

Title: Retention time and mild coagulation for obtaining a coagulated tuber protein product

5 **Field of the invention**

The invention is directed to a method for obtaining a coagulated tuber protein product from a tuber fruit juice. The invention is further directed to a coagulated tuber protein product and to a food product comprising the coagulated tuber protein product.

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Background

There is an increasing demand for vegetarian and vegan alternatives for animal-derived products. In order to provide such alternatives, the industry is taking interest in plant-based proteins.

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One example is the widely available potato protein. Potato proteins are conventionally isolated from starch production streams. The isolation may be performed by a variety of means, such as diafiltration, bed adsorption or chromatography. The potato protein may be isolated in a native or coagulated form.

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A method for isolation of native tuber protein is for instance described in WO2020/242302. Herein, tuber processing water is subjected to a pretreatment step and a step of diafiltration. The tuber protein isolate may be obtained as the diafiltration retentate.

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US2010/0048873 describes a method for obtaining a tuber protein fraction with a high molecular weight, by precipitating a high-molecular-weight tuber plant protein fraction, separating this precipitated fraction and subsequently a further precipitation of a medium-molecular weight coagulated tuber plant protein fraction, which is also separated. However, this method does not provide small particles of coagulated tuber protein fractions, which are preferred as they provide a better mouthfeel.

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Currently, there are some other drawbacks associated with the isolation of potato proteins. Namely, the isolated potato proteins typically have a dark colour that is undesirable for use in food products. Further, the quality of the protein products may be insufficient to be directly usable for food applications, therefore requiring further processing steps that are time and energy consuming. For instance, the mouthfeel is not sufficient.

Figures

Figure 1 - Particle size distributions of sample 1 after varying amounts of shear. ■: no shear applied, ▼: 3000 rpm for 30 s, ▲: 9000 rpm for 30 s, ●: 15000 rpm for 30 s.

Figure 2 - Particle size distributions of sample 2 after varying amounts of shear. ◆: no shear applied, ■: 3000 rpm for 30 s, ▼: 9000 rpm for 30 s, ●: 15000 rpm for 30 s.

Detailed description

The inventors realized that a coagulated tuber protein product may be obtained wherein one or more of the above-mentioned drawbacks has been overcome, when using a method comprising at least two coagulation steps and wherein relatively mild coagulation conditions are employed and/or the (lean) tuber fruit juice is maintained at a coagulation temperature for a retention time in the range of 5 to 40 minutes during the first coagulation step, and optionally during the second coagulation step.

Accordingly, the invention is directed to a method for obtaining a coagulated tuber protein product from a tuber fruit juice. The method comprises:

- subjecting the tuber fruit juice to a first coagulation step to form a coagulated low-grade fraction;
- separating at least part of the coagulated low-grade fraction from the tuber fruit juice to obtain a lean tuber fruit juice;

- subjecting the lean tuber fruit juice to a second coagulation step comprising adjusting the temperature of the lean tuber fruit juice to form a coagulated tuber protein product;

- collecting at least part of the coagulated tuber protein product.,

5 wherein the first coagulation step comprises adjusting the temperature of the tuber fruit juice in the first coagulation step to at most 60 °C and maintaining the temperature for a retention time in the range of 5 – 40 minutes and wherein the temperature of the lean tuber fruit juice in the second coagulation step is adjusted to at least 60 °C; and/or

10 wherein the temperature of the lean tuber fruit juice in the second coagulation step is adjusted to a temperature between 65 – 75 °C and preferably wherein the pH of the tuber fruit juice in the first coagulation step is in the range of 5 – 6.5.

 It is an advantage of the present methods that coagulated tuber
15 protein product may be obtained with one or more of a superior mouthfeel, a superior digestibility, high efficiency, low protein loss, low costs, low environmental burden and minimal waste streams.

 In particular, it was found that with a method according to the invention, wherein the first coagulation step comprises adjusting the
20 temperature of the tuber fruit juice in the first coagulation step is adjusted to at most 60 °C and maintaining the temperature for a retention time in the range of 5 – 40 minutes and wherein the temperature of the lean tuber fruit juice in the second coagulation step is adjusted to at least 60 °C, preferably at least 70 °C, a coagulated protein product may be obtained with
25 a superior mouthfeel.

 Alternatively or additionally, it was found that with a method according to the invention wherein the temperature of the lean tuber fruit juice in the second coagulation step is adjusted to a temperature between 65 – 75 °C, preferably between 70 – 75 °C and preferably wherein the pH of

the tuber fruit juice in the first coagulation step is in the range of 5 – 6.5, a coagulated protein product may be obtained with superior digestibility.

The method may further comprise providing the tuber fruit juice (herein also referred to as tuber juice, juice or fruit juice). It may be appreciated that the tuber fruit juice may be obtained by a processing step of processing at least one tuber. This processing may comprise *e.g.* pulping, mashing, rasping, grinding, pressing and/or cutting the tuber, optionally in combination with water. The processing may also comprise at least partial starch removal, for example by decanting, cycloning and/or filtering.

Tuber is herein used to refer to structures which may also be called root. Tuber inherently comprises proteins, but may also be rich in starch.

The type of tuber is not particularly limiting. For instance, the tuber may comprise potato (*Solanum tuberosum*), sweet potato (*Ipomoea batatas*), cassava (including *Manihot esculenta*, syn. *M. utilissima*, also called manioc, mandioca or yuca and also including *M. palmata*, syn. *M. dulcis*, also called yuca dulce), yam (*Dioscorea spp*), and/or taro (*Colocasia esculenta*). Preferably, the tuber comprises potato, sweet potato, cassava or yam, more preferably the tuber comprises potato, sweet potato or cassava, even more preferably the tuber comprises a potato or sweet potato, most preferably the tuber comprises potato. Potato is the tuber from the plant *Solanum tuberosum*. There exists a plurality of varieties of potatoes, that are all suitable for the present method. This includes varieties intended for the starch industry (starch potatoes), as well as varieties intended for human consumption (consumption potatoes). Accordingly, it is particularly preferred that the tuber fruit juice is a potato tuber fruit juice.

Tuber varieties comprise native tuber protein. Native potato protein, for example, can be divided into three classes (i) the patatin family, highly homologous acidic 43 kDa glycoproteins (40-50 wt.% of the potato proteins), (ii) basis 5-25 kDa protease inhibitors (30-40 wt.% of the potato

proteins) and (iii) other proteins mostly high molecular weight proteins (10-20 wt.% of the potato proteins)

The tuber fruit juice may optionally be defoamed. This may be achieved by *e.g.* running the juice over a hydrocyclone (such as a 15 mm hydrocyclone). The density of the fruit juice may also be adjusted. This may be done by any means known in the art, such as by feeding the underflow from the hydrocyclone to a defoaming tank until the desired density is reached. This density may be approximately 950-1000 kg/m³, such as 980 kg/m³.

The tuber fruit juice is subjected to a first coagulation step to form a coagulated low-grade fraction (herein also referred to as the low-grade fraction).. Preferably, this first coagulation step comprises adjusting the temperature of the tuber fruit juice to at most 60 °C.

Advantageously, by forming the low-grade fraction, one or more impurities *i.e.* compounds that are undesirable in the coagulated tuber protein product may be removed. Namely, the low-grade fraction typically has a dark colour and comprises the impurities, *i.e.* compounds that are undesirable in the coagulated tuber protein product. The dark colour is believed to originate from the interaction of iron ions with phenolic components such as chlorogenic acid and its polymers. Advantageously, by extracting the iron from the tuber fruit juice into the low-grade fraction, the coagulated tuber protein product (herein also referred to as tuber protein product or tuber product) typically has less (*e.g.* a whiter) colour, which is preferred for food applications. The low-grade fraction may alternatively or additionally comprise lipids and/or phenolic compounds. It may be appreciated that the low-grade fraction may be of commercial value. For instance, the low-grade fraction may find its use in animal foods, such as pet foods.

The temperature of the first coagulation step is generally sufficient to coagulate the desired low-grade fraction. The components for

this fraction are typically not very heat stable. Preferably, the temperature is not too high, as this would result in more protein coagulation. This is generally undesired as the proteins preferably remain in the lean tuber fruit juice (herein also referred to as lean fruit juice, lean juice or lean tuber juice). Accordingly, the first coagulation step comprises adjusting the temperature of the tuber fruit juice to at most 60 °C. Typically, a lower temperature allows for a lower energy input, which is both economically advantageous and environmentally friendly. It is preferred that the first coagulation step comprises adjusting the temperature of the tuber fruit juice to at most 55 °C, preferably at most 50 °C.

It may be appreciated that the temperature is preferably not too low, as this would likely not provide sufficient coagulation to form the coagulated low-grade fraction. Therefore, the temperature is preferably at least 30 °C, more preferably at least 35 °C. It was found that an optimal balance for the temperature is found at a temperature in the range of 30 – 50 °C, more preferably 35 – 45 °C, such as about 40 °C.

In an aspect, this temperature is preferably maintained for a retention time. The retention time may be achieved in various ways, for instance by keeping the fruit juice in a heated and/or isolated vessel and/or by leading the tuber fruit juice through a path of heated and/or isolated tubes, for instance (isolated) stainless steel pipes. Preferably, the retention time is controlled, more preferably by means of a residence time tube. In such a set-up, the flow rate determines the time that the heated liquid spends in a tube at a controlled temperature. This ensures that the time under which the juice is exposed to heat is exact by avoiding the need to ramp up the temperature in a stirred vessel during heating and the need to ramp down the temperature again upon completing the coagulation. As the skilled person will appreciate, the properties of residence time tube will depend on various aspects of the method, such as the scale of the experiment, the retention time that is envisaged, and the properties of the

fluid. It is within the capabilities of the skilled person to select a residence time tube of appropriate volume and length in order to arrive at a predefined retention based on common general knowledge and the information provided herein.

