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(54) Title: HYDROTHERMAL PROCESS FOR CONVERTING BERRY AND FRUIT MATERIALS TO BIOACTIVE FRACTIONS

(57) Abstract: The invention relates to process for converting berry and/or fruit materials to fractions comprising bioactive compounds, said process comprising the steps, where at least one berry material or fruit material selected from berries, fruits, by-products, side streams and waste materials originating from berries or fruits, and any combinations thereof is sieved whereby a seed fraction is separated from a skin fraction, the seed fraction is subjected to sanding, followed by separation of a seed coat fraction comprising surface layer of the seeds, which is subjected to hydrothermal extraction.

HYDROTHERMAL PROCESS FOR CONVERTING BERRY AND FRUIT MATERIALS TO BIOACTIVE FRACTIONS

FIELD OF THE INVENTION

The present invention relates to a hydrothermal process for converting berry and fruit materials to fractions comprising bioactive compounds. The invention also relates to fractions comprising bioactive compounds, originating from berries and fruit, obtainable by the process. The invention further relates to the use of said fractions comprising bioactive compounds in cosmetics, hygiene products, nutraceuticals, food products and food supplements, feeds, packages and in pharmaceutical products.

BACKGROUND OF THE INVENTION

In the industry dealing with processing of berries and fruits significant amounts of waste materials, side streams and by-products are formed. Food industry uses wild berries, cultured berries and fruits in the manufacture of wide range of products, such as pastes, beverages, alcohol products, jams, conserves, milk based products, sweets and the like. The use of berry and fruit fractions has also become very popular in cosmetic products and for example cloudberry seed oil, rich in polyunsaturated fatty acids, is regarded as a valuable component in cosmetic preparations.

Large volumes of waste are produced in the processing of berries and fruit, particularly in the food industry, which waste material is utilized to a very small extent. Most of the waste material is currently discarded or transported to landfill or dumping area, thus increasing the environmental burden. Some of this waste material is subjected to drying followed by extraction of seed oils, however only a very small portion of the material is used.

Typically, in the processing, berries and fruit are mechanically cleaned, followed by removing of the juice, pulp or paste by suitable methods, such as pressing. The remaining wastematerial, such as pomace, berry cake, fruit cake or press cake comprises berry or fruit skins, peels, seeds and pith, which contain bioactive phenolic compounds, fibers and other bioactive compounds.

FI 122664 B discloses a method for fractionating berries and separating nutrients from the fractions formed, in which method berry raw material is dried and ground lightly, so that the seeds of the berry separate from the fruit flesh and skin portion without breaking, followed by a second light grinding, which is carried out on the formed seedless fruit flesh

and skin fraction, whereby a fine powder is formed, which is screened or classified. Seeds are not fractionated by this method. Seed are discarded and peels are further fractionated.

US 2013/0040005 A1 relates to an antihypertensive agent comprising boysenberry seed extract as active ingredient and to a method for obtaining said agent. In said method boysenberry pomace is dried, crushed and sieved to separate the seed, followed by grinding the seed to fine powder, which is extracted with water or organic solvent, followed by contacting the extraction solution with a polyphenol absorbent and eluting with alcohol based solvent to obtain the target extract.

Based on the above it can be seen that there still exists a need to provide improved methods for utilizing berry and fruit materials, particularly waste materials, side streams and by-products for providing fractions comprising valuable bioactive compounds contained in said materials.

SUMMARY

In the present invention it was found that fractions comprising bioactive compounds, particularly ellagic acid and/or derivatives thereof, originating from berry and/or fruit seeds, can be enriched with a simple and environmentally safe method.

The present invention is particularly based on studies on hydrothermal processing and converting of pomace, berry cake, fruit cake and press cake obtained from berries and fruit, and the use of the obtained products. The invention provides convenient and effective means particularly for utilizing waste materials, side streams and by-products from berry and fruit industry, such as pomace, press cake, berry cake and fruit cake, in the manufacture of fractions comprising bioactive compounds useful in cosmetics, hygiene products, food supplements, food products, feeds, packages and in pharmaceutical products, particularly as natural antimicrobials or natural preservatives.

