



- (51) **International Patent Classification:**
A23L 1/03 (2006.01) A23L 1/212 (2006.01)
- (21) **International Application Number:**
PCT/EP2013/074967
- (22) **International Filing Date:**
28 November 2013 (28.11.2013)
- (25) **Filing Language:** English
- (26) **Publication Language:** English
- (30) **Priority Data:**
12197948.8 19 December 2012 (19.12.2012) EP
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(81) **Designated States** (unless otherwise indicated, for every kind of national protection available): AE, AG, AL, AM, AO, AT, AU, AZ, BA, BB, BG, BH, BN, BR, BW, BY, BZ, CA, CH, CL, CN, CO, CR, CU, CZ, DE, DK, DM, DO, DZ, EC, EE, EG, ES, FI, GB, GD, GE, GH, GM, GT, HN, HR, HU, ID, IL, IN, IR, IS, JP, KE, KG, KN, KP, KR, KZ, LA, LC, LK, LR, LS, LT, LU, LY, MA, MD, ME, MG, MK, MN, MW, MX, MY, MZ, NA, NG, NI, NO, NZ, OM, PA, PE, PG, PH, PL, PT, QA, RO, RS, RU, RW, SA, SC, SD, SE, SG, SK, SL, SM, ST, SV, SY, TH, TJ, TM, TN, TR, TT, TZ, UA, UG, US, UZ, VC, VN, ZA, ZM, ZW.

(84) **Designated States** (unless otherwise indicated, for every kind of regional protection available): ARIPO (BW, GH, GM, KE, LR, LS, MW, MZ, NA, RW, SD, SL, SZ, TZ, UG, ZM, ZW), Eurasian (AM, AZ, BY, KG, KZ, RU, TJ, TM), European (AL, AT, BE, BG, CH, CY, CZ, DE, DK, EE, ES, FI, FR, GB, GR, HR, HU, IE, IS, IT, LT, LU, LV, MC, MK, MT, NL, NO, PL, PT, RO, RS, SE, SI, SK, SM, TR), OAPI (BF, BJ, CF, CG, CI, CM, GA, GN, GQ, GW, KM, ML, MR, NE, SN, TD, TG).

Published:

— with international search report (Art. 21(3))

(54) **Title:** PREPARATIONS OF PLANT PARENCHYMA CELLS

(57) **Abstract:** The invention relates to a preparation of plant parenchyma cells, wherein at least some of the plant parenchyma cells have an intact and hydrated cell wall and comprise at least 10 wt% hydrophobic substance. The invention further relates to food products comprising such preparations. The invention also relates to a method for preparing said preparations.



PREPARATIONS OF PLANT PARENCHYMA CELLS

Field of the invention

The present invention relates to preparations of plant parenchyma cells comprising
5 hydrophobic substance, food products comprising such preparations and a method of preparing such preparations.

Background of the invention

Organoleptic properties are the aspects of food as experienced by the senses,
10 including taste, sight, sound, smell and touch. These properties play an important role in how consumers perceive a food product, like for example freshness, in addition to e.g. nutritional value or price thereof.

The texture of a food product is an important part of the overall eating experience and
15 appreciation of the food product. Crispness and crunchiness are two well-known textural aspects. Crispness can be described as the gustatory sensation of brittleness in the mouth, such that the food item shatters immediately upon mastication. Two types of crispness can be distinguished. Dry crisp, like e.g. potato chips, and wet crisp, like e.g. fresh fruit and vegetables (Varela, P. and Fiszman, S. (2012) Appreciation of Food
20 Crispness and New Product Development, in Food Oral Processing: Fundamentals of Eating and Sensory Perception (eds J. Chen and L. Engelen), Wiley-Blackwell, Oxford, UK Chapter 15). Crunchiness is the gustatory sensation of muffled grinding of a foodstuff. Examples include carrots, mints and peanuts. Crispness differs from crunchiness in that a crunchy food continues to provide its material sensation after a
25 few chews. A crispy food quickly loses the 'taut' equilibrium of its material.

In Western countries, the attributes crispy and crunchy top the list of all liked textural characteristics. However, to design and produce a crispy and/or crunchy food and to maintain its textural features during storage involves many technical and practical
30 challenges.

Textural properties like crispness and/or crunchiness together with taste, including flavor and aroma, play an important role in the perceived freshness of fruit and

- vegetables. For example, a fresh apple will be very crisp, but apples quickly lose crispness during storage or under refrigerator or freezer cabinet conditions and will be perceived as less crispy, drier and less fresh. This poses a problem for food products comprising pieces of fruits, like for example pieces of apple, that are stored for some time, sometimes under freezing conditions, before being consumed, such as ice cream or pudding, as over time the diminishing crispness of the fruit pieces (e.g. apple) negatively influence the consumer perceived freshness and/or fruitiness of the food product.
- 10 Consumers use known and expected organoleptic properties like e.g. texture to get confirmation of the quality of a food product, such as the freshness of an apple. However, some consumers may be interested in new organoleptic experiences that include new or unexpected textural properties in a food product.
- 15 Dongowski et al (Biotechnol. Prog. 1999, 15, 250-258) describe dietary fibers of the 'cellan' type consisting mainly or exclusively of undestroyed plant cells. It suggests that oil-loaded cellans consisting of closed cells might be carriers or absorbers of lipophilic substances, e.g. vitamins, drugs, and gases, in aqueous physiological, pharmaceutical, or biotechnological systems. The cellans are produced by a series of solvent exchange steps including the use of octane.
- 20

There is thus a need for alternative and/or improved ways to provide lasting or new textural properties to food products, like for example crispness and/or crunchiness and/or freshness and/or fruitiness. Especially so for food products that have been frozen, for example frozen storage; and frozen products, i.e. food products that are consumed in frozen condition like e.g. ice cream and water ice.