5 The present inventors surprisingly found that the retention time allows for a coagulated tuber product with a certain particle size distribution that provides a good mouthfeel, little grittiness and/or little horniness (*vide infra*). The retention time further advantageously allows for provision of an optimal size of the coagulates in the low-grade fraction,
10 allowing for easier separation of the low-grade fraction from the lean tuber fruit juice.

 It was found that a retention time below 5 minutes does not significantly affect the coagulated tuber product. For instance, no significant difference in particle size distribution was obtained compared to no
15 retention times. Long retention times may unnecessarily prolong the processing times, while the properties of the coagulated tuber product remain essentially unchanged compared to shorter retention times.

 Therefore, the retention time is preferably between 5 – 40 minutes. Shorter retention times are preferred for quicker processing.
20 Accordingly, the retention time is preferably between 5 – 20 minutes. Better results were obtained for a retention time between 8 – 16 minutes. The optimal retention time was found at a time between 10 – 14 minutes, such as approximately 12 or 13 minutes.

 The tuber fruit juice is typically an aqueous tuber fruit juice. The
25 pH of the tuber fruit juice also affects the coagulation. For instance, a high pH may result in a less effective coagulation, indicating that less impurities are included in the low-grade fraction. A low pH may result in more protein coagulation, thereby reducing the yield of the coagulated tuber protein product. The pH of the tuber fruit juice is preferably in the range of 5 – 7,
30 more preferably 5.5 – 6.5. Most preferably, the tuber fruit juice has an

endogenous pH (*i.e.* a pH that has not been externally altered by any means). The endogenous pH of typical fruit juice, in particular potato fruit juice, is in the range of 5.7 – 6.2.

If it is desired to have another pH than the endogenous pH, the
5 pH of the tuber fruit juice may be adjusted before the first coagulation step and/or in the first coagulation step. The tuber fruit juice may, for example, be contacted with a fruit juice buffer.

The fruit juice buffer is a buffer which is added to the fruit juice. The buffer may comprise an acid or a base, as is known in the art. Suitable
10 bases are sodium or potassium hydroxide, ammonium chloride, sodium or potassium carbonate, oxides and hydroxides of calcium and magnesium. Preferably, the fruit juice buffer comprises an acid. This may allow for some acid coagulation. More preferably the acid is sulfuric acid, phosphoric acid, lactic acid, formic acid, citric acid, acetic acid and/or hydrochloric acid. For
15 economical reasons, sulfuric acid and/or hydrochloric acid may be preferred. Due to corrosion and water treatment systems, hydrochloric acid may not be preferred. Accordingly, sulfuric acid is most preferred.

At least part of the formed coagulated low-grade fraction, preferably essentially all (*e.g.* more than 95 wt.%, more than 98 wt.%, or
20 more than 99 wt.%, such as 100 wt.% based on the total weight of the low-grade fraction), is separated from the tuber fruit juice to obtain a lean tuber fruit juice. The low-grade fraction may be collected. The separation methods are known in the art and not particularly limiting. A suitable method may comprise filtration (*e.g.* micro-, nano- or ultrafiltration), centrifugation, such
25 as a high-speed centrifugal separator, cycloning, decanting, belt filter and/or using a disc-stack separator. In case a high-speed centrifugal separator is used, it may be preferred to operate it at low capacities. This may allow for the fruit juice to experience high g-forces over a long time, resulting in higher separation yields. A disc-stack separator may also be preferred as

this is associated with higher separation yields. Even more preferably, a self-discharging disc-stack separator.

The obtained lean tuber fruit juice is subjected to the second coagulation step. This second coagulation step comprises heating the
5 temperature of the lean tuber fruit juice to at least 60 °C. This temperature is accordingly higher than the temperature used during the first coagulation step. This indicates that generally any proteins and components that have not coagulated during the first coagulation step, at the lower temperature, may coagulate in this second coagulation step. Typically, this entail the
10 coagulation of tuber proteins. As an example, these tuber proteins may *i.a.* be patatin and/or protease inhibitor, preferably potato patatin and/or protease inhibitor.

Protease inhibitor, as defined herein, is a root or tuber protein, preferably a potato protein, which is in its native form capable of inhibiting
15 the protease activity of proteases.

Patatin, as defined herein, is a root or tuber protein, preferably a potato protein, which is an acidic glycoprotein which functions as a storage protein in the tuber.

The temperature of the second coagulation step is at least 60 °C, preferably at least 65 °C, in particular at least 70 °C.
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Also for this second coagulation step, it may be preferred that the temperature is not too high for economic and environmental reasons. Preferably the temperature is less than 100 °C, or even less than 90 °C, such as less than 80 °C. Such temperatures were found to be sufficiently high for
25 the coagulation of the tuber protein product. Accordingly, the second coagulation step preferably comprises adjusting the temperature of the lean tuber fruit juice to a temperature in the range of 60 – 100 °C, preferably 60 – 90 °C, more preferably 60 – 80 °C, such as 65 – 75 °C.

The temperature of the second coagulation step allows for forming
30 a coagulated tuber protein product with superior digestibility (*vide infra*). It

was found that even better results were obtained wherein the second coagulation step comprises adjusting the temperature of the lean tuber fruit juice to a temperature in the range of 65 – 74 °C, preferably 65 – 73 °C, more preferably 65 – 71 °C. Preferably the temperature is between
5 66 – 69 °C, such as about 67 °C or about 68 °C.

This temperature of the second coagulation step is preferably maintained for a retention time. The retention time may be achieved in various ways, as described herein above. The retention time is preferably between 5 – 40 minutes. Shorter retention times are preferred for quicker
10 processing. Accordingly, the retention time is preferably between 5 – 20 minutes. Better results were obtained for a retention time between 5 – 10 minutes, such as approximately 7 or 8 minutes.

The pH of the lean tuber fruit juice is not of particular relevance. Therefore, the pH is typically not adjusted in the second coagulation step.
15 Accordingly, the pH of the lean tuber fruit juice may be endogenous and thus typically between 5.7 – 6.2. Nonetheless, the pH may be slightly different, due to the removal of the low-grade fraction. Accordingly, the pH of the lean tuber fruit juice in the second coagulation step is typically in the range of 5 – 7, preferably 5.5 – 6.5, more preferably 5.6 – 6.0.

20 To increase the efficiency of the coagulation, it may be preferred to contact the lean fruit juice with a coagulation buffer. This coagulation buffer may be used to add coagulants and/or anti-oxidants, in particular sulfite. Sulfite is typically used for providing the coagulated tuber protein product with a lighter and/or more whitish colour. The use of coagulants
25 may result in slight foaming. Therefore, it may be advantageous to add an anti-foamer. Accordingly, the coagulation buffer preferably comprises a mild coagulants, sulfite and/or an anti-foamer. Suitable coagulants are known in the art. One example is calcium. Calcium is typically associated to remove impurities. However, calcium is also known to be associated with fouling
30 and stimulation of microbial proliferation. Therefore, it may also be

preferred to use no or little calcium. Calcium may be present in a salt complex, such as CaCl_2 or a hydrate thereof. It is preferred that at most 3 g of calcium or a calcium salt complex per liter coagulation buffer is present, more preferably at most 2.5 g/L, such as at most 2 g/L or at most 1 g/L.

- 5 Suitable anti-foamers may include, but are not limited to, antifoams commercially available from Struktol and/or Dispelair® anti-foams.

At least part of the coagulated tuber protein product, preferably essentially all (*e.g.* more than 95 wt.%, more than 98 wt.%, or more than 99 wt.%, such as 100 wt.% based on the total weight of the coagulated tuber protein product), is collected from the lean tuber fruit juice.

The coagulated tuber protein product preferably advantageously has a small particle size. This small particle size is associated with a better mouthfeel, less grittiness and less horniness. The coagulated tuber protein product typically has a d90 particle size distribution of at most 40 μm , preferably at most 30 μm , more preferably at most 28 μm , such as at most 25 μm .

The d10, d50 and d90 are common parameters to express particle size distribution. The d50 is the volume median particle size, and indicates the diameter, in μm , that splits the distribution into two equal fractions, wherein half of the particle volume has a diameter above the median diameter, and wherein half of the particle volume has a diameter below the median diameter.

Similarly, the d10 indicates the diameter, in μm , that splits the particle size distribution into two (volume) portions, wherein 10 % of the particle volume has a diameter below the d-10, and wherein 90 % of the particle volume has a diameter above the d-10.

The d90 is defined in a similar manner, and indicates the diameter that splits the particle size distribution into two (volume) portions, wherein 90 % of the particle volume has a diameter below the d90, and wherein 10 % of the particle volume has a diameter above the d90.

The particle size distribution may be determined by a variety of means, such as laser diffraction methods. Preferably, the particle size distribution is determined with HELOS (commercially available from Sympatec) using laser diffraction. The samples may be measured in process
5 water with Quixel dispersion system (commercially available from Sympatec). The data is typically evaluated with the Fraunhofer diffraction equation.

Most advantageous, this particle size distribution is generally obtained without the need of grinding, milling and/or crushing. It was found
10 that the retention time allows for such particle size distributions. Accordingly, essentially no grinding, milling and/or crushing is performed on the coagulated tuber protein product.

Alternatively or additionally, the coagulated tuber protein product preferably advantageously has a good digestibility. This digestibility is
15 typically at least 1 mol amine / kg dry coagulated tuber protein product, preferably at least 2, more preferably at least 3, even more preferably at least 3.5 mol amine / kg dry coagulated tuber protein product. The digestibility is measured according to Infogest 2.0 as described by Brodkorb, A., Egger, L., Alming, M. *et al.* in *Nat. Protoc.* 2019, Apr; 14(4): 991-1014.