Thus an object of the invention is to provide a process for converting berry and fruit materials to fractions comprising bioactive compounds, particularly ellagic acid and/or derivatives thereof.

A further object of the invention is to utilize waste materials, side streams and by-products from berry industry and/or fruit industry without the need to discard said materials.

A further object of the invention is to provide fractions comprising bioactive compounds originating from berries and fruits.

A still further object of the invention is the use of said fractions comprising bioactive compounds in cosmetics, hygiene products, nutraceuticals, food products, food supplements, feeds, packages and in pharmaceutical products.

The invention is directed to a process for converting berry and fruit materials to fractions comprising bioactive compounds, which process comprises the steps, where
in the first step at least one berry material or fruit material selected from berries, fruits, by-products, side streams and waste materials originating from berries or fruits selected from the genus *Rubus, Sorbus, Rosa, Empetrum, Aronia* and *Hippophae* and from combinations thereof, and the fruit are selected from the genus *Vitis, Punica, Pyrus, Citrullus, Benincasa, Cucumis, Momordica, Olea, Malus and from species Argania spinosa,* and combinations thereof, said berry material or fruit material having water content not more than 15 wt%, is sieved, whereby a seed fraction is separated from a skin fraction,
in the second step the seed fraction is subjected to sanding where 2-40 wt% of the seed is removed and a seed coat fraction comprising surface layer of the seeds and a sanded seed fraction comprising the sanded seeds are obtained,
in the third step the seed coat fraction is mixed with an aqueous medium in a ratio from 1:3 to 1:80 by weight, at a temperature from 15 to 95°C for 20 min to 10 hours to obtain a mixture comprising bioactive compounds, and the mixture is subjected to separation whereby a hydrothermal extract is separated from a hydrothermal residue.

The bioacative compounds particularly comprise ellagic acid and/or derivatives thereof.

The invention is further directed to the use of the fractions comprising bioactive compounds, in cosmetics, hygiene products, nutraceuticals, food products, food supplements, feeds, packages and in pharmaceutical products.

Accordingly, the present invention provides simple and economic means for utilizing waste materials, side streams and by-products originating from berry industry and fruit industry in the manufacture of fractions comprising bioactive compounds, whereby dumping of berry or fruit waste can be avoided or at least substantially decreased.

The characteristic features of the invention are presented in the appended claims.

BRIEF DESCRIPTION OF THE DRAWINGS

Figure 1 illustrates one embodiment of the process of the invention.

Figure 2 illustrates another embodiment of the process of the invention, where berry or fruit material is pretreated prior to sieving.

Figure 3 (3A-3E) shows antimicrobial activity of the fractions comprising bioactive compounds, obtained with the process.

Figure 4 shows table 5 presenting consumer feed-back of cloudberry pearling cream.

Figure 5 presents HPLC profiling and fatty acid content of hydrothermal extraction residue of cloudberry seed coat powder.

DEFINITIONS

Unless otherwise specified, the terms, which are used in the specification and claims, have the meanings commonly used in the field of food industry. Specifically, the following terms have the meanings indicated below.

The term "berry" is understood here to mean all wild and cultivated berries comprising internal seeds, which berries belong to the genus *Rubus, Sorbus, Empetrum, Rosa, Aronia* or *Hippophae,* including all hybrid berries of these genera. Sea buckthorn is an example of the genus *Hippophae.* Raspberries, blackberries, arctic bramble, dewberries and cloudberries are examples of the *Rubus* species. As the *Rubus* species readily interbreed and are apomicts, the parentage of the hybrid plants is often highly complex, but it is generally agreed to include in the definition cultivars of blackberries and raspberries. Examples of said hybrid berries include loganberry, boysenberry, veitchberry, marionberry, silvanberry, tayberry, tummelberry and hildaberry.

The term "fruit" is understood here to mean all wild and cultivated fruit belonging to the genus *Vitis*, *Punica, Pyrus, Malus, Citrullus, Benincasa, Cucumis, Momordica, Olea and species Argania spinosa,* including all hybrid fruit of these genera or species.