25

Summary of the invention

It was surprisingly found that a specific preparation of plant parenchyma cells, wherein at least some of the plant parenchyma cells have an intact and hydrated cell wall and comprise at least 10 wt% hydrophobic substance, provide textural aspects to food products like crispness and/or crunchiness. The textural aspect is stable over storage and/or under refrigerator or freezer cabinet conditions.

30

Accordingly the present invention relates a preparation of plant parenchyma cells, wherein at least some of the plant parenchyma cells have an intact and hydrated cell wall and comprise at least 10 wt% hydrophobic substance, wherein at least part of the hydrophobic substance is of a different origin than the plant cells and wherein the hydrophobic substance is at least partially liquid at 40 degrees Celsius, and wherein the smallest dimension of the preparation is from 1.5 millimeter to 2 centimeter.

The invention also relates to a food product comprising the plant parenchyma cell preparation of the invention.

The invention further relates to a method for preparing the plant parenchyma cell preparations of the invention.

15 **Detailed description of the invention**

Weight percentage (wt%) is calculated on the total weight of the composition unless otherwise stated. The terms 'fat' and 'oil' are used interchangeably. Where applicable the prefix 'liquid' or 'solid' is added to indicate if the fat or oil is liquid or solid at ambient temperature as understood by the person skilled in the art. The term 'structuring fat' refers to a fat that is solid at ambient temperature. Ambient temperature is a temperature of about 20 degrees Celsius.

The preparation of plant parenchyma cells of the invention is made by incorporating a hydrophobic substance in plant cells with intact cell walls. It was surprisingly found that when the cell walls of these loaded plant cells are hydrated an audible and/or textural sensory effect is obtained during mastication.

A preparation

A preparation according to the invention is a preparation of plant parenchyma cells, i.e. more than one plant parenchyma cell, like for example at least 10 plant cells. It will be appreciated that the plant cells form a particle in the sense that the plant cells are associated with one another, as e.g. can be found in plant tissue, and not merely form a loose collection of plant cells. Preferably the preparation is an edible preparation, that

is to say, the preparation is suitable for human consumption. The edible preparation thus consists of food grade ingredients. Such ingredients may be of natural origin, nature identical, naturally derived or chemically derived. Preferably at least part and more preferably all ingredients are of natural origin, nature identical or naturally
5 derived. Preferably the preparation only consists of food grade ingredients of natural origin. For the purpose of the invention 'naturally derived' means that the ingredient is of 'natural origin' but has subsequently been chemically modified. 'Natural origin' specifically excludes ingredients that are modified by chemical means, but may include natural modification processes like fermentation.

10

Preferably the preparation comprises from 25 to 1,000,000 plant cells.

The number of cells in a preparation of plant parenchyma cells (N) can suitably be calculated by taking the volume of the preparation (V) and the average distance of the
15 center of a cell to the center of the neighboring cells (d). The number of cells is then calculated using $N = V/(d*d*d)$. Alternatively the number of cells can be counted using micro X-ray computed tomography (G. van Dalen, "A study of Bubbles in Foods by X-Ray Microtomography and Image Analysis", Microscopy and Analysis, pp:S8-S12, 2012), after critical point drying suitably known to the skilled person. In hydrated
20 systems, the number of cells can be counted using synchrotron radiation tomography (H.K. Mebatsion, P. Verboven, A.M. Endalew, J. Billen, Q.T. Ho, B.M. Nicolai A novel method for 3-D microstructure modeling of pome fruit tissue using synchrotron radiation tomography images. J. Food Eng., 93 (2009), pp. 141–148).

25 The preparation of plant parenchyma cells can have any shape, including spherical, cubic, rod-like, elongated and lamellar. The smallest dimension of the preparation is from 1.5 millimeter to 2 centimeter. Preferably the smallest dimension of the preparation is from 2 millimeter to 1,5 centimeter, more preferably from 2.5 millimeter to 10 millimeter, like 3 millimeter to 10 millimeter, and even more preferably from 3.5
30 millimeter to 10 millimeter. In the context of the present invention the size of such a preparation is defined as the length of the smallest dimension of the preparation. This means for example that the size of a lamellar shape is defined as the thickness of the lamella, and that the size of a rod like structure is the diameter of its smallest circular

cross-section. In irregular shaped preparations, the size is the smallest obtainable distance when held between a pair of calipers.

For example a cubic preparation of 2x2x2 millimeter obtained from apple tissue will
5 comprise about 1000 plant cells as apple cells have an average distance of the center of one cell to the center of the neighboring cells of about 200 micrometer

Plant parenchyma cells

The source of the plant parenchyma cells may be any plant that contains plant
10 parenchyma cells having a cellulose skeleton. A plant cell wall contains cellulose and hemicellulose, pectin and in many cases lignin. This contrasts with the cell walls of fungi (which are made of chitin), and of bacteria, which are made of peptidoglycan. Primary plant cell walls contain lignin only in minor amounts, if at all. The cell wall of the plant parenchyma cells of the preparation according to the present invention may
15 comprise some lignin, like less than 10 wt% calculated on total amount of cell wall material, but preferably do not contain substantial amounts of lignified tissue. Preferably the cell wall of the plant cells consist essentially of non-lignified tissue as understood by the skilled person in the area of plant biology.