20 Most advantageous, this digestibility is obtained by the mild temperatures of the second coagulation step. Accordingly, there is often no or little need to perform any further chemical or physical processes on the coagulated tuber protein product.

Preferably, said coagulated tuber protein product is subjected to a
25 heating step to at most 90 °C to form a stabilized coagulated tuber protein product. The present inventors surprisingly found that said heating step allows for obtaining a coagulated tuber product with low stickiness, a relatively narrow particle size distribution, excellent separability of the coagulated tuber protein product from the lean tuber fruit juice, excellent
30 emulsifying capacity and improved shear stability.

Preferably the temperature is at least 70 °C, more preferably at least 75 °C, even more preferably at least 80 °C. Accordingly, the temperature is preferably in the range of 80 °C to 90 °C, more preferably in the range of 82 °C to 88 °C, such as approximately 85 °C or 86 °C.

5 Said coagulated tuber protein product is preferably maintained for a retention time. The retention time is preferably between 5 – 40 minutes. Shorter retention times are preferred for quicker processing. Accordingly, the retention time is preferably between 5 – 20 minutes. Better results were obtained for a retention time between 8 – 16 minutes. The
10 optimal retention time was found at a time between 10 – 14 minutes, such as approximately 12 or 13 minutes.

Preferably, a fluid comprising said coagulated protein is heated under a flow, more preferably a turbulent flow. As used herein, the term fluid refers to a liquid or mixtures of liquids, or mixtures of a liquid and at
15 least one other phase, such as suspensions. Typically, said turbulent flow has a Reynolds number of at least 3000, more preferably at least 4000, even more preferably at least 5000, most preferably at least 8000. Preferably, said Reynolds number is in the range of 3000 to 100,000, preferably in the range of 4000 to 50,000, such as in the range of from 5000 to 25,000.

20 As is known, the Reynolds number can be determined using the following formula:

$$Re = \frac{\rho \cdot D \cdot v}{\mu}$$

wherein ρ is the density of the fluid, D is the hydraulic diameter (which usually corresponds to the diameter of the pipe, if a tube heat
25 exchanger is used), v is the mean fluid velocity and μ is the dynamic viscosity of the fluid.

Usually, the density of a fluid comprising coagulated tuber protein product is at least 1000 kg/m³, preferably at least 1500 kg/m³, more preferably a in the range of between 1000 and 2500 kg/m³. Typically, the

viscosity of a fluid comprising coagulated tuber protein product is at least 1 mPa · s, preferably at least 10 mPa · s, more preferably at least 50 mPa · s. Typically, said fluid has a viscosity in the range of between 1 and 10,000 mPa · s, such as between 50 and 1000 mPa · s.

5 Usually, the velocity of a fluid comprising coagulated tuber protein product in a heating body is at least 0.5 m/s, preferably at least 1 m/s, even more preferably at least 2 m/s. Preferably, the velocity of a fluid comprising coagulated tuber protein product in a heating body is in the range of between 0.5 and 5 m/s, preferably in the range of 1 and 2.5 m/s.

10 Usually, the (hydraulic) diameter of the heating body, preferably heat exchanger, more preferably tubular heat exchanger, is at least 10 mm, more preferably at least 19 mm, even more preferably at least 25 mm, such as at least 40 mm. Typically, the diameter of the heating body is in the range of between 10 mm and 55 mm, in particular between 12 mm and
15 51 mm, such as between 19 mm and 26 mm.

 Alternatively or additionally, said heating step to at most 90 °C comprises shear conditions. As used herein the term shear conditions refer to conditions wherein a force per unit area is exerted by a fluid on a heating body (also referred to as shear stress), which typically arises when moving a
20 fluid through a heating body. As is known, factors that impact the amount of shear stress exerted by a fluid include fluid velocity, fluid density and/or fluid viscosity, flow regime and heating body geometry. Herein, shear stress usually increases with increasing fluid velocity and higher fluid density or viscosity. Likewise, shear stress typically increases with decreasing
25 diameter of the heating body through which the fluid moves. For many geometries the equations for shear stress are described in the textbooks, such as for example Barnes "Handbook of elementary rheology".

 Typically, the shear stress during said heating step is at least 10 Pa, preferably at least 20 Pa, more preferably at least 30 Pa, more
30 preferably at least 40 Pa, in particular at least 50 Pa. Usually, shear stress

is in the range of between 10 Pa and 200 Pa, preferably between 20 Pa and 100 Pa, more preferably between 30 Pa and 50 Pa. Shear stress can be determined empirically or analytically using any suitable approach known in the art, for example using the Darcy-Weisbach equation.

5 The shear conditions may be achieved in various ways, for instance by leading a fluid comprising coagulated tuber protein product through a (tubular) heat exchanger operated at a temperature of at most 90 °C. Collecting the tuber protein product typically comprises separation of at least part, preferably essentially all, of the coagulated tuber protein
10 product from the lean tuber fruit juice. It may be appreciated that the coagulated tuber protein product may comprise some residual lean tuber fruit juice. The means for collection may be similar to the separation means for separating the low-grade fraction from the tuber fruit juice. Accordingly, collecting at least part of the coagulated tuber protein product may comprise
15 filtration (*e.g.* micro-, nano- or ultrafiltration), centrifugation, such as a high-speed centrifugal separator, cycloning, decanting, using a belt filter and/or using a disc-stack separator.

 In case a high-speed centrifugal separator is used, it may be preferred to operate it at low capacities. This may allow for the fruit juice to
20 experience high g-forces over a long time, resulting in higher separation yields.

 It is typically required to use a gentle collection means, in particular for coagulated tuber protein products with small particle size distributions. Accordingly, a disc-stack separator, such as a self-discharging
25 disc-stack separator is preferred.

 Even more preferably, a ultrahigh g-force decanter, such as the Sedicanter ® commercially available from Flottweg SE, is most preferred.

 After collection of at least part of the coagulated tuber protein product, the coagulated tuber protein fraction may at least be partially
30 dried. For instance, at least 90 wt.%, at least 95 wt.%, at least 97 wt.%, at

least 98 wt.% or at least 99 wt.%, such as 100 wt.% of the lean tuber fruit juice may be removed. The method for drying is not particularly limiting. Suitable means are known in the art and include for instance freeze drying or drying in a vortex-dryer. It was found that while using a vortex-drying, short residence times and/or dry solid matter contents above 85 %, such as above 90 % or even above 95 % are preferred due to the small particle size. Typical residence time are for instance in the order of seconds, such as 1-30 seconds, preferably 1-15 seconds, more preferably 1-10 seconds. This is in the art sometimes also referred to as flash drying. Dry solid matter is herein used to refer to the amount as determined by *e.g.* thermogravimetry, for instance using a Mettler Toledo HR83 Moisture Analyzer device.

Alternatively, or additionally the method may comprise at least partially washing the tuber protein fraction. Washing may be performed in order to increase the purity of the coagulated tuber protein product. For instance, washing typically removes or at least minimizes the amounts of glycoalkaloids.

Glycoalkaloids herein refer to glycosylated alkaloids, defined as the total of solanine and chaconine derivatives. This quantity can also be referred to as the total glycoalkaloid content (TGA) and can be determined according to the method of Laus *et al.* (Laus M.C., Klip G. & Giuseppin M.L.F. (2016) Food Anal. Methods 10(4) "Improved Extraction and Sample Cleanup of Tri-glycoalkaloids α -Solanine and α -Chaconine in Non-denatured Potato Protein Isolates"). Glycoalkaloids are known to be poisonous to humans, for which reasons their presence is preferably limited in the tuber protein product.

The washing method is not limited and suitable means are known in the art. It is most preferred to wash the coagulated tuber protein fraction in a washing medium. Preferably, the washing medium comprises an acidic aqueous solution, such an aqueous solution comprising sulfuric acid. The best results were obtained for a washing medium having a pH of 3 – 5,

preferably 3.5 – 4.5. For sufficient washing it is typically preferred to have a washing mixture (*i.e.* the washing medium and the coagulated tuber protein fraction) having 1 – 7 % solid matter, such as 2 – 6 %, or 2 – 5 %. A higher dry solid matter content typically results in a too viscous washing mixture, compromising on the washing quality. The optimum balance between flow characteristics and process efficiency was found at 3 %.

The coagulated tuber protein product typically comprises at least 85 wt.% protein, preferably at least 90 wt.%, more preferably at least 95 wt.%. Advantageously, the obtainable tuber protein product thus comprises a high amount of (coagulated) proteins. Accordingly, a high yield with little impurities may be obtained. This allows for usage for a variety of applications.

Herein, the amount of protein is defined as Kjeldahl Nitrogen multiplied by 6.25 and the weight percentage is based on the total dry weight of the coagulated tuber protein product. The Kjeldahl method is preferably performed according to analytical procedure ANAL-10005 at Nutricontrol BV, under accreditation by the Dutch Accreditation Council (Certificate LO53).

Particularly advantageous is a method for obtaining a coagulated tuber protein product from a tuber fruit juice, said method comprising:

- subjecting the tuber fruit juice to a first coagulation step comprising adjusting the temperature of the tuber fruit juice to at most 60 °C and maintaining said temperature for a retention time in the range of 5 -20 minutes to form a coagulated low-grade fraction;
- separating at least part of the coagulated low-grade fraction from the tuber fruit juice to obtain a lean tuber fruit juice;
- subjecting the lean tuber fruit juice to a second coagulation step comprising adjusting the temperature of the lean tuber fruit juice to at least 70 °C and maintaining said temperature for a retention time in the range of 5 – 10 minutes to form a coagulated tuber protein product;

- preferably wherein a fluid comprising said coagulated tuber protein product is heated under a turbulent flow having a Reynolds number of 3000 or more to at most 90 °C to form a stabilized coagulated tuber protein product; and
- 5 - collecting at least part of the coagulated tuber protein product or stabilized coagulated tuber protein product.

