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(54) IMPROVEMENTS IN OR RELATING TO THE PREPARATION OF FODDER

(71) We, LICENCIA TALALMAN-YOKAT ERTEKESITO VALLALAT, of 10, József nádor tér, Budapest V., Hungary, a body corporate duly organized under the laws of Hungary, do hereby declare the invention, for which we pray that a patent may be granted to us, and the method by which it is to be performed, to be particularly described in and by the following statement:—

The invention relates to a process for the production of a green plant concentrate of high nutritional value in a detoxicated form which can be used as concentrated fodder or feedstuff, as well as in preparations containing this concentrate.

An aim of research into concentrated green fodders has been to obtain products which are nearly as good as or approach at least the nutritional value of protein fodders of animal provenance (e.g. fish-meal) and special protein fodders made of plant seed (e.g. soya, ground-nut) and which at the same time can be economically produced from cultivated plants and possibly even from common weeds.

According to known processes, the comminuted matter of the domestic plants which have attained full growth, i.e. the so-called generative stage, or their parts are dried by means of flue gas of 600°C to 700°C inlet temperature and milled if desired. (Advances in Agronomy, Vol. II., 1950). The drying process carried out in this way produces a considerable decrease in nutritional value, increasing thus the damage caused by the other operations. The product developed in this way has a disadvantageously high fiber content.

Also it has been recommended, though not put into practice, to convert a pulp containing 12 to 13% dry matter obtained from green domestic plants by means of fine milling either into a nutriment of protein suitable for use as human food or into fodder by segregation therefrom or the protein by means of heat coagulation (J. Sci. Food e.a. Agric., 1961., pp. 502—512., Premier congrès international des industries agricoles et alimentaires des zones tropicales et subtropicales, Abidjan, 13—

19 décembre 1964.), or by drying the milled product (Agrártudomány I., 9.). Experiments have shown that these methods are likely to cause a considerable loss in the nutritionally valuable substances the final product being devoid not only of the polypeptides having a molecular weight less than 10,000, but also of free amino acids and other extremely valuable plant substances such as water-soluble vitamins, minerals and carbohydrates. In general the average raw protein content of present concentrated fodders originating from green plants is at least 17%. 50% of the original protein and other nutriment content of the harvested plant or of the plant particles is lost in the course of the processing, e.g. during the natural drying process.

Further there is a great number of plants which either contain components having a useful physiological or sensory effect in small amount or contain small amounts of components having a deleterious physiological or sensory effect and thus cannot be used or whose use is significantly restricted for nutritive, i.e. feeding purpose.

The present invention seeks to provide a process for preparing a green fodder concentrate in a substantially fiberless form.

This invention also seeks to provide a process for the preparation of a green fodder concentrate which contains all essential, important and useful ingredients except the fiber content of the green plant employed as raw material and at the same time to eliminate any deleterious accompanying substances or to reduce their concentration in the obtained fodder to a tolerable extent not influencing the utility of the product.

This invention further seeks to provide a process for the preparation of a green fodder concentrate which is suitable due to its low fiber content or in a substantially fibreless form for foddering monogastric animals and at the same time preserving all essential nutritional components of the green plant.

Still another aim of the present invention is to provide a process which can be employed

[Price 25p]

for the working up of various green plants or even weeds which have been considered previously unsuitable of foddering purposes.

5 This invention seeks, in addition, to provide a process to obtain green fodder concentrate which is rather simple to carry out from a technological point of view and at the same time ensures a considerable economic efficiency.

10 The most commonly-found substances detrimental for foddering purposes and present in most of the green plants used for foddering purposes are e.g. as follows:

15 a) Saponines, a part thereof having hemolytic effect of warm blooded animals (alfalfa);
b) mustard oils, in free form or in form of glucosides (rape, field kale);
c) coumarine and glucosides thereof (melilot);

20 d) alkaloids, as parts of the nitrogen fraction of the green plants, in addition to nitrates, due to the effect of fertilizers;

25 e) aromatic substances and volatile oils, having in themselves no toxic effect, however, undesirable from foddering viewpoints.