20 The plant cells can be sourced from various parenchymous tissues. Preferred sources are apple cells, pear cells, peach cells, pumpkin cells, onion cells, as well as cells from other plants from the Allium genus in the Alliaceae family of plants, carrot cells, potato cells, or sugar beet cells. The plant material can be fresh or dried, as long as the plant cells are still intact. Also other non-lignified tissues could be used to make a
25 preparation according to the invention, but preferably, the plant cells are selected from apple, onion, pear, peach, pumpkin, potato, carrot, sugar beet and combinations thereof. Apple is preferred because it is readily available.

Middle lamella

30 In plant tissue plant cells are hold together by the so-called 'middle lamella'. Middle lamella is a pectin layer that cements the cell walls of two adjoining cells together. Plants need this to give them stability and so that they can form plasmodesmata between the cells. It is the first formed layer that is deposited at the time of cytokinesis.

The cell plate that is formed during cell division itself develops into middle lamella or lamellum. The middle lamella is made up of calcium and magnesium pectates. In plants, the pectins form a unified and continuous layer between adjacent cells. When middle lamella dissolves, the cells get isolated from each other. If enzymes degrade
5 the middle lamella, the adjacent cells will separate.

Preparations according to the invention preferably have an intact middle lamella as this may result in preparations having better crispness and/or crunchiness. The textural impression of the preparation can be manipulated by partly weakening or strengthening
10 the middle lamella. Weakening can suitably be done using known techniques, like for example cooking optionally in the presence of enzymes that degrade the middle lamella. It will be appreciated that care has to be taken not to degrade the complete middle lamella as this will result in single plant cells. Strengthening can suitably be done for example by low temperature blanching through activating pectin methyl
15 esterase (Lee, C. Y., Bourne, M. C. and Van Buren, J. P. (1979), Effect of blanching treatments on firmness of carrots. Journal of Food Science, 44: 615–616.).

Cell wall

A plant cell wall contains a framework of cellulose microfibrils. These microfibrils form a
20 dense and irregular network having small pores between the microfibrils. The microfibrils consist of a multitude of aligned cellulose macromolecules that are oriented to form a microfibril. These microfibrils have a diameter of between 1 and 10 nanometer, more in particular between 2 and 5 nanometer.

25 At least some of the plant parenchyma cells in the preparation of plant parenchyma cells of the invention have an intact and hydrated cell wall. In the context of the invention an intact cell wall is a cell wall that surrounds the cell cavity without large pores or ruptures. Preferably the cell wall network contains pores between the cellulose microfibrils having a size smaller than 200 nanometer, more preferably of between 1
30 and 100 nanometer, even more preferably between 2 and 50 nanometer, and still more preferably between 5 and 40 nanometer.

The intactness of cell walls can easily be determined using 3D-Confocal Laser Scanning Microscopy down to a scale of 200 nanometer or alternatively with Scanning Electron Microscopy down to a scale of 10 nanometer.

- 5 In the context of the invention a 'hydrated cell wall' is defined as a cell wall of which water molecules fill the pores such that the cell wall is covered with a film of water.

A liquid hydrophobic substance that is located in a plant parenchyma cell with an intact and hydrated cell wall cannot easily escape from the cell due to the lack of large pores
10 and the hydrophilic nature of the rehydrated cell wall.

Preferably the amount of plant parenchyma cells comprising hydrophobic substance and having an intact and hydrated cell wall in the preparation of plant parenchyma cells of the invention is at least 20 %, more preferably at least 40 %, even more preferably at
15 least 60 %, still more preferably at least 80 % and even still more preferably the preparation of plant parenchyma cells of the invention essentially consists of plant parenchyma cells comprising hydrophobic substance and having an intact and hydrated cell wall.

20 Hydrophobic substance

At least some of the plant parenchyma cells in the preparation of plant parenchyma cells of the invention have an intact and hydrated cell wall and comprise at least 10 wt% hydrophobic substance wherein at least part of the hydrophobic substance is of a different origin than the plant cells and wherein the hydrophobic substance is at least
25 partially liquid at 40 degrees Celsius.

It has been found that plant cells that have an intact and hydrated cell wall comprise a hydrophobic substance that is at least partially liquid at 40 degrees Celsius provide a sensory effect, i.e. crispiness and/or crunchiness, when consumed (i.e. during
30 mastication). Preferably the hydrophobic substance is at least partially liquid at 37 degrees Celsius, more preferably at 30 degrees Celsius, even more preferably at 20 degrees Celsius and still even more preferably at 10 degrees Celsius. For example a vegetable oil like sunflower is essentially liquid at ambient temperature. A vegetable fat

like palm oil, sometimes also called a structuring fat, will be mostly solid at e.g. 10 degrees Celsius, but will liquefy at higher temperatures, like for example 40 degrees Celsius.

- 5 At least 10 wt% of the hydrophobic substance is of a different origin than the plant cells. So for example, if apple cells are used at least 10 wt% of the hydrophobic substance needs to be of non-apple origin, like for example a vegetable oil like sunflower oil, rapeseed oil, canola oil, soybean oil or combinations thereof. For the avoidance of doubt, the amount of hydrophobic substance of a different origin than the
10 plant cells is calculated on the total amount of hydrophobic substance.

Preferably at least 50 wt% of the hydrophobic substance is of a different origin than the plant cells, more preferably at least 70 wt%, even more preferably at least 90 wt% and still more preferably essentially all.

15

The hydrophobic substances are preferably lipid compounds. These lipid compounds are preferably compounds such as vegetable oils or fats (e.g. sunflower oil, rapeseed oil, canola oil, soybean oil, olive oil, palm oil, coconut oil), animal oils or fats (such as dairy fats like butter oil, fish oil), or essential oils.