The present invention is further directed to a coagulated tuber protein product having a d90 particle size distribution of at most 30 µm, preferably at most 28 µm, such as at most 25 µm. This allows for a tuber
10 protein product with superior mouthfeel, less grittiness and/or less horniness compared to larger particle size distributions. As detailed herein above, such coagulated tuber protein product may be obtainable by the method as detailed herein, in particular a method wherein the first coagulation step comprises adjusting the temperature of the tuber fruit juice
15 in the first coagulation step is adjusted to at most 60 °C and maintaining the temperature for a retention time in the range of 5 – 40 minutes, preferably 5-20 minutes.

The present invention is further directed to a coagulated tuber protein product having a digestibility of at least 1 mol amine / kg dry
20 coagulated tuber protein product, preferably at least 2, more preferably at least 3, even more preferably at least 3.5 mol / kg dry coagulated tuber protein product. As detailed herein above, such coagulated tuber protein product may be obtainable by the method as detailed herein, in particular wherein the temperature of the lean tuber fruit juice in the second
25 coagulation step is adjusted to a temperature between 65 – 75 °C, preferably between 70 – 75 °C and preferably wherein the pH of the tuber fruit juice in the first coagulation step is in the range of 5 – 6.5.

It was advantageously found that the coagulated tuber protein product may have a good emulsifying activity index. This is particularly
30 beneficial as effective emulsifiers are economic to use. The coagulated tuber

protein product may therefore find purpose as emulsifier in *e.g.* the food industry. The coagulated tuber protein typically has an emulsifying activity index of at least 1 m²/g dry coagulated tuber protein product, preferably at least 2 m²/g. The emulsifying activity may be determined by the following
 5 protocol.

A sample of the coagulated tuber protein product is dispersed in demineralised water at 2 wt.% dry matter and thoroughly mixed. 12 mL of sunflower oil (Reddy) is added to 8 mL of this dispersion. The oil and water phases are emulsified by means of an Ultra-Turrax equipped with a S 25 N
 10 dispersing tool operating at 16 kRPM for 30 seconds.

25 µL of these emulsions are added to 5 mL of 0.1 wt.% of sodium dodecyl sulphate (SDS) solution. The absorbance at 500 nm is recorded against an SDS-solution blank in a 1-cm cuvet. All measurements are typically done in triplicate. The Emulsifying Activity Index (EAI) may then
 15 be calculated from the absorbances by equation 1.

$$EAI \left(\frac{m^2}{g} \right) = \frac{2 \cdot 2.303 \cdot A_{500} \cdot DF}{c \cdot (1 - \theta) \cdot 10,000} \quad (\text{Equation 1})$$

Where A₅₀₀ is the absorbance at 500 nm, the dilution factor is 250,
 20 c is the protein concentration in wt.%, and θ is the fraction of oil that was used to form the emulsion (0.67).

Alternatively, or additionally, the coagulated tuber protein product may have a CIELAB L*-value of at least 72, preferably at least 73. CIELAB is known in the art and sometimes also referred to as L*a*b. It is a
 25 colour space defined by the International Commission on Illumination that is specifically intended to provide reproducible representations of colours. The colours are herein expressed as L* for perceptual lightness and a* and b* for the unique colours of human vision; red, green, blue and yellow.

Preferably, the colours of the coagulated tuber protein product are measured on a Hunterlab Colorflex EZ set for D65/10° in a high-precision cup in the CIELAB colour space.

Typically, the coagulated tuber protein fraction comprises 1 – 10 g crude fat per kg dry coagulated protein product, preferably 2 – 9 g, more preferably 3 – 8 g, even more preferably 3 – 5 g per kg dry coagulated protein product. The crude fat content may be determined by extraction of the fats using an extraction medium. In particular, the crude fat content may be determined by extracting the crude fats by Soxhlet extraction after acid hydrolysis, using petroleum ether and gravimetric detection. Most preferably, the crude fat content is determined according to EG 205-2009.

Alternatively, or additionally, the coagulated protein product comprises less than 60 mg sulfite per kg dry coagulated protein product, preferably less than 40 mg, more preferably less than 20 mg, such as 8 – 10 mg. A lower sulfite content is preferred as this allows for a higher protein content and direct suitability in the products. The sulfite contents are preferably measured according to the optimized Monier-Williams method (AOAC Official Method 990.28 Sulfites in food).

Alternatively, or additionally, the coagulated tuber protein product comprises more than 800 g protein / kg dry coagulated tuber protein product, preferably more than 850 g/kg, wherein the protein is defined as Kjeldahl Nitrogen multiplied by 6.25. This high protein content is advantageous for the food applications. Further, such a high amount of protein indicates that little contaminants are present.

Alternatively, or additionally, the coagulated tuber protein product comprises less than 300 mg glycoalkaloids / kg dry coagulated tuber protein product, more preferably below 150 mg/kg, most preferably below 50 mg/kg.

The invention is further directed to a food product, such as a plant-based or non-dairy product, *e.g.* plant-based milk, plant-based

yoghurt, plant-based cream, comprising the coagulated tuber protein product.

For the purpose of clarity and a concise description, features are described herein as part of the same or separate embodiments, however, it
5 will be appreciated that the scope of the invention may include embodiments having combinations of all or some of the features described.

The invention may further be illustrated by the following non-limiting examples.

10 Examples

Lipid contents

Crude fat content (lipids) were extracted by Soxhlet extraction after acid hydrolysis, using petroleum ether and gravimetric detection
15 (according to EG 205-2009).

Total Nitrogen

Nitrogen content was determined according to the Kjeldahl method performed according to analytical procedure ANAL-10005 at
20 Nutricontrol BV, under accreditation by the Dutch Accreditation Council (Certificate LO53).

Dry matter

Dry solid contents were determined by thermogravimetry, using a
25 Mettler Toledo HR83 Moisture Analyzer device.

Protein composition by Maurice S

Protein Molecular Weights (MW) were determined using a Maurice S capillary electrophoresis device (CE-SDS, Bio-Techne
30 ProteinSimple).

Protein samples were diluted to approximately 2 mg/mL in sample buffer from the Maurice CE-SDS PLUS Reagent Kit and denatured at 70 °C in the presence of β -mercaptoethanol. The samples were run at 10 °C, using a 30-minute run at 5,750 V. Proteins were detected
5 spectrophotometrically at 280 nm.

MW were calculated by comparing the electrophoresis plots against those of a Pierce™ Unstained Protein MW Marker #26610 (14.4kDa to 116kDa).

Equal signal-to-concentration conversion was ensured by
10 including a 10 kDa internal standard in each run.

Sulfite determination via Monier-Williams

Residual sulfite contents were measured according to the optimized Monier-Williams method (AOAC Official Method 990.28 Sulfites
15 in food).

Colour Measurements

The colours of the dried potato powder samples were measured on a Hunterlab Colorflex EZ set for D65/10° in a high-precision cup in the
20 CIELAB colour space.

Particle Size Distribution via Sympatec Helos

The particle size distribution has been measured with the Sympatec Helos using laser diffraction. Protein samples were measured in
25 process water with the Quixel dispersion system.

The resulting data were evaluated with the Fraunhofer diffraction equation and expressed as d10, d50 and d90 in microns.

Ash

Ash was determined by incineration of a known quantity of the sample at 550 °C and weighing the residue.

5 *Determination of glycoalkaloid level*

Glycoalkaloids (total glycoalkaloids or TGA) were determined essentially according to the method of Laus and coworkers (Laus M.C., Klip G. & Giuseppin M.L.F. (2016) Food Anal. Methods 10(4) “Improved Extraction and Sample Cleanup of Tri-glycoalkaloids α -Solanine and
10 α -Chaconine in Non-denatured Potato Protein Isolates”).

Briefly, samples were dissolved or diluted in 5 % acetic acid solution containing 20 mM of heptane sulfonic acid sodium salt (VWR 152783K) for at least 2 hours at 37 °C . Insoluble materials were removed by centrifugation at 9000 g at ambient temperature (Heraeus Multifuge 1 SR,
15 rotor 75002006) and the supernatant was filtered over a GHP Acrodisc 13 mm Syringe Filter with 0.45 μ m GHP Membrane (PALL PN 4556T) directly into a 1.5 mL HPLC vial (VWR 548-0004) and capped with an aluminium ci 11 mm, rubber/butyl/TEF cap (VWR 548-0010). Samples were introduced automatically onto a SPE column (Oasis HLB prospect-2
20 /Symbiosis cartridge 2.0 x 10 mm particle size 30 μ m) via a Vanquish Core Online SPE system (Thermo). The glycoalkaloids were eluted onto a Thermo Accucore C18 (250 mm x 4.6 mm, 2.5 μ m) column with a Guard Holder (PN 850-00) Accucore C18 (10 x 4.6mm) guard column and separated using 60% acetonitrile / phosphate buffer pH 7.0. Analytes were detected using a
25 DAD detector at 202 nm (Thermo) and quantified on a calibration curve prepared from purified glycoalkaloids (α -solanine, Carl Roth 4192,1 and α -chaconine Carl Roth 2826,1).

Minerals

Elemental compositions were determined via X-ray fluorescence (XRF) for all elements with an atomic number above that of sodium via a Rigaku CG ED-XRF (Rigaku).

5

Determination of the Emulsifying Activity Index

Protein sample were dispersed in demineralised water at 2 wt.% dry matter and thoroughly mixed. 12 mL of sunflower oil (Reddy) were added to 8 mL of this dispersion. The oil and water phases were then
10 emulsified by means of an Ultra-Turrax equipped with a S 25 N dispersing tool operating 1t 16 kRPM for 30 seconds.