Fruit of the genus *Vitis* mean grapes comprising internal seeds, including all hybrid grapes, which are primarily crosses between *V. vinifera* and another grapevine. Grapes are used for making wine, jam, beverages, jelly, seed extract, raisins, vinegar, and grape seed oil. *Vitis* is a genus of about 60 vining plants in the family *Vitaceae*.

Fruit of the genus *Citrullus, Benincasa, Cucumis, Momordica* mean melons comprising internal seeds, including all hybrid melons.

Fruit of the genus *Olea* means olives, particularly *Olea europaea*.

Fruits refers also to fruits of fruit trees, such as pomegranate (*Punica granatum*), pear tree *(Pyrus communis)* and apple tree (*Malus* family) and all hybrids thereof.

The terms "berry industry" and "fruit industry" refer to industry dealing with the development, refining and manufacture of products and processes relating to wild and cultivated berries and fruit.

The term "antimicrobially active" compound refers here to compounds being able to kill microorganisms or inhibit their growth. Examples of these compounds are phenolic compounds, such as ellagic acid and ellagitannins. In addition to antimicrobial and preservative activity, these compounds often have other biological activities, particularly antioxidant activity.

The term "sanding of seeds" refers here to mechanical, abrasive treatment where the surface layer of the seeds is removed as powder. Sanding is understood to also include polishing and pearling.

The term "hydrothermal" processing means here processing in the presence of water at elevated temperatures.

Hygiene products include here particularly diapers, female hygiene pads, tampons, etc, incontinence pads and products, and the like.

DETAILED DESCRIPTION OF THE INVENTION

The invention provides a convenient process for converting berry materials and fruit materials to fractions comprising valuable bioactive compounds, particularly to fractions comprising significant amounts of ellagic acid and/or derivatives of ellagic acid, particularly ellagitannins. Ellagitannins are esters of glucose with ellagic acid which, when hydrolysed, yield ellagic acid.

The invention is based on studies on the processing and converting of waste materials, side streams and by-products originating from processing of berries and fruit, such as pomace and press cake, for providing means for utilizing these waste materials and by-products in the manufacture of fractions comprising bioactive compounds, useful for

example in cosmetics, hygiene products, nutraceuticals, food products, food supplements, feeds, packages and in pharmaceutical products, as natural preservatives.

It was found that particularly the surface layer (seed coat fraction or seed peel layer) of the seeds of berries of the genus *Rubus, Sorbus, Empetrum, Rosa, Aronia* and *Hippophae* and seeds of the fruits of the genus *Vitis, Punica, Pyrus, Malus, Citrullus, Benincasa, Cucumis, Momordica, Olea,* and *species Argania spinosa*, contains high concentrations of ellegic acid and/or derivatives thereof, particularly ellagitannins. The fractions comprising ellagitannins show strong antimicrobial activity for example against the human pathogen *Staphylococcus aureus.* The seed coat fractions contain high concentrations of bioactive phenolic compounds. Particularly ellagic acid, ellagitannins and derivatives thereof are comprised in said fractions of *Rubus* berries.

Recent studies have shown that ellagic acid and ellagitannins possess many interesting biological activities and thus they may play preventive role in disease prevention.

Ellagic acid appears to have anti-cancer properties, especially against prostate cancer. In addition, pre-clinical work suggests a possible anti-inflammatory effect in obesity-related diseases. Ellagitannins and ellagic acid may also have positive effects on glucose metabolism.

Remarkable amounts of biologically active phenolic compounds, particularly ellagic acid and/or ellagitannins and their derivatives remain in the waste material, which is currently not utilized. Ellagic acid and ellagitannins are natural antioxidants and preservatives having strong antimicrobial effect, thus useful as natural preservatives and antioxidants in various applications in the field of cosmetics, hygiene products, food industry and feed industry, as well as in packaging industry and pharmaceutical industry.

In skin care and hygiene products the invention may be utilized to convert berry and fruit materials to fractions that stabilize, balance and protect healthy skin microbiome, as these fractions are known to inhibit the growth of many pathogenic microbes without effecting the growth of beneficial microbes.