30 In addition to the detrimental substances mentioned above some green plants contain oxalic acid and oxalate (e.g. spinach, sorrel). The plant *Galega officinalis*, which has a high protein content, contains in considerable amounts guanidine and galegine (isoamylene-guanidine) which hinder its use as fodder raw material.

35 Attempts to reduce the amount of detrimental substances present in plants by the so-called plant breeding method have, however, been unsuccessful in solving the problem.

40 According to the known method for processing green plants by means of drying, neither the fibreless form of the fodder, nor the reduction of the detrimental substances can be attained without considerable losses. Thus the presently known methods are unsuitable for preparing green plant extracts or concentrates in substantially fibreless form having high nutritional value containing all the essential ingredients of the green plant, while removing detrimental substances.

45 British Patent No. 705,369 discloses a process for the treatment of vegetable or animal matter by subjecting the raw material without comminution to pressure, separating the pressed liquid and coagulating this to recover the protein content thereof. The coagulated protein fraction is used as animal fodder, this fraction however contains only a part of the valuable ingredients inherent in a green plant. Therefore it cannot be considered as a full grade animal fodder.

60 U.S. Patent No. 2,600,903 relates only to the treatment of alfalfa and is in fact not adaptable to a wide range of green plant fodder material. According to this method freshly harvested alfalfa is pressed, the separated juices adjusted to an alkaline pH value

and the liquid and solids components recovered thereafter separated. The juice obtained is promptly heated to inactivate the enzymes contained therein and then the suspended solids are separated again and concentrated. 70 The process provides the isolation of certain substances such as xanthophylls, carotenoid acids, and mineral substances. However, all nutritionally important components cannot be recovered in the main product and deleterious components are not removed although alfalfa contains some of them e.g. saponines and/or nitrates. 75

The processes known in the art start in general with a pressed juice obtained from green plants which contains the important nitrogen content of the green plants in the form of several compounds. The nitrogen content of a green plant is commonly expressed as the raw protein content of the plant, which can be calculated by multiplying the determined nitrogen content by 6.25. This raw protein content of the green plant includes the following fractions containing nitrogen: 80

a) true protein fraction (chloroplasts) containing polypeptides having a molecular weight of greater than 10,000, which can be coagulated under the action of heat, acid or heavy metal salts; 85

b) "amide-fraction" containing polypeptides having a molecular weight lower than 10,000, such as free amino acids and amides such as asparagine and glutamin; 90

c) the "amide-fraction" containing the inorganic nitrates and ammonium salts which are also determined as part of the raw protein content of the starting green plant. 100

The "amide-fractions" present in green plants is not converted to a more valuable protein fraction according to conventional procedures although the heat-coagulable true protein fraction only constitutes about 30—50% of the total amount of nitrogen containing compounds in the green plant. The process of the present invention, as described below, converts the "amide fractions" mentioned at (b) and (c) above into useful substances. 105

The present invention provides a process for producing green plant extract of high nutritional value which process comprises comminuting green plant material which has not yet reached the generative stage, pressing the comminuted plant material to force liquid therefrom, adding antioxidant to the separated liquid and coagulating the true protein fraction thereof, separating the coagulated protein fraction from the liquid phase containing nitrogen sources which are substantially free from true protein, washing the coagulated precipitate with water or dilute acid, combining the liquid phase with the washing liquids, inoculating and aerobically fermenting the combined liquid phase with a micro-organism capable of utilizing nitrogen in the form of nitrate and ammonia until substantially all the nitrogen 120 125 130

sources are exhausted, concentrating the nitrogen-containing matter in the liquid phase and combining it with the coagulated true protein fraction and drying the resulting product.

- 5 The coagulation of the true protein fraction is effected according to preferred method at 80—85°C.

- 10 As a microorganism capable of utilizing nitrogen in the form of nitrate and ammonia preferably a microorganism selected from the genus *Hansenula*, *Candida*, *Saccharomyces*, *Ascomycetes*, *Phycomycetes* and mixtures thereof is used.

- 15 The concentration of the liquid phase which contains nitrogen containing matter substantially free from true protein may be effected by means of separation of the suspended micro-organism from the liquid phase. The concentration may also be carried out by means of evaporating the water content of the liquid phase under vacuum.