20

Preferably the hydrophobic substance is selected from vegetable oils, essential oils and combinations thereof.

- 25 Preferably the plant parenchyma cells comprise at least 20 wt% hydrophobic substance, more preferably 30 wt%, even more preferably 40 wt% and still more preferably at least 50 wt%. For the avoidance of doubt, the amount of hydrophobic substance is calculated on total amount of the preparation of plant parenchyma cells.

- 30 If for example 1 gram of dried apple pieces are loaded with 20 grams of sunflower oil and hydrated with 29 grams of de-mineralized water, the weight percentage of oil contained in these cells is $20/(1+20+29)*100\% = 40\%$. Percentage of hydrophobic substance comprised in the plant parenchyma cells can be measured using proper solvent extraction and gravimetric analysis, as known by a person skilled in the art.

The hydrophobic substance that is at least partially liquid at 40 degrees Celsius, or other preferred temperatures as stated elsewhere, may comprise further hydrophobic ingredients that are not partially liquid at the defined temperature. Such further
5 ingredients, also called actives, include fat-soluble vitamins like A, D, E and K. Other suitable further ingredients are hydrophobic compounds like the carotenoids (e.g. alpha-carotene, beta-carotene, lycopene, lutein, zeaxanthin), hydrophobic flavors and beneficial actives like theobromine.

10 Inclusion of such further ingredients is suitably done by dissolving/mixing such ingredients in/with the hydrophobic substance. This may require elevated temperatures to promote dissolution or melt the further ingredients. After inclusion of the hydrophobic substance comprising the further ingredients the further ingredients may stay in solution or may solidify.

15

Water-soluble flavor

The preparation of plant parenchyma cells may further comprise water-soluble flavor. This is suitably introduced by using water comprising such water-soluble flavor in the rehydration step when preparing the plant cells according to the invention.

20

It has been found that the use of water-soluble flavors aids the sensorial impression, like for example freshness. Preferably the water-soluble flavor is a fruit derived acid like citric acid and/or malic acid.

25 Food products

Preparations of plant parenchyma cells of the invention are suitable to impart textural aspects to food products like crispness and/or crunchiness as well as an impression of freshness and/or fruitiness. The invention thus also concerns a food product comprising the preparation according to the invention.

30

Preferred food products are those that are frozen at least during part of their life cycle. For example, a food product may be frozen upon storage but defrosted when consumed. Preferably the food product is a frozen product, i.e. a food product that is

consumed in frozen condition, like for example ice cream, water ice and frozen drinks (i.e. slush puppy). Preferred food products include ice cream, optionally coated with for example chocolate. Preferably the food matrix comprising the preparation comprises water, oil or combinations thereof, more preferably comprises a water and oil emulsion, and even more preferably is a water-in-oil or an oil-in-water emulsion. The food matrix is defined as the part of the food product comprising the preparation of plant parenchyma cells. For example, in a chocolate-coated ice cream bar wherein the ice cream comprises the plant parenchyma cells, the chocolate-coated ice cream bar is defined as the food product and the ice cream is defined as the food matrix.

Preferably the hardness of the food matrix comprising the preparation of plant cells is less the hardness of the preparation of plant cells. This will provide for an enhanced textural impression. Therefore, preferably the food matrix comprising the preparation has a Young's modulus at 37 degrees Celsius of from 0 to 10 MPa.

Young's modulus, also known as the tensile modulus, is a measure of the stiffness of an elastic material and is a quantity used to characterize materials. It is defined as the ratio of the uniaxial stress over the uniaxial strain within the limits of elasticity. It can be experimentally determined from the slope of a stress-strain curve created during tensile tests conducted on a sample of the material.

Method for preparing

The invention also concerns a method for preparing the preparation of plant parenchyma cells comprising the steps,

- a) providing the plant parenchyma cell material;
- b) contacting the material with a polar solvent;
- c) contacting the material with liquid carbon dioxide;
- d) subsequently bringing the carbon dioxide into a supercritical state followed by decrease of the pressure to release gaseous carbon dioxide;
- e) contacting the material with the hydrophobic substance in liquid form;
- f) contacting the material with water.

- In step a) the plant parenchyma cell material is provided by selecting the preferred source or sources, like for example apple or a combination of apple and pear. The source of the plant parenchyma cell wall material is preferably used as harvested, i.e. the tissue is not dried. The plant parenchyma cell material is provided in the required size of the material, like for example pieces comprising more than one plant cell like for example a piece of apple of 2x2x2 millimeter comprising about 1000 apple cells. At least some of the plant parenchyma cells have to remain intact before proceeding with the method according to the invention.
- 10 To manipulate the texture impression it may be preferred to weaken the interactions between the plant cells by partially dissolving the pectin middle lamellae and other compounds present in the cell walls by heating the plant parenchyma cell material in a solvent, preferably water. It will be appreciated that the required heating period will depend on the type of material and temperature used. Preferably, hydrated plant parenchyma cell material is in most cases not heated, or heated at a temperature below 75°C. Preferably most hydrated plant parenchyma cell material is not or less than 1 minute heated above 75°C. Preferably the pH is the natural pH of the aqueous suspension of the plant cell material. The heating may be done in combination with the presence of enzymes, which may degrade compounds present in between the cellulose microfibrils. Enzymatic treatment may be used to improve extraction of cell wall contents and to loosen the cells. Optionally, the plant cell material is separated from the solvent by any suitable method, for example by filtration, centrifugation, decanting or a combination thereof.
- 25 In step b) plant parenchyma cell material provided under a) is contacted with a polar solvent to wash the material with the solvent. Preferably this washing step is done for a period of between 10 seconds and 72 hours. Preferably two or more washing steps are applied, during a period of between 10 minutes and 24 hours for each step. Preferably each washing step will last between 1 hour and 10 hours, preferably between 1 hour and 5 hours.

