actual measured TME content (2997 kcal/kg) in the half press PKC++ was about 200 kcal less than the theoretically calculated value, based on fat and improved protein availability.

In conclusion the improved metabolisable energy content of the half press PKC++ is explained by a higher fat content and an improved protein digestibility as a result of a less harsh processing.

CLAIMS

1. A method for improving the nutritional value of mannan-containing cake comprising the steps of
 - (i) providing a mannan-containing half press cake with an oil content from 8% to 16% (w/w%),
 - (ii) forming an aqueous slurry comprising the mannan-containing cake of step (i),
 - (iii) contacting the slurry of step (ii) with one or more enzymes to produce a soluble hydrolysate,
 - (iv) contacting the soluble hydrolysate of step (iii) with a fermenting organism,
 - (v) and thereby obtaining a mannan-containing cake with improved nutritional value,wherein the mannan-containing half press cake of step (i) is palm kernel half press cake, wherein the mannan-containing cake with improved nutritional value is half press upgraded palm kernel cake (PKC++).
2. The method according to claim 1, wherein the mannan-containing cake with improved nutritional value has a mannose content from 2% to 30%, such as from 2% to 20%, such as from 2% to 18%, such as from 2% to 16%, such as from 2% to 14%, such as from 2% to 12%, such as from 2% to 10%, such as from 2% to 8%, such as from 3% to 7%, such as from 4% to 7% w/w% in a 90 w/w% dry matter composition.
3. The method according to any of claims 1 to 2, wherein the mannan-containing cake with improved nutritional value has a content of non-starch polysaccharide from 2% to 40%, such as from 2% to 30%, such as from 2% to 28%, such as from 2% to 24%, such as from 2% to 22%, such as from 2% to 20%, such as from 5% to 20%, such as from 10% to 20% and such as from 15% to 20% w/w% in a composition with 90 w/w% dry matter.
4. The method according to any of claims 1 to 3, wherein the mannan-containing cake with improved nutritional value has an acid detergent fiber bound nitrogen (ADF-N) selected from the group consisting of from 5% to 50%, such as from 5% to 35%, such as 5% to 30%, such as 5% to 25% w/w% in a composition with 90 w/w% dry matter.
5. The method according to any of claims 1 to 4, wherein the mannan-containing cake with improved nutritional value has a crude fat content of from 8% to 16%, such as from 8% to

14%, such as from 10% to 14% and such as from 12% to 14% w/w% in a composition with 90 w/w% dry matter.

- 5 6. The method according to any of claims 1 to 5, wherein the mannan-containing cake with improved nutritional value has a true metabolisable energy of 2400 kcal/kg to 4000 kcal/kg such as a true metabolisable energy of 2400 kcal/kg to 3800 kcal/kg, such as a true metabolisable energy of 2400 kcal/kg to 3800 kcal/kg, such as a true metabolisable energy of 2400 kcal/kg to 3600 kcal/kg, such as a true metabolisable energy of 2400 kcal/kg to 3400 kcal/kg and such as a true metabolisable energy of 2400 kcal/kg to 3200 kcal/kg.
- 10 7. The method according to any of claims 1 to 6, wherein the one or more enzymes in step iii) in claim 1 have one or more of the enzyme activities selected from the group consisting of cellulase, mannanase and mannosidase.
- 15 8. A mannan-containing cake with improved nutritional value comprising metabolizable energy of at least 2400 kcal/kg and one or more of the features selected from the group consisting of:
- 20 (i) a content of less than 30% mannose w/w% in a composition with 90 w/w% dry matter,
- (ii) a content of less than 40% non-starch polysaccharide w/w% in a composition with 90 w/w% dry matter,
- (iii) a content of less than 40% acid detergent fiber bound nitrogen w/w% in a composition with 90 w/w% dry matter,
- 25 (iv) improvement in digestible amino acid content of at least 4% compared to full press PKC++,
- (v) improvement in digestible cysteine content of at least 50% compared to full press PKC++,
- (vi) improvement in digestible glycine content of at least 5% compared to full press PKC++, and
- 30 (vii) improvement in digestible lysine content of at least 10% compared to full press PKC++,
wherein the mannan-containing cake is half pressed PKC++.
9. An animal feed or an animal feed additive comprising the mannan-containing cake with improved nutritional value according to claim 8.

10. The animal feed or an animal feed additive according to claim 9, wherein the animal feed or an animal feed additive is an animal feed or an animal feed additive for a mono-gastric animal.

5 11. Use of mannan-containing cake with improved nutritional value according to claim 8 or the animal feed or the animal feed additive according to any of claims 9 to 10 for feeding a mono-gastric animal.