The fermentation can be carried out in two steps employing the same or different micro-organisms.

- 25 According to a preferred method of the present invention in order to increase the nitrogen content of the liquid phase in the first step of the fermentation, the fermentation is performed until the nitrogen sources present in the liquid phase are substantially exhausted. Then further nitrogen sources in form of ammonia or nitrate are added to the liquid phase and these are subjected to a repeated fermentation until the energy sources in the liquid phase are substantially exhausted. The nitrogen containing matters of the liquid phase after fermentation are then combined with the coagulated true protein fraction and dried.

- 40 The basis of the process according to the invention is the recognition that it is possible to produce a green fodder extract which is substantially fully equivalent to the afore-said protein-carriers, that is to fish-meal, soya and ground nuts and which—regarding its protein composition and nutritional value—is of even higher quality and can be stored for a long time without substantial decrease in value. According to the present process there is used cultivated plants or even weeds or plant portions which are in a growing or vegetative stage, since it is in this stage that their protein content and quality come nearest to the animal proteins and also their content of nutritionally important substances is most suitable. The nutritionally valuable complex substances may be recovered therefrom by a method in which the valuable substances are spared and the proteins are preserved to the maximum extent and the fiber content of the plant matter is substantially removed.

- 60 The process in general consists in that the green plant raw material, plants harvested prior to the generative stage or portions thereof, is subjected to a coarse mechanical comminution, then the material thus comminuted

is pressed in one or more steps, preferably with the first cake moistened with water. Generally the cake is wetted with water and again pressed to separate further liquid and the liquid thus obtained combined with that separated during formation of the press cake. The material subjected to repeated pressing is moistened with water in a quantity preferably equivalent to 20 to 30% of the raw material. The pressing process is carried out preferably in 2 or 3 steps and the press cake is moistened with water once or twice. The liquid is then separated for example by means of filtering from the fiber particles. In some cases the pure liquid separated from the liquid obtained after the first pressing operation may be applied as washing liquid via second pressing. After the removal of the filamentary material (cellulose, hemicellulose, lignine) of the plants or plant portions, the resulting liquids—which are preferably combined—form a practically homogeneous or a similar phase. Antioxidant in a rate equivalent to 0.5 to 4% of the dry content based on the finished product is dissolved or dispersed in the liquid phase. As antioxidant, preferably dimethylethoxyquinoline is used. The liquid phase is treated to cause the coagulation of the true protein content thereof. The coagulation is preferably carried out by heating the liquid to a temperature of 80°C to 85°C. The heat treatment exerts a double effect. On the one hand the pasteurization of the liquid can be effected and on the other hand the true protein content in the liquid is coagulated. The coagulated precipitate is separated from the liquid. The heat treatment may be carried out by introducing steam directly into the liquid or by indirect heating in pasteurization equipment. After the coagulation and separation the liquid is substantially free from true protein fraction and the nitrogen content thereof is only in form of the "amide fraction" referred to above. The original nitrogen content of the liquid obtained by means of pressing green plants varies to a considerable extent depending on the nature of the green plant processed and also on the growth stage of the green plant. The limits of the nitrogen content are as follows:

1. Plants containing carbohydrate in a considerable amount such as sorghum, sweet sorghum, wheat, Sudan grass (*Sorghum vulgare sudanese*) contain raw protein in a quantity of about 12—20% by weight calculated on the dry matter content, of which 6—10% by weight (that is 30—35% by weight of the raw protein content) is true protein.

2. Plants containing proteins in a considerable amount such as alfalfa contain raw protein in a quantity of about 17—25% by weight calculated on the dry matter content.

Generally the heat coagulable true protein fraction of the total raw protein quantity is about 30—50% by weight. Most of the con-

ventional procedures utilize only the heat coagulable fraction of the green plants and the remaining and valuable nitrogen containing matters are not utilized or transformed into more valuable nitrogen sources.