It is possible to provide the plant parenchyma cell material in the medium, like for example water, in which it was heated and replace the medium by the polar solvent.

Preferably the exchange of the medium with the polar solvent is done by at least one washing step with the solvent.

5 Preferably the polar solvent is miscible with water, and suitable for use in processes for making foods. Preferably the solvent comprises ethanol or acetone, or mixtures thereof. By contacting the plant material with the polar solvent the cell walls are cleaned and the natural contents of the cells are at least partly removed. Compounds that are soluble in the solvent will be washed out from the cell walls.

10 To remove the solvent used in step b) it is contacted in step c) with liquid carbon dioxide to replace the polar solvent with liquid carbon dioxide and subsequently in step d) the carbon dioxide is brought into a supercritical state. The polar solvent used in the previous step is miscible with liquid carbon dioxide; therefore the liquid carbon dioxide replaces the polar solvent. By adding liquid carbon dioxide, the polar solvent is diluted
15 and washed away. At least one washing step with liquid carbon dioxide is required; preferably at least two washing steps are applied. Carbon dioxide behaves as a supercritical fluid above its critical temperature (31.1°C) and critical pressure (73.8 bar). After or during replacing the polar solvent with liquid carbon dioxide, the liquid carbon dioxide is brought in the supercritical state.

20

This is followed by decrease of the pressure to release gaseous carbon dioxide, because carbon dioxide becomes gaseous again after release of the pressure to atmospheric pressure. The pressure release needs to be slow enough not to disrupt the cell walls due to sudden expansion. During the pressure release, the temperature
25 should be above the critical temperature, like at 40 degrees Celsius, to prevent passing the boundary from liquid to gas. Therewith the so obtained plant cells are released from water, solvent and carbon dioxide. Moreover some of the natural elements of the cell interior may be removed during this step. The volume of the cell is largely retained by this method.

30

To fill the so obtained plant parenchyma cells, they are contacted in step e) with the hydrophobic substance in liquid form. This may require the use of elevated temperatures to get the hydrophobic substance in liquid form and could be combined

with pressure to lower the melting temperature. It has been found that the heating at this stage does not affect the firmness of the plant cells.

For example, the hydrophobic substance may be in liquid form at room temperature,
5 like for example sunflower oil, or it may be a fat or oil, which has been heated to liquefy the lipid material, like for example coconut fat. In the latter case step d) would be performed at a temperature higher than room temperature, in order to keep the hydrophobic substance in a liquid state.

10 If the hydrophobic substance is a mixture of a compound that is liquid at room temperature, like for example sunflower oil, and a compound that is substantially solid at room temperature, like for example a carotenoid, the latter can first be dissolved in sunflower oil at room temperature before the mixture is contacted with the plant parenchyma cells. In such a case the solid compound may remain dissolved.

15 Alternatively the solid compound may crystallize inside the plant cell.

Preferably step e) is carried out in a vessel which can be closed and put under overpressure or underpressure (compared to atmospheric pressure). Preferably the pressure of the gas headspace above the hydrophobic substance (usually air) is lower
20 than atmospheric pressure, in order to prevent entrapping of gas bubbles from the headspace into the hydrophobic substance and/or plant cells.

The temperature at which step e) is performed is preferably such that the hydrophobic substance remains liquid during the entire step e).

25

Before contacting the plant cell material with water in step f) it may be preferred or required to remove the hydrophobic substance that has not been absorbed by the plant cells.

30 This separation may be done by any suitable method, such as centrifugation and decanting of the free hydrophobic material. Alternatively the free hydrophobic material may be washed away by rinsing with a suitable solvent. Another method may be to

filter the materials obtained on a filter which retains the filled plant cells and not the free liquid hydrophobic material.

By contacting the plant cells with water the cell wall is hydrated and the hydrophobic
5 substance inside the plant cells is trapped.

Use of the preparation of plant parenchyma cells

The invention also concerns the use of the preparation according to the invention to impart a textural aspect to a food product.

10

The invention is now illustrated by the following non-limiting examples.

Examples

15 Example 1: Preparation of plant parenchyma cells

Fresh apples (cv Jonagold) in a ripe stage (firm, with a red and green to red and yellow color) were obtained from the supermarket. Cubic particles of approx. 7mm size without skin or core were cut out of the apple. These apple particles were immersed in ethanol (food grade, 96%), with approx. 300 ml of particles in 700 ml of ethanol.

20 Ethanol was renewed after 1, 4, and 16 hours. Ethanol was replaced by liquid CO₂ (food grade) in a Jumbo Critical Point Dryer Unit (SPI Supplies, West Chester, PA USA) at 10°C and approx. 50 bar. Liquid CO₂ was renewed 9 times in a period of 3 hours, by releasing the free liquid while keeping the pressure, and subsequent refilling with liquid CO₂. Then the CO₂ was brought in the supercritical state by raising the
25 temperature to 43°C. CO₂ was subsequently released while maintaining the temperature, leaving dried particles with intact cell volumes. The thus dried particles were immersed in refined olive oil (from supermarket) with a vacuum (by rotary pump) above the oil. Once loaded with oil, which was judged by the fact of sinking, the particles were immersed in drinking water for at least 2 hours.