25 uL of these emulsions were added to 5 mL of 0.1 wt.% of sodium dodecyl sulphate (SDS) solution. The absorbance at 500 nm was recorded against an SDS-solution blank in a 1-cm cuvet. All measurements
15 were done in triplicate. The Emulsifying Activity Index (EAI) was then calculated from the absorbances by Equation 1.

$$EAI \left(\frac{m^2}{g} \right) = \frac{2 \cdot 2.303 \cdot A_{500} \cdot DF}{c \cdot (1 - \theta) \cdot 10,000} \quad \text{Equation 1}$$

20 Where A_{500} is the absorbance at 500 nm, the dilution factor is 250, c is the protein concentration in wt.%, and θ is the fraction of oil that was used to form the emulsion (0.67).

Infogest digestion

25 Digestibility of different potato protein prototypes and reference material was performed according to the INFOGEST method. All measurements used 5 wt.% potato protein dispersions as starting material and were performed in duplicate. Pepsin was P6887 from SigmaAldrich, Pancreatin was P7545 from SigmaAldrich. Digestions were performed

without lipase or amylase because no fat or starches were present in the samples.

Upon completion of the in-vitro digestion, aliquots were withdrawn from the reaction mixtures and inactivated by heating to 90 °C for 10 minutes. Precipitates were removed by centrifugation (10 minutes at 14 krpm in an Eppendorf centrifuge). The amount of free amines was quantified by the ortho-phthaldehyde method.

OPA reagent was prepared by dissolving 20 mg of ortho-phthaldehyde (OPA, 00681 Fluka) in 200 uL of ethanol and adding 20 uL of beta-mercaptoethanol (8.05740 Merck). The total volume was then adjusted to 40 mL with OPA buffer (100 mM sodium carbonate at pH 10.5). The OPA reagent was stored in the dark until used and was prepared fresh within two hours of the analysis.

The samples were diluted between 10 and 20 times in OPA buffer. 20 uL aliquots were incubated with 180 uL of OPA reagent for one-and-a-half minutes after which the absorbance at 340 nm was read. Absorbances were converted into free amine concentrations by means of a calibration curve of 0 – 2.5 mM glutamine (Applichem).

The most critical factor in determining the digestibility of potato protein is the pH in the simulated intestinal fluid, with higher pH values giving a more thorough digestion.

Assuming an average reduced molecular weight of ~ 100 Da per amino acid, the maximum possible digestibility of a kg of protein is around 10 moles.

25

Example 1 - Improved method for controlled pre-coagulation

Potato fruit juice comprising roughly 150 mg/L of sulfite was recovered from a potato starch factory and defoamed by running the juice over a 15 mm hydrocyclone. The underflow was fed into a defoaming tank

until the density reached 980 kg/m³. The resulting liquid was either kept *as is* or heated to 40 °C by means of a heat exchanger and fed into an insulated stainless-steel pipe to form a residence time loop at different incubation times according to the tables below.

5 The resulting juice was passed immediately over a high-speed centrifugal separator operating at low capacity. The separator was discharged every 200 seconds to ensure maximal removal of the low-grade fraction. The low capacity ensured that the juice experienced high g-force over a long period. 2-liter and 50 mL aliquots were taken of the lean fruit
10 juice; the 2-liter samples were immediately coagulated in a water bath at 75° C into coagulated potato protein product. These products were transferred to flasks and lyophilized into fine powders, using gentle pressure to break up the lumps that result from the freeze-drying process.

For this example, it is noted that the method of coagulating in a
15 water bath is inherently less efficient than coagulation at larger industrial scale but serves here to demonstrate the improved nature of the juice from a controlled pre-coagulation process in a laboratory setting. Similarly, the freeze-drying process results in lighter powders than industrial drying so colour-data should only be compared within the freeze-dried group.

20 From the sample that was heated to 40 °C at 13 minutes of retention time the discharge of the separator was collected and analyzed via the same procedure as the other juices and proteins.

 The 50 mL samples were analyzed for Brix (Bx), pH, conductivity, protein content and suspended solids. The dried protein samples were
25 analyzed for colour.

Table 1: Properties of the process liquor and products of the improved pre-coagulation procedure

Juice temperature	Residence time	High Speed separator	pH	Conductivity mS/cm	Bx	% v:v suspended solids	wt. % Protein
as is	none	no	5,95	11,89	5,0	2,2	1,82
as is	none	yes	5,95	11,85	5,0	0,15	1,64
40 C	none	yes	5,85	12,03	4,9	0,08	1,58
40 C	5 minutes	yes	5,95	12,14	4,9	0,06	1,51
40 C	13 minutes	yes	5,89	12,06	4,8	0,05	1,50
40 C	13 minutes	discharge	5,71	5,15	3,9	20	2,41

Table 2: Properties of the powder

Juice temperature	Residence time	High Speed separator	L*	a*	b*
as is	none	no	80,03	0,73	13,2
as is	none	yes	72,62	1,65	20,2
40 C	none	yes	86,75	0,01	10,5
40 C	5 minutes	yes	88,67	-0,2	8,4
40 C	13 minutes	yes	85,03	-0,1	11,6
40 C	13 minutes	discharge	45,40	3,89	14

5

The L value in the CIELAB colour space corresponds to “lightness” of the colour, with higher values corresponding to a lighter product and lower values corresponding to a darker product. The a^* and b^* values correspond to chromaticity and are preferably close to zero,

The results demonstrate the effectiveness of the removal of the low-grade fraction in removing colour bodies and suspended solids from the potato juice. The protein that is then coagulated from this lean potato juice is superior in terms of colour over protein that is coagulated from untreated juice. The separator discharge (*i.e.* the low-grade fraction), representing the fraction that is removed *via* the controlled pre-treatment, is darker than the other fractions.

Example 2 - Production of potato protein prototypes with controlled pre-coagulation compared to uncontrolled pre-coagulation

5 The method according to the present invention was applied to industrial potato juice to produce two prototypes for evaluation of PSD (particle size distribution) and composition. This method was not yet optimized for glycoalkaloids removal and washing, resulting in relatively high ash- and alkaloid residues.

10

Production of uncontrolled reference sample

Potato juice comprising roughly 150 mg/L of sulfite was recovered from a potato starch factory and defoamed by running the juice over a 15 mm hydrocyclone. The underflow was fed into a defoaming tank until the
15 density reached 980 kg/m³. The resulting liquid was heated to 35 °C by means of a single-tube heat exchanger. The coagulated fraction was removed over a disk stack centrifuge and the supernatant was pumped to a storage tank where it cooled down to 25 °C. The pH was adjusted to 5.6 and the juice was introduced into a jet cooker operating at 104 °C by direct
20 steam injection. The resulting protein coagulate was collected in a storage tank and dewatered on a CA 360 decanter with a 15° cone. The coagulate was then slurried to a final concentration of 4.5 % using hot water (80 °C) and adjusted to pH 3.0 with sulfuric acid to remove glycoalkaloids. The protein coagulate was collected again via the same CA 360 decanter and
25 washed twice with hot water to remove excess acid, resulting in a coagulated protein cake. The cake was then dried on a ultrarotor drier operating at an inlet temperature of 160 °C. The final protein powder was sieved over a 350 micron safety sieve.

Production of prototypes via controlled pre-coagulation (i.e. the coagulation of a low-grade fraction)

Potato juice comprising roughly 150 mg/L of sulfite was recovered from a potato starch factory and defoamed by running the juice over a
5 15 mm hydrocyclone. The underflow was fed into a defoaming tank until the density reached 980 kg/m³. The resulting liquid was heated to 40 °C by means of a heat exchanger and fed into an insulated stainless steel pipe to form a residence time loop of 13 minutes (*i.e.* a retention time of 13 minutes). The resulting coagulated juice was passed immediately over a
10 high speed centrifugal separator operating at low capacity. The separator was discharged every 200 seconds to ensure maximal removal of low-grade fraction and the discharge was discarded. The low capacity ensured that the juice experienced high g-force over a long time period. The juice that was produced in this way was split into two fractions. One was coagulated in a
15 tubular heat exchanger operating at 85 °C (“mildly coagulated prototype”). The other was introduced into a jet cooker for coagulation via direct steam injection at 85 °C (“DSI prototype”).

The resulting protein coagulates were collected in a storage tank and dewatered on a SCE 306 decanter with a 15° cone. The coagulates were
20 then slurried to a final concentration of 4.5 % using water and adjusted to pH 3.0 with sulfuric acid to remove glycoalkaloids. The protein coagulates were collected again via the same SCE 306 decanter resulting in coagulated protein cakes. Some aliquots of these decanter cakes were kept, the rest was dried. The mild coagulated protein material was dried on a spray-drier
25 operating at an inlet temperature of 180 °C. The DSI protein material was dried on a pneumatic drier.

Sample evaluation

Both prototypes, the uncontrolled reference sample as well as two commercial reference samples were analyzed for their chemical composition, colour, particle size distribution.

5 The results show that the controlled pre-treatment results in higher protein-contents, reduced lipid contents and smaller particles. In the CIELAB colour space, the L-value corresponds to the “lightness” of the colour. In the case of potato protein, whiter powders are preferred, corresponding to higher L-values. Such “whiter” proteins are highly
10 preferred in plant-based dairy. Both the DSI- and mild-coagulation prototypes are substantially whiter than commercial potato proteins, or a reference sample that was produced *via* uncontrolled pretreatment.