The coagulated true protein fraction is separated from the liquid and is washed either with water or with dilute acid. The washing liquids are combined with the liquid and cooled to a temperature of about 28—30°C. This liquid is subjected to fermentation in a fermentation reactor suitable for aerobic fermentation and equipped with a stirrer and aerating units. The liquid is inoculated with a microorganism capable of utilizing nitrogen in form of nitrate and ammonia. As microorganism generally a yeast is used either in the form of a stock culture or the yeast separated from a previous principal fermentation step. The yeast is propagated under aerobic conditions utilizing the nitrogen sources present in the liquid. The microorganism is forced due to the absence of the true protein fraction, to utilize the nitrogen sources having a lower biological value, i.e. the so-called "amide fraction". Thus the "amide fraction" can be transformed into a more valuable nitrogen containing substance which has increased nutritional value. The propagation of the yeast under aerobic conditions is the main process taking place in the course of which the growth factors necessary for the propagation are taken from the liquid. As energy sources for the propagation of the microorganism e.g. yeast serve the carbohydrate fractions of the liquid, especially the reducing sugars. Plant acids may also serve as energy sources. In the course of the fermentation the deleterious substances present in the liquid such as saponines and mustard oils are also utilized as energy sources, therefore the amounts thereof can be reduced to an acceptable level. During the fermentation a foam reducing additive such as silicone oil or sulfonated and neutralized sunflower oil can be incorporated in the liquid in an amount 50—100 ml cubic metre.

In the case of green plants containing a large amount of carbohydrate, such as Sorghum species, the carbohydrate content serves as an energy source. In the case of green plants containing large amounts of protein, such as alfalfa and field kale, the carbon fraction, e.g. plant acids, serves as the energy

source. The mineral substances and other necessary components for the propagation of yeast are present in the original pressed liquid.

The aeration of the liquid (fermentation broth) may be carried out continuously, in the first stage of the fermentation, applying 30 cubic metres air per cubic metre fermentation broth. Later, the amount of air necessary for the fermentation is regulated according to the requirements of the micro-organism employed.

During the propagation of the micro-organism the nitrogen content of the fermentation broth is reduced. When the amount of nitrogen containing substances is reduced to a certain point, the propagation of yeast finishes and autolysis of the microorganism begins. The fermentation generally is continued until the beginning of the autolysis of the micro-organism, because this ensures, in the case of suitable aeration of the broth, consumption of substantially all the nitrogen sources present. According to our experience when 60% of the original nitrate in the liquid has been consumed, then the quantity of the undesired substances, such as saponines and mustard oils, is reduced to 10—15% of the starting value. Of course, it is necessary that toxic substances should be eliminated entirely from the liquid: hence the actual fermentation is continued until substantially all the nitrogen is exhausted. This period of time is defined as the detoxication period. The reduction in the original nitrate content indicates the consumption of undesired substances and at the same time the conversion of the "amide fraction" present at the starting of the fermentation into more valuable nitrogen fraction.

The fermentation can be accomplished with one microorganism species or with a mixture of various microorganism species. Thus e.g. species of the genus *Saccharomyces* and *Candida* can be applied together. Sometimes it proves useful to start the fermentation with a certain yeast species and finish it with another yeast species. The selection of the appropriate microorganism species depends on the nature of the green plant to be processed and those skilled in the art can choose the most appropriate microorganism species for each green plant.

The average composition of the liquid phase before and after fermentation is illustrated in the following:

TABLE

		Before	After Fermentation
110	Substance		
	NH ₂ -nitrogen g/l	0.25—0.40	0.04—0.06
	NH ₄ -nitrogen g/l	0.12—0.20	0.015—0.025
	Nitrate nitrogen mg/l	150—300	50—90
	Reducing sugars g/l	4.0—50.0	0.5—2.5
115	Yeast dry matter content g/l	traces	7.00—25.00
	Saponines mg/l	0.12—0.20	0.01—0.02
	Mustard oils mg/l	2.0—4.0	0.01—0.02

Provided no nitrogen sources are added to the broth the original raw protein content of the starting liquid does not increase in an absolute sense as a result of the fermentation. However, the liquid after fermentation, that is after consumption of the original nitrogen sources, may still contain a considerable quantity of energy sources such as carbohydrates. In order to utilize these energy sources, and to transform them to a more valuable nitrogen fraction, further nitrogen sources in the form of ammonia or ammonium salts may be added to the fermentation broth, thus the ammonia may be converted into protein. Thus the final protein content of the product can be increased. A feature of the present process is that the nitrogen content of the green plant having a low nutritional value may be converted in an extent to 60—80% into a more valuable nitrogen fraction from the viewpoint of foddering.