30

A tasting panel of 7 members did the textural assessment of the so obtained particles (i.e. preparation of plant parenchyma cells according to the invention). The particles were taken into the mouth and repetitively crushed between the upper and lower front

teeth. All 7 members of the tasting panel reported a clear crunch that was still present after repeated biting. The crunch was described as a multitude of audible cracks when crushing the particles between the teeth.

5 Example 2: Water ice comprising a preparation of plant parenchyma cells

A preparation of plant parenchyma cells according to example 1 was prepared with the exception that a standard water ice matrix (20% sucrose dissolved in drinking water) was used instead of plain drinking water during the aqueous immersion of the particles.

10 Two samples were prepared:

Example 2A: 5ml Water ice matrix with 1 particle of fresh apple.

Example 2B: 5ml Water ice matrix with 1 particle of the preparation of plant parenchyma cells (i.e. oil loaded apple).

15 The two samples were frozen in a blast freezer at -30°C and after 1 hour transferred to a storage freezer at -28°C and stored overnight.

The following day a panel consisting of 7 members tasted the samples. During tasting, the water ice was taken into the mouth and allowed to thaw. The particle was then bitten with the front teeth and firmness and crunch of the samples were compared.

20

All 7 panelists judged example 2B firmer and crunchier than example 2A.

Example 3

A preparation of plant parenchyma cells according to example 1 was prepared.

25

Four samples were prepared:

Example 3A: particles of fresh apple.

Example 3B: the preparation of plant parenchyma cells (i.e. oil loaded apple).

Example 3C: Example 3A after a freeze-thaw cycle.

30 Example 3D: Example 3B after a freeze-thaw cycle.

The freeze-thaw cycle was applied as follows:

10 particles were immersed in 10 ml water in a plastic laboratory vial of 25ml. The vials were frozen in a blast freezer at -30°C and after 1 hour transferred into a storage freezer at -28°C and stored overnight. The following day the vials were placed on a laboratory desk at room temperature. After thawing, the vials were placed in a water bath of 20°C, to equilibrate to room temperature. Within 2 hours after thawing, the samples were tested as described below.

Mechanical testing was performed using a texture analyzer (TA-XT Plus, Stable Micro Systems Ltd., Surrey, UK). A sample particle was placed on a flat table. A puncture probe was moved into the sample particle with a fixed speed of 0.4 mm/s, starting 10 mm above the table and ending at 0.0 mm (total 25 seconds). The puncture probe was a cylindrical rod (0.75 mm diameter) with a flat end, puncturing through the center of the sample particle, down to the table.

Simultaneously with the force, the sound emitted during puncturing was recorded using an acoustic sensor (Bruel & Kjaer 4189 prepolarized free-field ½" microphone plus a 2671 Deltatron preamplifier microphone) with a frequency band of 6.3 Hz up to 20 kHz and a sensitivity of 50 mV/Pa. The set up used for the simultaneous force and acoustic measurement has been described extensively by Castro-Prada et al. (Castro-Prada, E. M., Luyten, H., Lichtendonk, W., Hamer, R. J. and Van Vliet, T. (2007), An improved instrumental characterization of mechanical and acoustic properties of crispy cellular solid. *Journal of Texture Studies*, 38: 698-724). A fixed distance of 5 cm from the particle to the microphone was used for sound recording. The analogue sound signal and the data of the texture analyzer were digitized using a Brüel & Kjaer Front-end A/D converter system (type number 2827) with a sampling rate of 65 kHz. To avoid interference with an external source of sound, sound tests were performed inside an anechoic and isolated chamber. Recording and initial signal analysis were performed using Brüel & Kjaer Pulse Labshop Software (version 7.0). More extensive signal analysis was done using Brüel & Kjaer Sound Quality type 7698 software. Three FFT filters were applied:

- 0-500 Hz, to remove low frequency noise
- 1000-5000 Hz, to remove high frequency noise
- 790-810 Hz, to remove noise from the texture analyzer

Further, a sound intensity threshold was applied at 0.0005 Pa² to identify significant sound events.

The number of significant sound events was recorded as a function of time.

5 Table 1

Example	Sound	Force
3A	Significant sound events along the whole path of the probe through the particle.	A continuous fluctuation of puncturing force between 5 and 40 grams along the whole path of the probe through the particle.
3B	A multitude of significant sound events during the last seconds of the path of the probe through the particle.	A gradual buildup of puncturing force to above 100 grams along the first millimeters of the path of the probe through the particle, often followed by a sudden decrease. Always a steep increase of the puncturing force to above 300 grams along the last millimeter of the path of the probe.
3C	Very few significant sound events along the whole path of the probe through the particle.	A continuous fluctuation of puncturing force between 0 and 30 grams along the whole path of the probe through the particle.
3D	A multitude of significant sound events during the last seconds of the path of the probe through the particle.	A gradual buildup of puncturing force to above 100 grams along the first millimeters of the path of the probe through the particle, often followed by a sudden decrease. Always a steep increase of the puncturing force to above 300 grams along the last millimeter of the path of the probe.

Significant sound events during the last 2.5 seconds of the tests were also counted. The table below gives the averages, standard deviations and the number of tested particles per sample.

5 Table 2

Example	Average	Standard deviation	Number of particles
3A	2.25	1.83	8
3B	9.4	4.67	8
3C	0.75	1.39	5
3D	8.2	4.1	5

The results show that the material of the invention largely retained its specific sound/texture properties over a freeze-thaw cycle, whereas fresh material lost both sound and texture properties over a freeze-thaw cycle.