15 Table 3 – properties of potato tuber proteins products obtained by the method according to the invention and of comparative potato tuber protein products

		Invention		Uncontrolled pretreatment	Commercial references	
Analyses	Units	DSI	Mild		Protastar	Finnamyl PP1
Kjeldahl nitrogen *6,25	g/kg as is	885	857	868	740	780
Loss-on-drying	g/kg as is	30	41	60	112	80
Ash residue	g/kg	2.0	24	13	23	38
Sulfite	mg/kg	14	12	140	67	80
Crude fat (acid hydrolysis)	g/kg	4	3	29	21	35
PSD d10	microns	2.9	3,0	16	40	18
PSD d50	microns	8.9	6,3	38	114	45
PSD d90	microns	19	23	76	437	111
Colour	L	75.36	73.47	71.87	61.29	71.07
	a*	0.58	0.41	0.73	3.03	4.68
	b*	5.26	9.09	14.84	20.33	27.83

Example 3 Emulsifying Activity Index (EAI) of potato protein prototypes that were prepared via controlled pre-coagulation.

The EAI is a measure for the amount of surface area of an emulsion, determined via absorbance, and is expressed as m²/g. Higher surface areas correspond with more effective emulsification. EAI values were determined for the DSI prototype and the mild-coagulation prototype from example 2 and are compared a commercial reference material, Finnamyl PP1.

Commercial coagulated reference protein has a very low EAI. The Mild coagulation prototype has a better EAI than the DSI prototype.

Table 4: Emulsifying Activity Index of potato protein prototypes and commercial reference materials

Sample		EAI m2/g
DSI Prototype	This invention	2.13
DSI Prototype	This invention	3.71
DSI Prototype	This invention	2.14
Mild coagulation Prototype	This invention	5.9
Mild coagulation Prototype	This invention	5.93
Mild coagulation Prototype	This invention	5.21
Finnamyl PP1	Coagulated potato protein reference	0.49

Example 4 Plant-based fruit yoghurt

Plant-based yoghurts were prepared from the mild-coagulation prototype of example 2 and from its corresponding decantercake, *i.e.* the same material before drying. Finnamyl PP1 was used as a commercial reference to prepare a yoghurt *via* the same recipe.

The plant-based yogurts were produced at lab scale using a Thermomix and Niro Soavi Twin Panda homogenizer. These yoghurts used several starches to aid in achieving the correct texture: Etenia 457, an

enzymatically modified potato starch; Eliane VE 580, a Hydroxy propylated, crosslinked waxy potato starch; and Eliane MC 160, an Instant OSA waxy potato starch. Frozen strawberries were purchased locally (supermarket).

5 All powders were premixed. The frozen strawberries were mashed in a thermomixer at speed 3. Water was preheated to 50 °C in a Thermomix. The coconut fat was added while mixing at speed 1, followed by addition of the dry ingredients at speed 3. The mixture was allowed to hydrate for 15 minutes at speed 2, while maintaining the temperature at 50 °C. The temperature was then raised to 70 °C and the blend was homogenized in 10 two stages at 150+50 bar. The resulting product from the prototype materials (Samples A and B) displayed emulsification, while the Finnamyl reference material (sample C) did not. Sample C tended to clog the homogenizer. The products were heated to 90 °C for 2 minutes at speed 3, filled out into sterile containers and stored at 4 °C. The yoghurts were then 15 inspected visually and analysed on a Brookfield viscometer.

Yoghurts A and B achieved satisfactory viscosity, while yoghurt C was thin and showed phase separation.

Table 5: Recipes for plant-based yoghurt

20

Ingredient, wt. %	A	B	C
Water	70,4	61,4	70,4
Mild coagulation prototype	1,0		
Mild coagulation decantercake		10	
Finnamyl PP1			1,0
Eliane VE 580	2,5	2,5	2,5
Etenia 457	1,5	1,5	1,5
Eliane MC 160	1,0	1,0	1,0
Kristal coconut fat	3,50	3,50	3,50
Salt	0,10	0,10	0,10
Sugar	5,00	5,00	5,00
Fruit puree strawberry	15,00	15,00	15,00
Viscosity (cP)	2.128	14.500	too low to measure

Example 5 - Improved method for controlled pre-coagulation

Potato fruit juice comprising roughly 150 mg/L of sulfite was recovered from a potato starch factory and defoamed by running the juice
5 over a 15 mm hydrocyclone. The underflow was fed into a defoaming tank until the density reached 980 kg/m³. The resulting liquid was either kept *as is* or heated to 40 °C by means of a heat exchanger and fed into an insulated stainless-steel pipe.

The resulting juice was passed immediately over a high-speed
10 centrifugal separator operating at low capacity. The separator was discharged every 200 seconds to ensure maximal removal of the low-grade fraction. The low capacity ensured that the juice experienced high g-force over a long period. 2-liter and 50 mL aliquots were taken of the lean fruit juice; the 2-liter samples were immediately coagulated in a water bath at
15 75 °C into coagulated potato protein product. These products were transferred to flasks and lyophilized into fine powders, using gentle pressure to break up the lumps that result from the freeze-drying process.

For this example, it is noted that the method of coagulating in a water bath is inherently less efficient than coagulation at larger industrial
20 scale but serves here to demonstrate the improved nature of the juice from a controlled pre-coagulation process in a laboratory setting. Similarly, the freeze-drying process results in lighter powders than industrial drying so colour-data should only be compared within the freeze-dried group.

The 50 mL samples were analyzed for Brix, pH, conductivity,
25 protein content and suspended solids. The dried protein samples were analyzed for colour and digestibility via the INFOGEST method.

Table 6: Properties of the lean fruit juice

Juice temperature	High Speed separator	pH	Conductivity mS/cm	Bx	% v:v suspended solids	wt. % Protein
as is	no	5.95	11.89	5.0	2.2	1.82
as is	yes	5.95	11.85	5.0	0.15	1.64
40 C	yes	5.85	12.03	4.9	0.08	1.58

Table 7: Properties of the protein product (powder)

Juice temperature		High Speed separator	L*	a*	b*	Digestibility mol/kg
as is		No	80.03	0.73	13.2	1.70
as is		Yes	72.62	1.65	20.2	1.49
40 C		Yes	86.75	0.01	10.5	2.10

The L value in the CIELAB colour space corresponds to

5 “lightness” of the colour, with higher values corresponding to a lighter product and lower values corresponding to a darker product. The a* and b* values correspond to chromaticity and are preferably close to zero.

The results demonstrate the effectiveness of the removal of the low-grade fraction in removing colour bodies and suspended solids from the potato

10 juice. The protein that is then coagulated from this lean potato juice is superior in terms of colour and digestibility over protein that is coagulated from untreated juice. The separator discharge (*i.e.* the low-grade fraction), representing the fraction that is removed *via* the controlled pre-treatment, is both darker and less digestible than the other fractions.

15

Example 6: Heating coagulated tuber protein product

Potato juice comprising roughly 150 mg/L of sulfite was recovered from a potato starch factory and defoamed by running the juice over a

15 mm hydrocyclone. The underflow was fed into a defoaming tank until the

20 density reached 980 kg/m³. The resulting liquid was heated to 40 °C by means of a heat exchanger and fed into an insulated stainless steel pipe. The resulting coagulated juice was passed immediately over a high speed

centrifugal separator operating at low capacity. The separator was discharged every 200 seconds to ensure maximal removal of low-grade fraction and the discharge was discarded. The low capacity ensured that the juice experienced high g-force over a long time period.

5 The juice that was obtained was fed into a 150 L stirred vessel which was kept at a temperature of between about 70 °C and about 75 °C and maintained for a retention time in the range of 5-10 minutes. The formed fluid was heated to between 80 and 90 °C under a turbulent flow having a Reynolds number of at least 8000 (achieved using Ultra-Turrax at
10 8000 rpm), to obtain a stabilized coagulated tuber protein product. The stabilized coagulated tuber product was perceived as having improved stability, in particular exhibiting lower stickiness, a more narrow particle size distribution, in particular having a particle size $D_{90} < 50 \mu\text{m}$, better separability, excellent emulsifying capacity and improved shear stability.

15 **Example 7: Effect of shear on coagulated potato protein**

Materials

The following protein materials were used:

- Sample 1 (coagulated potato protein obtained with the method described in Example 1)
- 20 - Sample 2 (coagulated potato protein obtained with the method described in Example 6).

Method – Particle size distribution determination using laser diffraction

25 To determine the effect of shear on the particle size distribution before and after heating step under shear conditions, 15 grams of the dispersed coagulated protein has been placed in a centrifuge tube. The samples is then sheared with an Ultra-Turrax (IKA T 18 digital Ultra-Turrax with S18N-10G dispersing element) at a shear rate of 0, 3000, 9000 or 15000 rpm for 30 s.

The particle size distribution is determined using laser diffraction (Sympatec Helos (H4308) combined with Quixel wet dispersion cell) using measurement ranges R4 and R7 (total range: 0,5 – 3500 µm). The optical concentration was aimed to be 15-20% to ensure optimal measurement conditions. The signal is translated to particle size distributions using the Fraunhofer diffraction equation. An overview of the system settings is shown in 8.

Table 8 – System settings of Sympatec Helos combined with Quixel wet dispersion cell

10	SYSTEM		TRIGGER CONDITION	
	Instrument	HELOS (H4308) & QUIXEL, R4+R7	Start	1 s after button+
	Software	PAQXOS 5.0.1	Valid	Always
			Stop	30 s real time
	DISPERSING METHOD			
15	Dispersant	Water	Temperature	21 ± 0,5 °C
	Cuvette size	2 mm	Sonication	no
	Fill level	High	Pump speed	400 rpm

Results

20 The results of the particle size distribution measurements can be found in Table **Error! Reference source not found.**9. Graphs of the measured particle size distributions can be found in Figure 1 and 2. $d_{10,3}$, $d_{50,3}$ and $d_{90,3}$ are the volume-based percentiles, i.e. for sample 1, wherein the coagulated protein has not been subjected to a step of heating under shear conditions, 25 10% of the total volume of particles has a maximum size of 37.52 µm, thus $d_{10,3}$ is 37.52 µm.