Experiments show that the fermentation process can be accomplished within 4—6 hours and during this period of time the undesired deleterious substances can be eliminated or at least the amount thereof can be reduced to an acceptable level. In some cases the pH value of the fermentation liquid should be adjusted before or during fermentation depending on the requirements of the micro-organism employed.

The working up of the fermented liquid can be carried out by separating e.g. the mass of yeast cells mechanically from the liquid, heating the separated portion to a temperature to about 85°C to promote thermolysis and recombining this with a coagulated true protein fraction. The separated liquid obtained after removal of the mass of yeast cells can be mixed either with the fiberless end product or with the fibrous press cake. According to a preferred embodiment of the present process, the fermented liquid is evaporated in vacuo and the residue obtained is recombined with the coagulated true protein fraction.

The recombined protein-rich fraction is finally granulated or pressed to a cake or to another usual press product either alone or together with other fodder and nutriment.

The fibrous press cake obtained as by-product is not valueless, because it can be utilized alone as low grade hay for the purpose of ruminants when completed preferably with urea.

The invention is illustrated by the following examples.

Example 1

10 tons of rye, harvested prior to corn formation and at latest two weeks after blossoming, was subjected to an aqueous washing in order to eliminate the stone and iron contaminant. The washed material was then coarsely comminuted. The comminuted material was then pressed.

The pressing, using partly batch-type presses, was accomplished as follows:

- a) Discontinuous type low-pressure press (e.g. winepress) was applied as preliminary press,
- b) Discontinuous or continuous-type high-pressure press (e.g. screw press for vegetable oils) was used as final press.

The comminuted material was charged into press a) having a basket volume of e.g. 40 hectoliters. About 2.5—3.0 metric tons of rye may be charged into a basket of this size. Thus, the raw material may be processed by means of four consecutive chargings. The material in the primary press a) was pressed until a liquid yield of about 50% had been reached starting with a low pressure of 1 kg per sq.cm. Pressing was continued until the yield in liquid falls below 5 to 10 l/min/ton, the press cake was then loosened and the subsequent pressing was effected at a higher pressure (2 to 3 kg per sq.cm.). This operation was repeated at a higher pressure.

The press-cake weighing about 1.2 to 1.5 metric tons was discharged from the machine in the following way: the loosened press-cake was sprinkled with 10 to 30% of water based raw material in two stages, in the present case 500 kg. After the treatment with water the material was aged preferably for an hour before further processing, then charged, after secondary comminution, into a press of similar type and working under similar service conditions as before.

To obtain an extract content yield exceeding 90% of the raw material excluding the fiber content, another water treatment followed by a pressing operation should be carried out. The press-cake discharged from the preliminary press, amounting to 25 to 30% of the raw material, was charged into a discontinuous or continuous-type high-pressure final press in order to recover—together with the nutritive materials—a part of the moisture content of about 50%. Thus, the moisture content of the press-cake was reduced to 14% without appreciable consumption of thermal energy. The temperature of the press-cake, having a moisture content of about 30% discharged from the high-pressure final press, was from 50 to 70°C, thus in further drying required practically only the power for ventilating.

By this method 8 to 10 tons of liquid could be obtained from 10 tons of rye. This liquid contains 90% of the non-fibrous dry content of the processed plant material. Thereafter, liquid was separated from the fiber particles, from the press, by passing these through a screen of 0.5—1 mm mesh. To the obtained liquid a small quantity of anti-oxidant was added first (0.5% based on the dry content),

and then the concentrate was adjusted to the anti-oxidant level as required.