10

Example 4

A preparation of plant parenchyma cells according to example 1 was prepared with the following exceptions. Instead of olive oil, colored oils were used in two different samples, using sunflower oil (refined, from supermarket) and beta-carotene suspension (1% beta-carotene in rapeseed oil). During the oil immersion step, the samples were heated to 85°C to dissolve all carotene.

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The following two samples were prepared:

Example 4A: preparation of plant parenchyma cells with beta-carotene suspension.

20

Example 4B: preparation of plant parenchyma cells with 1 part beta-carotene suspension diluted in 9 parts sunflower oil.

The initially white plant cells showed a bright orange color with a higher intensity for Example 4A.

25

Example 5

A preparation of plant parenchyma cells according to example 1 was prepared with the following exceptions. Instead of olive oil, flavored oils were used in three different

samples, using sunflower oil (refined, from supermarket) and limonene (97% pure, Sigma Aldrich, food grade):

Example 5A: preparation of plant parenchyma cells with limonene.

- 5 Example 5B: preparation of plant parenchyma cells with 2 parts limonene diluted in 3 parts sunflower oil (w/w).

Example 5C: preparation of plant parenchyma cells with 1 part limonene diluted in 49 parts sunflower oil (w/w).

- 10 The initially flavorless plant cells obtained a limonene flavor at different concentrations.

Example 6: Ice Cream comprising a preparation of plant parenchyma cells.

A commercial dessert ice cream (Hertog Slagroomijs, Unilever, Rotterdam, The Netherlands) was used as Ice Cream matrix.

15

Particles of 7mm were prepared according to example 1 with the following exceptions.

Instead of olive oil, sunflower oil was used, containing 2% apple flavour (Symrise 290211 Apple Natural Flavouring, Symrise, Holzminden, Germany) and 0.4% colourant (Sensient 506087-0010 Fusion Mint Green, Sensient Colors Europe GmbH,

- 20 Geesthacht, Germany)

Instead of plain drinking water, water containing 20% sucrose, 4% citric acid, and 2% apple flavour, was used during the aqueous immersion of the particles.

- 25 After 4 hours of aqueous immersion, the particles were shortly blotted on filter paper and frozen in a blast freezer at -30°C and after 1 hour transferred to a storage freezer at -30°C and stored overnight.

The samples were prepared as follows:

- 30 Ice Cream matrix was brought to a temperature of -5°C and cut into 1cm pieces. Each popsicle was made by spooning 8 particles through 30ml ice cream matrix, loading this mixture into a 50ml Falcon Tube, and inserting a wooden popsicle stick halfway into the mixture. After making the popsicles, being still below 0°C, they were deep frozen in a blast freezer at -30°C and after 1 hour transferred to a storage freezer at -30°C.

Two sets of samples were prepared:

Example 6A: 30ml popsicle containing Ice Cream matrix with 8 particles of fresh apple.

Example 6B: 30ml popsicle containing Ice Cream matrix with 8 particles of the
5 preparation of plant parenchyma cells (i.e. 7mm colored and flavored apple pieces).

After 7 days a panel of 3 members tasted the samples. Same paneling was done by 4 other persons, individually, after 5 weeks of storage.

10 All 7 panelists judged particles in example 6B much crunchier than example 6A.
Furthermore, with frozen particles, the panelist noted that 6A was icy and hard, but 6B had a texture comparable to original fresh material. After thawing, 6A turned soft and slimy, while 6B still resembled structure of fresh material, still with crunch.
Furthermore particles in example 6B were judged to have a more intense apple flavor,
15 a more intense sourness, and a more intense color than example 6A.

So, the material according to the invention had improved 'fresh like' sensory attributes.

Example 7: Strawberry Ice Cream comprising a preparation of plant parenchyma cells

20 Ice Cream popsicles were prepared according to example 6 with the following exceptions.

Instead of using apple flavored particles, strawberry flavored particles were prepared as follows.

25 Particles of 7mm were prepared according to example 1 with the following exceptions.
Instead of 7 mm apple particles, particles of about 5-10 mm of strawberry were used.
Instead of apple flavor, 3.1% strawberry extract was used (Symrise 620264 Strawberry Natural Flavouring)
Instead of colorant, 0.06% Carmine (Sensient Carmine 25% L-OD, 503203) plus 0.6%
30 paprika extract (Sensient Paprika extract L-OS, 503107) were used.

Two sets of samples were prepared:

Example 7A: 30ml popsicle containing Ice Cream matrix with 8 particles of fresh strawberry.

Example 7B: 30ml popsicle containing Ice Cream matrix with 8 particles of the
5 preparation of plant parenchyma cells (i.e. colored and flavored strawberry pieces).

Pieces in Example 7A were hard and icy, while pieces in 7B had a softer, more preferred texture.

10 Example 8: Multifruit Ice Cream comprising a preparation of plant parenchyma cells

Ice Cream popsicles were prepared according to example 6 with the following exceptions.

Instead of using 8 apple flavored pieces, different pieces were used, all originating from apple tissue, but now with different flavors and colorants:

15 2 Green particles: Colored with Fusion Mint Green 0.4%, and flavored with 2% Apple Flavour.

2 Red pieces: Colored with Carmine 0.08% and Paprika Extract 0.6%, and flavored with 2.0% strawberry.

2 Orange particles: Colored with beta-carotene 0.7% (Sensient Beta-Carotene 0.3% L-
20 OS 503001), and flavored with 2.1% peach flavor (Symrise 159489 Peach Natural Flavouring).