With the process of the invention berry skins or fruit skins can be separated from the seeds and the seed surface layer can then be removed and subjected to hydrothermal extraction to obtain fractions having high antimicrobial activity. Further, optionally enzymes may be used in the hydrothermal extraction.

The hydrothermal residue separated after the hydrothermal extraction from the hydrothermal extract may also be used particularly in nutraceuticals, food products, food supplements and feeds because of high contents of ellagic acid, C18 fatty acids, lignin and fibers. Ellagic acid is more easily absorbed in the GI tract than ellagitannins typically present in the natural berries and fruit.

Berries

In the present invention, all wild berries, cultivated berries and all hybrid berries of the genus *Rubus*, *Sorbus, Empetrum, Rosa, Aronia* and *Hippophae* and any combinations thereof may be used. Raspberries, blackberries, arctic bramble (synonym arctic raspberries), dewberries and cloudberries, and hybrid berries including loganberry and boysenberry are examples of the *Rubus* species suitable for the invention. Rowanberry is an example of *Sorbus* species, crowberry of *Empetrum* species, rose hip and dog rose of *Rosa* species, chokeberry of *Aronia* species and sea buckthorn berry of *Hippophae* species suitable for the invention.

Cloudberries are valuable wild berries having high aroma content and they contain also valuable seed oil. Cloudberries are used in food, liqueur and cosmetic industry, however only the seed oil is presently utilized from the waste material remaining after pressing the berries. Ellagic acid content found in cloudberry is the following: berry fruit (fruit + seeds) 0.6 mg/g dry weight, skins 20.3 mg/g dry weight, sanded seed coat power 19.6 mg/g dry weight and polished seeds 12.6 mg/g dry weight. Ellagitannin content found in cloudberry is the following: berry fruit 24,6 mg/g dry weight, skins 11,0 mg/g dry weight, sanded seed coat powder 18,6 mg/g dry weight and polished seeds 10,9 mg/g dry weight. In the seeds ellagitannins are concentrated in the sanded seed coat fraction.

Arctic brambles contain very high ellagic acid and ellagitannin contents and thus they are also particularly suitable as raw material for the process of the invention. From the ecological point of view, wild berries, such as cloudberries and arctic bramble which have grown without any manmade fertilizers or pesticides, are particularly suitable.

Fruits

All fruits of the genus *Vitis, Punica, Pyrus, Malus, Citrullus, Benincasa, Cucumis, Momordica, Ole,* and of *species Argania spinosa* are suitable for the process of the invention. All grapes and all hybrid grapes, containing seeds and belonging to the genus *Vitis* may be used in the present invention. Typically huge amounts of waste material are

obtained from processing of grapevines, such as from pressing grapevines and thus grapes offer also a particularly suitable raw material source for the present invention.

Fruits of fruit tree, such as pomegranate (*Punica granatum*), pear tree *(Pyrus communis)* and apple tree (*Malus* family), including all hybrids thereof are also suitable raw material source. Remarkable amounts of waste material are obtained during processing of these fruits.

Berry materials and fruit materials

The berry materials and fruit materials suitable for the process of the invention may be selected from whole berries, whole fruits, by-products, side streams and waste materials, originating from processing of berries or fruit. Examples of such by-products, side streams and waste materials are press cakes, pomaces, berry cakes, fruit cakes and fractionating residues. Said by-products, side streams and waste material typically comprise berry or fruit skins or peels, seeds, some pulp, occasionally some leaves, arbors and conifer needles, depending also how well the berries or fruit are cleaned mechanically before processing.

Processing of berries or fruit may be carried out for example at a facility carrying out processing or refining or fractionating of berries or fruit, or at a facility in the food or feed processing industry, from the manufacture of beverages, pastes, purees, wines, jams, conserves, sweets and the like. Particularly preferably by-products, side streams and waste materials are used in the present invention.

Typically in a juicing line, berries or fruit are pressed and the remaining press cake is frozen and stored at approx. -20°C for further use, or alternatively it may be dried.