The separated liquid was heated to a temperature of 85°C and the coagulated true protein fraction containing the chloroplasts was separated. Having started with 8—10 tons of pressed juice 1.5—2 tons of wet precipitate could be obtained having a dry matter content of about 20—25% by weight. The precipitate was washed with water and dilute acid in a quantity of about 10—15% by weight calculated on the amount of the precipitate and the washing liquids were combined with the juice obtained after separation of the true protein fraction (liquid phase). The liquid phase was adjusted to a pH value of 4.5—5 and was aerated with mixing. The liquid phase was cooled to about 28°C in a heat exchanger and was inoculated with a yeast called *Candida utilis*, taken either from a stock culture or from a *Candida utilis*—containing broth from a previous fermentation. The quantity of the inoculum was 1 kg yeast dry matter calculated on each ton of the liquid phase. After inoculation of the broth the aeration was started with a large excess of air which was 30 cubic metres air per cubic metre of broth per hour. The rate of aeration was reduced one hour

after inoculation of the broth according to the required growth. As foam reducing additive, a sulfonated and neutralized sunflower oil was used. The pH value of the broth was controlled during a period of 4 hours after the starting of yeast propagation. In the sixth hour of the fermentation the increase in number of yeast cells begins to stagnate, the pH value of the broth increases and the reducing sugar content in the broth is reduced to as low as 2 g/l. The aerated broth was then heated in order to promote thermolysis of the yeast. Evaporation of the broth was then carried out at a temperature of 50—140°C until the residue contained 60% by weight dry matter. The residue was recombined with the coagulated true protein fraction and spray dried in a usual manner.

According to another variation of the process the fermentation, in order to consume all energy sources present in the broth, 2 g/l ammonia or ammonium salt were added to the broth in order to prevent the autolysis of the yeast and the increase of the pH value.

The composition of the dried products obtained according to the described two variations of the process is illustrated in the following table.

Ingredients	Without fermentation	According to the 1st variation (fermentation period 6—8 hours)	According to the 2nd variation (consuming all energy sources)
Raw protein %	15—20	30—35	40—45
True protein % (from raw protein)	7—11	18—22	28—32
Raw fibers %	1	1	1
Raw fat %	1,5	2,0	2,5
N-free extract %	60—65	40—45	35—40
Mineral substances %	10—13	12—18	15—20

The yield obtained in dried product was about 0.8—1.4 tons depending on the rate of the biological conversion.

thereof. The composition of the dried end product is illustrated herebelow:

Example 2

10 tons of alfalfa was harvested before blossoming and was processed according to the method described in Example 1. The pH value of the pressed juice was adjusted to about 6—7 and inoculated with *Hansenula anomala*. The fermentation was continued until the original nitrogen sources have been exhausted, then the broth was heated to about 85°C and the mass of yeast cells separated. During the separation a wet solid matter was obtained which was intermixed with a coagulated true protein fraction whereas the dilute juice arising from the separation has been evaporated and the obtained concentrate was added to the fiber containing by-product before drying

Ingredients	Without fermentation	With fermentation
Raw protein %	35.0	40—45
True protein % (from raw protein)	16—18	28—32
Raw fiber %	1.0	1.0
Raw fat %	3.0	3.5
N-free extract %	40—45	35—40
Ash %	15—20	12—18

The yield obtained in dried product was about 1.2—1.6 tons.

Example 3

10 tons of field kale (*Brassica oleracea*) was processed according to the method described in Example 1. The pressed juice was fermented after inoculation with a yeast called *Candida utilis*. When the reducing sugar con-

tent of the juice during fermentation had been reduced to about 5 g/l, which coincided with the point of time when the pH change occurred, the mass of yeast cells accumulated in the broth were separated and after heat treatment they were intermixed with the coagulated true protein fraction. The pH value of the separated juice was adjusted thereafter to about 6.5 and inoculated with a yeast called *Hansenula suaveolens* and the aeration of the broth was started. The quantity of the yeast employed as inoculum was 1 kg per cubic metre broth. The fermentation was continued for 3 hours thereafter and the mass of yeast cells were separated and again intermixed with the true protein fraction. The separated juice was evaporated and the residue recombined with the fiber containing by-product before drying.

The recombined protein fractions were spray dried.