2 Yellow particles: Colored with Lutein 0.07% (Sensient Lutein 2.5% L-OS 3026),
Turmeric root extract 0.3% (Sensient Turmeric root extract L-OS 503081), Beta-
carotene 0.34%, and Flavored with 2.1% Lemon Flavor (Symrise 206006 Lemon Lime
25 MRDR376139 Flavouring).

The resulting popsicles contained vividly colored particles in different colors on a white background. In an assessment session the participants judged the particles to be non-icy, with crunchy texture, still having a firm structure after thawing. Furthermore, the
30 different types of particles delivered clearly distinct flavor contrasts within the popsicle during consumption.

Claims

1. Preparation of plant parenchyma cells, wherein at least some of the plant parenchyma cells have an intact and hydrated cell wall and comprise at least 10 wt% hydrophobic substance, wherein at least part of the hydrophobic substance is of a different origin than the plant cells and wherein the hydrophobic substance is at least partially liquid at 40 degrees Celsius, and wherein the smallest dimension of the preparation is from 1.5 millimeter to 2 centimeter.
2. Preparation according to claim 1 wherein the amount of plant parenchyma cells comprising hydrophobic substance and having an intact and hydrated cell wall is at least 20 %, more preferably at least 40 %, even more preferably at least 60 %, still more preferably at least 80 % and even still more preferably essentially consisting of primary plant cells comprising hydrophobic substance and having an intact and hydrated cell wall.
3. Preparation according to claim 1 or claim 2 wherein the plant parenchyma cells comprise at least 20 wt% hydrophobic substance, more preferably 30 wt%, even more preferably 40 wt% and still more preferably at least 50 wt%.
4. Preparation according to any one of claims 1 to 3 wherein at least 50 wt% of the hydrophobic substance is of a different origin than the plant cells, more preferably at least 70 wt%, even more preferably at least 90 wt% and still more preferably essentially all.
5. Preparation according to any one of claims 1 to 4 wherein the hydrophobic substance is selected from vegetable oils, essential oils and combinations thereof.
6. Preparation according to any one of claims 1 to 5 wherein the plant cells are selected from apple, onion, pear, peach, pumpkin, potato, carrot, sugar beet and combinations thereof.
7. Preparation according to any one of claims 1 to 6 wherein the preparation comprises from 25 to 1,000,000 plant cells.

8. Preparation according to any one of claims 1 to 7 wherein the smallest dimension of the preparation is from 2 millimeter to 1.5 centimeter, more preferably from 2.5 millimeter to 10 millimeter and even more preferably from 3.5 millimeter to 10 millimeter.
9. Preparation according to any one of claims 1 to 8 further comprising water-soluble flavor.
10. Food product comprising the preparation according to any one of claims 1 to 9.
11. Food product according to claim 10 wherein the food matrix comprising the preparation comprises water, oil or combinations thereof, more preferably comprises a water and oil emulsion, and even more preferably is a water-in-oil or an oil-in-water emulsion.
12. Food product according to claim 10 or claim 11 wherein the food product is a frozen food product and preferably is an ice cream or water ice.
13. Food product according to anyone of claims 10 to 12 wherein the food matrix comprising the preparation has a Young's modulus at 37 degrees Celsius of from 0 to 10 MPa.
14. Method for preparing the preparation according to any one of claims 1 to 9 comprising the steps,
 - a) providing the plant parenchyma cell material;
 - b) contacting the material with a polar solvent;
 - c) contacting the material with liquid carbon dioxide;
 - d) subsequently bringing the carbon dioxide into a supercritical state followed by decrease of the pressure to release gaseous carbon dioxide;
 - e) contacting the material with the hydrophobic substance in liquid form;
 - f) contacting the material with water.

15. Use of the preparation according to any one of claims 1 to 9 to impart a textural aspect to a food product.

INTERNATIONAL SEARCH REPORT

International application No
PCT/EP2013/074967

A. CLASSIFICATION OF SUBJECT MATTER
INV. A23L1/03 A23L1/212
ADD.

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

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)
A23L

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

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

EPO-Internal, FSTA, WPI Data

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	G. DONGOWSKI ET AL: "Binding of Water, Oil, and Bile Acids to Dietary Fibers of the Cellan Type", BIOTECHNOLOGY PROGRESS, vol. 15, no. 2, 5 April 1999 (1999-04-05), pages 250-258, XP055053455, ISSN: 8756-7938, DOI: 10.1021/bp990014c	1-13,15
Y	cited in the application the whole document ----- -/--	14



Further documents are listed in the continuation of Box C.



See patent family annex.

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"O" document referring to an oral disclosure, use, exhibition or other means

"P" document published prior to the international filing date but later than the priority date claimed

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Date of the actual completion of the international search

19 February 2014

Date of mailing of the international search report

25/02/2014

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INTERNATIONAL SEARCH REPORT

International application No

PCT/EP2013/074967

C(Continuation). DOCUMENTS CONSIDERED TO BE RELEVANT		
Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	DONGOWSKI G ET AL: "[Composition and properties of dietary fibre of the cellan type]", ZEITSCHRIFT FUER ERNAHRUNGSWISSENSCHAFT, STEINKOPF VERLAG, DARMSTADT, DE, vol. 36, no. 1, 1 January 1997 (1997-01-01), page 85, XP008161940, ISSN: 0044-264X	10-13,15
A	the whole document	1-9,14
Y	RU 2 330 430 C1 (KVALENKOV OLEG IVANOVICH [RU]) 10 August 2008 (2008-08-10)	14
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INTERNATIONAL SEARCH REPORT

Information on patent family members

International application No

PCT/EP2013/074967

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