It can be derived from table 9 that there is a general tendency towards a decrease in particle size ($d_{10,3}$, $d_{50,3}$ and $d_{90,3}$) with increasing shear rate. It follows that sample 1 shows a much larger decrease in particle size 30

distribution upon application of shear than sample 2, indicating that the step of heating under shear conditions improves the shear stability of the coagulated potato protein.

5 *Table 9 – Overview of samples including applied shear and particle size distributions*

<i>Sample</i>	<i>Shear (rpm)</i>	<i>Shear time (s)</i>	<i>d_{10,3} (μm)</i>	<i>d_{50,3} (μm)</i>	<i>d_{90,3} (μm)</i>
<i>1</i>	-	-	37,52	104,56	189,28
<i>1</i>	3000	30	32,05	93,26	168,31
<i>1</i>	9000	30	15,65	44,34	91,28
<i>1</i>	15000	30	9,34	24,92	93,57
<i>2</i>	-	-	11,99	33,95	84,09
<i>2</i>	3000	30	11,39	32,46	81,88
<i>2</i>	9000	30	9,93	26,93	73,81
<i>2</i>	15000	30	8,92	24,95	111,06

10 **Example 8: Emulsifying capacity measurements according to the method described in US2010/0048873 A1**

Materials

The following protein materials were used:

- 15 - Sunflower oil (Olitalia. Batch: L 168230070);
- Potato protein (coagulated potato protein obtained with the method described in Example 6)
- Potato protein (coagulated potato protein obtained with the method described in Example 2).

The coagulated potato protein obtained with the method described in Example 6 is spray dried using a spray drier (Sanco Processing BV) with an inlet temperature of 200 °C and outlet temperature of 60 °C before determination of the emulsifying capacity.

5

Method - Emulsifying capacity

The method described below is equal to the method described in US2010/0048873 A1.

25 g of protein was suspended in 100 g water using an Ultra-turrax (IKA T
10 18 digital Ultra-Turrax with S18N-19G dispersing element) at 8.000 RPM. Subsequently, sunflower oil is added slowly and in portions while continuing dispersing until the emulsion breaks. The oil-binding and emulsifying capacity is indicated with regard to the concentration of the 3 substances with the maximum oil-binding capacity. 1:4:6 means that a mixture of 1 part
15 protein and 4 parts water can bind 6 parts of oil, i.e., after adding more than 150 g oil to the above mentioned suspension the test emulsion will break.

Results

The results are shown in table 10.

Table 10 - Overview of samples and results.

Sample no.	Sample	Parts protein	Parts water	Parts oil
1.	Coagulated potato protein according to Example 6 (Avebe; Spray dried)	1	4	1
2.	Coagulated potato protein according to Example 6 (Avebe; Spray dried)	1	8	≥ 14
3.	Coagulated potato protein according to Example 2 (Avebe)	1	4	7

5 Sample 1 became too viscous to properly emulsify the sunflower oil during addition of the first part of oil. Therefore, a sample containing 12,5 g of protein suspended in 100 g of water was prepared. Sunflower oil was added according to the previously described method. After adding 14 parts of oil, sample 2
10 became too viscous to be properly processed with an Ultra-turrax, however, the emulsion was not broken. Therefore, it was concluded that the emulsifying capacity is at least 1:8:14. Sample 3 broke after adding oil when already 7 parts of sunflower oil were added. Therefore, the emulsifying capacity of this sample is 1:4:7.

Both sample 2 and sample 3 have a larger emulsifying capacity than 1:4:4 to
15 1:4:6.

The invention further relates to the following items:

1. Method for obtaining a coagulated tuber protein product from a tuber fruit juice, said method comprising:
 - subjecting the tuber fruit juice to a first coagulation step comprising
 - 5 adjusting the temperature of the tuber fruit juice to at most 60 °C and maintaining said temperature for a retention time in the range of 5 – 40 minutes to form a coagulated low-grade fraction;
 - separating at least part of the coagulated low-grade fraction from the tuber fruit juice to obtain a lean tuber fruit juice;
 - 10 - subjecting the lean tuber fruit juice to a second coagulation step comprising adjusting the temperature of the lean tuber fruit juice to at least 60 °C to form a coagulated tuber protein product;
 - collecting at least part of the coagulated tuber protein product.
- 15 2. Method according to the previous item, wherein the retention time is between 5 – 20 minutes, preferably 8 – 16 minutes, more preferably 10 – 14 minutes, such as approximately 13 minutes.
3. Method according to any of the previous items, wherein the first
- 20 coagulation step comprises adjusting the temperature of the tuber fruit juice to at most 55 °C, preferably to a temperature in the range of 30 – 50 °C, more preferably 35 – 45 °C, such as about 40 °C and/or wherein the pH of the tuber fruit juice in the first coagulation step is in the range of 5 – 7, preferably 5.5 – 6.5, more preferably 5.7 – 6.2.
- 25 4. Method according to any of the previous items, further comprising contacting the tuber fruit juice with a fruit juice buffer, preferably wherein said fruit juice buffer comprises sulfuric acid, phosphoric acid, lactic acid, formic acid, citric acid, acetic acid and/or hydrochloric acid.

5. Method according to any of the previous items, wherein the second coagulation step comprises adjusting the temperature of the lean tuber fruit juice to a temperature in the range of 60 – 100 °C, preferably 60 – 90 °C, more preferably 60 – 80 °C, such as 65 – 75 °C and/or wherein
5 the pH of the lean tuber fruit juice in the second coagulation step is in the range of 5 – 7, preferably 5.5 – 6.5, more preferably 5.6 – 6.0.
6. Method according to any of the previous items, wherein the lean tuber fruit juice is contacted with a coagulation buffer, preferably wherein
10 said coagulation buffer comprises sulfite, an anti-foamer and/or a mild coagulant.
7. Method according to any of the previous items, further comprising washing and/or drying of the coagulated tuber protein product.
15
8. Method according to any of the previous items, wherein the coagulated low-grade fraction comprises lipids, iron and/or phenolic compounds.
- 20 9. Method according to any of the previous items, wherein the coagulated tuber protein product has a d90 particle size distribution of at most 40 µm, preferably 30 µm, more preferably at most 28 µm, such as at most 25 µm.
- 25 10. Method according to any of the previous items, wherein separating at least part of the coagulated low-grade fraction from the tuber fruit juice and/or collecting at least part of the coagulated tuber protein product comprises filtration, centrifugation, cycloning, decanting, using a belt filter and/or using a disc-stack separator, preferably decanting and/or
30 using a disc-stack separator.

11. Method according to any of the previous items, wherein essentially no grinding, milling and/or crushing is performed on the coagulated tuber protein product.
- 5
12. Method according to any of the previous items, wherein the tuber protein product comprises at least 85 wt.% protein, preferably at least 90 wt.%, more preferably at least 95 wt.%, wherein protein is defined as Kjeldahl Nitrogen multiplied by 6.25 and said weight percentage is based on
- 10 the total dry weight of the coagulated tuber protein product.
13. Coagulated tuber protein product having a d90 particle size distribution of at most 30 μm , more preferably at most 28 μm , such as at most 25 μm , preferably wherein said coagulated tuber protein product is
- 15 obtainable by the method according to any of the previous claims.
14. Coagulated tuber protein product according to the previous item, - having an emulsifying activity index of at least 1 m^2/g dry coagulated tuber protein product, preferably at least 2 m^2/g , and/or;
- 20 - a CIELAB L*-value of at least 72, preferably at least 73; and/or wherein said coagulated tuber protein comprises:
- 1 – 10 g crude fat per kg dry coagulated protein product, preferably 2 – 9 g, more preferably 3 – 8 g, even more preferably 3 – 5 g;
 - less than 60 mg sulfite per kg dry coagulated protein product, preferably

25 less than 40 mg, more preferably less than 20 mg, such as 8 – 10 mg; - more than 800 g protein / kg dry coagulated tuber protein product, preferably more than 850 g/kg, wherein the protein is defined as Kjeldahl Nitrogen multiplied by 6.25; and/or
 - less than 300 mg glycoalkaloids / kg dry coagulated tuber protein product

30 protein, more preferably below 150 mg/kg, most preferably below 50 mg/kg.

15. Food product comprising the coagulated tuber protein product as defined in any one of items 13-14.

- 5 16. Method for obtaining a coagulated tuber protein product from a tuber fruit juice, said method comprising:
- subjecting the fruit juice to a first coagulation step to form a coagulated low-grade fraction;
 - separating at least part of the coagulated low-grade fraction from the tuber
 - 10 fruit juice to obtain a lean tuber fruit juice;
 - subjecting the lean tuber fruit juice to a second coagulation step comprising adjusting the temperature of the lean tuber fruit juice to a temperature between 65 – 75 °C to form a coagulated tuber protein product;
 - collecting at least part of the coagulated tuber protein product;
 - 15 wherein the pH of the tuber fruit juice in the first coagulation step is in the range of 5 – 6.5.

17. Method according to the previous item, wherein the first coagulation step comprises adjusting the temperature of the tuber fruit juice
- 20 to a temperature of at most 60 °C, preferably to at most 55 °C, preferably to a temperature in the range of 30 – 50 °C, more preferably 35 – 45 °C, such as about 40 °C and/or wherein the pH of the tuber fruit juice in the first coagulation step is in the range of 5.5 – 6.5, more preferably 5.7 – 6.2.

- 25 18. Method according to any of items 16 to 17, further comprising contacting the tuber fruit juice with a fruit juice buffer, preferably wherein the fruit juice buffer comprises sulfuric acid, phosphoric acid, lactic acid, formic acid, citric acid, acetic acid and/or hydrochloric acid.