The yield obtained from 10 tons of field kale was about 0.7 tons dried product. The composition of the dried end product was as follows:

Ingredients	Without fermentation	With fermentation
Raw protein %	28—32	45—50
true protein %		
(from raw protein)	12—15	34—38
Raw fiber %	1	1
Raw fat	1.0—1.5	1.5—2.0
N-free extract %	45—50	35—40
Ash %	18—20	10—15

Example 4

10 tons of *Sorghum saccharatum* was processed according to the method described in Example 1. The pressed juice was inoculated with two species of yeast called *Saccharomyces cerevisiae* and *Candida utilis* in a quantity of 1 kg/cubic metre juice. When the reducing sugar content of the broth had been reduced to below 1 g/l the broth was heated to about 85°C, the yeast cells were separated and recombined with the true protein fraction after separation.

The yield obtained was about 1.2—1.4 tons of dried endproduct having a raw protein content of about 35—40% by weight.

WHAT WE CLAIM IS:—

1. A process for preparing green plant extract of high nutritional value which process comprises comminuting green plant material which has not yet reached the generative state, pressing the comminuted plant material to force liquid therefrom, separating the liquid from the pressed cake, adding antioxidant to the separated liquid and coagulating the true protein fraction thereof, separating the coagulated protein fraction from the liquid phase containing nitrogen sources which are substantially free from protein, washing the coagu-

lated precipitate with water or dilute acid, combining the liquid phase with the washing liquid, inoculating and aerobically fermenting the recombined liquid phase with a microorganism capable of utilizing nitrogen in the form of nitrate and ammonia until substantially all the nitrogen sources are exhausted concentrating the nitrogen-containing matter in the liquid phase and combining it with the coagulated true protein fraction and drying the resulting product.

2. A process according to claim 1 wherein the pressed cake is wetted with water and again pressed to separate further liquid, the liquid thus obtained is combined with the liquid separated during the formation of the press cake and the antioxidant added to this mixture.

3. A process according to claim 1 or 2 wherein the coagulation of the true protein fraction is effected at 80—85°C.

4. A process according to any one of claims 1 to 3 wherein the microorganism is selected from the genus *Hansenula*, *Candida*, *Saccharomyces*, *Ascomycetes*, *Physomycetes*, and mixtures thereof.

5. A process according to any one of claims 1 to 4 wherein the concentration of the liquid phase after fermentation is effected by separation of the microorganism cells accumulated in the liquid phase.

6. A process according to any one of claims 1 to 4 wherein the concentration of the liquid phase after fermentation is effected by evaporation the water content of the liquid phase under vacuum.

7. A process according to any one of claims 1 to 6 wherein the aerobic fermentation is carried out in two steps employing the same or different microorganisms.

8. A process for preparing substantially fibreless green plant extract of high nutritional value in a detoxicated form which process comprises comminuting green plant material which has not yet reached the generative state, pressing the comminuted plant material to force liquid therefrom, separating the liquid from the pressed cake, wetting the pressed cake with water, again pressing the pressed cake to separate further liquid, adding antioxidant to the liquid and coagulating the true protein fraction thereof, separating the coagulated protein fraction from the liquid phase containing nitrogen sources which are substantially free from true protein, washing the coagulated precipitate with water or dilute acid, combining the liquid phase with the washing liquid, inoculating and aerobically fermenting the combined liquid phase with a microorganism capable of utilizing nitrogen in form of nitrate and ammonia until the nitrogen sources contained therein are substantially exhausted, adding further nitrogen sources to the liquid phase in the form of ammonia or ammonium salts, subjecting the liquid phase to



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tent of the juice during fermentation had been reduced to about 5 g/l, which coincided with the point of time when the pH change occurred, the mass of yeast cells accumulated in the broth were separated and after heat treatment they were intermixed with the coagulated true protein fraction. The pH value of the separated juice was adjusted thereafter to about 6.5 and inoculated with a yeast called *Hansenula suaveolens* and the aeration of the broth was started. The quantity of the yeast employed as inoculum was 1 kg per cubic metre broth. The fermentation was continued for 3 hours thereafter and the mass of yeast cells were separated and again intermixed with the true protein fraction. The separated juice was evaporated and the residue recombined with the fiber containing by-product before drying.

The recombined protein fractions were spray dried.

The yield obtained from 10 tons of field kale was about 0.7 tons dried product. The composition of the dried end product was as follows:

Ingredients	Without fermentation	With fermentation
Raw protein %	28—32	45—50
true protein %		
(from raw protein)	12—15	34—38
Raw fiber %	1	1
Raw fat	1.0—1.5	1.5—2.0
N-free extract %	45—50	35—40
Ash %	18—20	10—15

Example 4

10 tons of *Sorghum saccharatum* was processed according to the method described in Example 1. The pressed juice was inoculated with two species of yeast called *Saccharomyces cerevisiae* and *Candida utilis* in a quantity of 1 kg/cubic metre juice. When the reducing sugar content of the broth had been reduced to below 1 g/l the broth was heated to about 85°C, the yeast cells were separated and recombined with the true protein fraction after separation.

The yield obtained was about 1.2—1.4 tons of dried endproduct having a raw protein content of about 35—40% by weight.

WHAT WE CLAIM IS:—

1. A process for preparing green plant extract of high nutritional value which process comprises comminuting green plant material which has not yet reached the generative state, pressing the comminuted plant material to force liquid therefrom, separating the liquid from the pressed cake, adding antioxidant to the separated liquid and coagulating the true protein fraction thereof, separating the coagulated protein fraction from the liquid phase containing nitrogen sources which are substantially free from protein, washing the coagu-

lated precipitate with water or dilute acid, combining the liquid phase with the washing liquid, inoculating and aerobically fermenting the recombined liquid phase with a microorganism capable of utilizing nitrogen in the form of nitrate and ammonia until substantially all the nitrogen sources are exhausted concentrating the nitrogen-containing matter in the liquid phase and combining it with the coagulated true protein fraction and drying the resulting product.

2. A process according to claim 1 wherein the pressed cake is wetted with water and again pressed to separate further liquid, the liquid thus obtained is combined with the liquid separated during the formation of the press cake and the antioxidant added to this mixture.

3. A process according to claim 1 or 2 wherein the coagulation of the true protein fraction is effected at 80—85°C.

4. A process according to any one of claims 1 to 3 wherein the microorganism is selected from the genus *Hansenula*, *Candida*, *Saccharomyces*, *Ascomycetes*, *Physomycetes*, and mixtures thereof.

5. A process according to any one of claims 1 to 4 wherein the concentration of the liquid phase after fermentation is effected by separation of the microorganism cells accumulated in the liquid phase.

6. A process according to any one of claims 1 to 4 wherein the concentration of the liquid phase after fermentation is effected by evaporation the water content of the liquid phase under vacuum.

7. A process according to any one of claims 1 to 6 wherein the aerobic fermentation is carried out in two steps employing the same or different microorganisms.

8. A process for preparing substantially fibreless green plant extract of high nutritional value in a detoxicated form which process comprised comminuting green plant material which has not yet reached the generative state, pressing the comminuted plant material to force liquid therefrom, separating the liquid from the pressed cake, wetting the pressed cake with water, again pressing the pressed cake to separate further liquid, adding antioxidant to the liquid and coagulating the true protein fraction thereof, separating the coagulated protein fraction from the liquid phase containing nitrogen sources which are substantially free from true protein, washing the coagulated precipitate with water or dilute acid, combining the liquid phase with the washing liquid, inoculating and aerobically fermenting the combined liquid phase with a microorganism capable of utilizing nitrogen in form of nitrate and ammonia until the nitrogen sources contained therein are substantially exhausted, adding further nitrogen sources to the liquid phase in the form of ammonia or ammonium salts, subjecting the liquid phase to

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5 further fermentation until the energy sources thereof are substantially exhausted, separating micro-organism cells obtained from the liquid, thermolysing the separated cells, combining the thermolysed cell with the coagulated true protein fraction and drying the resulting product.

10 9. A process according to claim 8 wherein the microorganism cells are separated before the addition of further nitrogen sources.

10 10. A process according to claim 1 substantially as herein described with reference to the specific Examples.

11. Green plant extract of high nutritional value prepared by the process claimed in any preceding claim. 15

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