19. Method according to any of items 16 to 18, wherein the second coagulation step comprises adjusting the temperature of the lean tuber fruit juice to a temperature in the range of 65 – 74 °C, preferably 65 – 73 °C, more preferably 65 – 71 °C, even more preferably 66 – 69 °C such as about
5 67 °C or about 68 °C and/or wherein the pH of the lean tuber fruit juice in the second coagulation step is in the range of 5 – 7, preferably 5.5 – 6.5, more preferably 5.6 – 6.0.

20. Method according to any of items 16 to 19, wherein the lean tuber
10 fruit juice is contacted with a coagulation buffer, preferably wherein said coagulation buffer comprises sulfite, an anti-foamer and/or a mild coagulant.

21. Method according to any of items 16 to 20, further comprising washing and/or drying of the coagulated tuber protein product.

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22. Method according to any of items 16 to 21, wherein the coagulated low-grade fraction comprises lipids, iron and/or phenolic compounds.

23. Method according to any of items 16 to 22, wherein the coagulated
20 tuber protein product has a digestibility of at least 1 mol amine / kg dry coagulated tuber protein product, preferably at least 2, more preferably at least 3, most preferably at least 3.5, wherein said digestibility is measured according to Infogest 2.0 as described by Brodkorb, A., Egger, L., Alminger, M. *et al.* in *Nat. Protoc.* 2019, Apr; 14(4): 991-1014.

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24. Method according to any of items 16 to 23, wherein separating at least part of the coagulated low-grade fraction from the tuber fruit juice and/or collecting at least part of the coagulated tuber protein product comprises filtration, centrifugation, cycloning, decanting, using a belt filter

and/or using a disc-stack separator, preferably decanting and/or using a disc-stack separator.

25. Method according to any of items 16 to 24, further comprising
5 processing a tuber, preferably a potato tuber, to obtain the tuber fruit juice.

26. Method according to any of items 16 to 25, wherein the coagulated
tuber protein product comprises at least 85 wt.% protein, preferably at least
90 wt.%, more preferably at least 95 wt.%, based on the total dry weight of
10 the coagulated tuber protein product.

27. Coagulated tuber protein product having digestibility of at least
1 mol amine / kg dry coagulated tuber protein product, preferably at least 2,
more preferably at least 3, most preferably at least 3.5, wherein said
15 digestibility is measured according to Infogest 2.0 as described by
Brodkorb, A., Egger, L., Alminger, M. *et al.* in *Nat. Protoc.* 2019, Apr; 14(4):
991-1014, preferably wherein said coagulated tuber protein product is
obtainable by the method according to any of the previous claims.

20 28. Coagulated tuber protein product according to the previous item,
wherein said coagulated tuber protein comprises:
- 1 – 10 g crude fat per kg dry coagulated protein product, preferably 2 –
9 g, more preferably 3 – 8 g, even more preferably 3 – 5;
- less than 60 mg sulfite per kg dry coagulated protein product, preferably
25 less than 40 mg, more preferably less than 20 mg, such as 8 – 10 mg;
- more than 800 g protein / kg dry coagulated tuber protein product,
preferably more than 850 g/kg, wherein the protein is defined as Kjeldahl
Nitrogen multiplied by 6.25; and/or
- less than 300 mg glycoalkaloids / kg dry coagulated tuber protein product
30 protein, more preferably below 150 mg/kg, most preferably below 50 mg/kg.

29. Coagulated tuber protein product according to any of the previous items 27 to 28, having an emulsifying activity index of at least 1 m²/g dry coagulated tuber protein product, preferably at least 2 m²/g and/or a
- 5 CIELAB L-value of at least 72, preferably at least 73.
30. Food product comprising the coagulated tuber protein product as defined in any one of items 27 to 29.

Claims

1. Method for obtaining a coagulated tuber protein product from a
5 tuber fruit juice, said method comprising:
 - subjecting the tuber fruit juice to a first coagulation step comprising adjusting the temperature of the tuber fruit juice to at most 60 °C and maintaining said temperature for a retention time in the range of 5 -20 minutes to form a coagulated low-grade fraction;
 - 10 - separating at least part of the coagulated low-grade fraction from the tuber fruit juice to obtain a lean tuber fruit juice;
 - subjecting the lean tuber fruit juice to a second coagulation step comprising adjusting the temperature of the lean tuber fruit juice to at least 70 °C and maintaining said temperature for a retention time in the range of
15 5 – 10 minutes to form a coagulated tuber protein product; and
 - collecting at least part of the coagulated tuber protein product.
2. Method according to the previous claim, wherein a fluid
comprising said coagulated tuber protein product is subjected to a heating
20 step under a turbulent flow having a Reynolds number of 3000 or more, preferably 8000 or more, to a temperature of at most 90 °C to form a stabilized coagulated tuber protein product.
3. Method according to any of the previous claims, wherein the pH of
25 the tuber fruit juice in the first coagulation step is in the range of 5 – 7, preferably wherein the pH of the tuber fruit juice in the first coagulation step is in the range of 5 – 6.5, more preferably in the range of 5.5 – 6.5, even more preferably in the range of 5.7 – 6.2.

4. Method according to the previous claim, wherein the retention time is between 8 – 16 minutes, more preferably 10 – 14 minutes, such as approximately 13 minutes.
- 5 5. Method according to any of the previous claims, wherein the first coagulation step comprises adjusting the temperature of the tuber fruit juice to at most 55 °C, preferably to a temperature in the range of 30 – 50 °C, more preferably 35 – 45 °C, such as about 40 °C.
- 10 6. Method according to any of the previous claims, further comprising contacting the tuber fruit juice with a fruit juice buffer, preferably wherein said fruit juice buffer comprises sulfuric acid, phosphoric acid, lactic acid, formic acid, citric acid, acetic acid and/or hydrochloric acid.
- 15 7. Method according to any of the previous claims, wherein the second coagulation step comprises adjusting the temperature of the lean tuber fruit juice to a temperature in the range of 60 – 100 °C, preferably 60 – 90 °C, more preferably 60 – 80 °C, such as 65 – 75 °C and/or wherein the pH of the lean tuber fruit juice in the second coagulation step is in the range
20 of 5 – 7, preferably 5.5 – 6.5, more preferably 5.6 – 6.0.
8. Method according to any of the previous claims, wherein the lean tuber fruit juice is contacted with a coagulation buffer, preferably wherein said coagulation buffer comprises sulfite, an anti-foamer and/or a mild
25 coagulant.
9. Method according to any of the previous claims, further comprising washing and/or drying of the coagulated tuber protein product.

10. Method according to any of the previous claims, wherein the coagulated low-grade fraction comprises lipids, iron and/or phenolic compounds.
- 5 11. Method according to any of the previous claims, wherein the coagulated tuber protein product has a d90 particle size distribution of at most 40 μm , preferably at most 30 μm , more preferably at most 28 μm , such as at most 25 μm and/or wherein the coagulated tuber protein product has a digestibility of at least 1 mol amine / kg dry coagulated tuber protein
- 10 product, preferably at least 2, more preferably at least 3, most preferably at least 3.5, wherein said digestibility is measured according to Infogest 2.0 as described by Brodkorb, A., Egger, L., Alminger, M. *et al.* in *Nat. Protoc.* 2019, Apr; 14(4): 991-1014.
- 15 12. Method according to any of the previous claims, wherein separating at least part of the coagulated low-grade fraction from the tuber fruit juice and/or collecting at least part of the coagulated tuber protein product comprises filtration, centrifugation, cycloning, decanting, using a belt filter and/or using a disc-stack separator, preferably decanting and/or
- 20 using a disc-stack separator.
13. Method according to any of the previous claims, wherein essentially no grinding, milling and/or crushing is performed on the coagulated tuber protein product.
- 25 14. Method according to any of the previous claims, wherein the tuber protein product comprises at least 85 wt.% protein, preferably at least 90 wt.%, more preferably at least 95 wt.%, wherein protein is defined as Kjeldahl Nitrogen multiplied by 6.25 and said weight percentage is based on
- 30 the total dry weight of the coagulated tuber protein product.

15. Coagulated tuber protein product having a d90 particle size distribution of at most 30 μm , more preferably at most 28 μm , such as at most 25 μm , preferably wherein said coagulated tuber protein product is obtainable by the method according to any of the previous claims.
- 5
16. Coagulated tuber protein product having digestibility of at least 1 mol amine / kg dry coagulated tuber protein product, preferably at least 2, more preferably at least 3, most preferably at least 3.5, wherein said digestibility is measured according to Infogest 2.0 as described by Brodkorb, A., Egger, L., Alminger, M. *et al.* in *Nat. Protoc.* 2019, Apr; 14(4): 991-1014, preferably wherein said coagulated tuber protein product is obtainable by the method according to any of the previous claims.
- 10
17. Coagulated tuber protein product according to any of claims 15-16,
- 15
- having an emulsifying activity index of at least 1 m^2/g dry coagulated tuber protein product, preferably at least 2 m^2/g , and/or;
 - a CIELAB L^* -value of at least 72, preferably at least 73; and/or
- wherein said coagulated tuber protein comprises:
- 20
- 1 – 10 g crude fat per kg dry coagulated protein product, preferably 2 – 9 g, more preferably 3 – 8 g, even more preferably 3 – 5 g;
 - less than 60 mg sulfite per kg dry coagulated protein product, preferably less than 40 mg, more preferably less than 20 mg, such as 8 – 10 mg;
 - more than 800 g protein / kg dry coagulated tuber protein product,
- 25
- preferably more than 850 g/kg, wherein the protein is defined as Kjeldahl Nitrogen multiplied by 6.25; and/or
- less than 300 mg glycoalkaloids / kg dry coagulated tuber protein product
- protein, more preferably below 150 mg/kg, most preferably below 50 mg/kg.
- 30
- 18 Food product comprising the coagulated tuber protein product as defined in any one of claims 15-17.