Berry and fruit pastes and purees are obtained for example by squeezing berries or fruit through a sieve and the remaining berry cake or fruit cake is frozen and stored at approx. -20°C, or alternatively it may be dried. The obtained frozen pomace, berry cake or fruit cake may contain from 40 to 70 % by weight of water, typically from 50 to 60 % by weight of water.

The by-products, side streams or waste material obtained from the berries of the genus *Rubus* or grapes from the genus *Vitis*, such as press cake or pomace contains predominantly seeds, skins or peels and some pulp.

Process

The present invention is directed a process for converting berry and fruit materials to fractions comprising bioactive compounds, which process comprises the steps, where in the first step at least one berry material or fruit material selected from berries, fruits, by-products, side streams and waste materials originating from berries or fruits selected from the genus *Rubus, Sorbus, Rosa, Empetrum, Aronia* and *Hippophae* and from combinations thereof, and the fruit are selected from the genus *Vitis, Punica, Pyrus, Malus, Citrullus, Benincasa, Cucumis, Momordica, Olea* and from *species Argania spinosa*, and combinations thereof, said berry material or fruit material having water content not more than 15 wt%, is sieved, whereby a seed fraction is separated from a skin fraction, in the second step the seed fraction is subjected to sanding where 2-40 wt% of the seed is removed and a seed coat fraction comprising surface layer of the seeds and a sanded seed fraction comprising the sanded seeds are obtained,
in the third step the seed coat fraction is mixed with an aqueous medium, in a ratio from 1:3 to 1:80 by weight, at a temperature from 15 to 95°C for 20 min to 10 hours to obtain a mixture comprising bioactive compounds, and the mixture is subjected to separation whereby a hydrothermal extract is separated from hydrothermal residue.

The bioactive compounds comprise ellegic acid and/or derivatives thereof, particularly ellagitannins. Preferably the bioactive compounds comprise ellagic acid and/or ellagitannins.

The berry material or fruit material having water content not more than 15 wt% means here dry berry material or fruit material.

In an embodiment in the third step the ratio of the seed coat fraction to the aqueous medium is from 1:5 to 1:50, respectively.

In an embodiment in the third step the ratio of the seed coat to the aqueous medium is from 1:5 to 1:40, respectively.

In an embodiment the seed coat fraction is mixed with the aqueous medium using mixing speed 100-1500rpm. Any suitable mixing devices may be used.

In an embodiment in the aqueous medium is selected from water or a mixture of water with an acid selected from ascorbic acid oxalic acid, citric acid, acetic acid, malic acid, benzoic acid and HCl, preferably ascorbic acid. The concentration of the acid in the aqueous

medium is from 0.01 to 0.2 % by weight, preferably from 0.05 to 0.1 % by weight. The mild acidic conditions have stabilizing effect on anthocyanins.

In an embodiment in the third step the mixing temperature may range from 20 to 95°C, preferably from 25 to 90°C.

In an embodiment in the third step at least one enzyme may be added to the aqueous medium. The enzyme is suitably selected from carbohydrate hydrolyzing enzymes, suitably from cellulose, pectinase, xylanase and combinations thereof. The enzymes are dosed based on their main activity (e.g. 100-200 nkat/g or 0.01-1 % by weight). The enzyme is diluted in water before mixing with the aqueous medium. Depending on the enzyme the treatments are carried out either at a pH optimal for each enzyme or at the intrinsic pH of the used material. When an enzyme is used, the third step is carried at a temperature of 40-47°C for 1-4 hours, and then from 30 min to 7 hours at 50-90°C.

In the third step the separation of the hydrothermal extract from the hydrothermal residue (solid materials), is carried out using suitable methods, such as centrifuging, filtration, decanting and the like.

In an embodiment the hydrothermal extract, obtained in the third step is dried. The drying may be carried out by freeze-drying, spray-drying, drying at elevated temperature, in drying cabinets, and the like. Suitably the drying is carried out at a temperature from 40 to 150oC, spray-drying at 80- 95°C.

The process of the invention is illustrated in Figure 1, where berry and/or fruit material 10 having water content not more than 15 wt%, is subjected to sieving 100, whereby a seed fraction 30 is separated from a skin fraction 20, the seed fraction 30 is subjected to sanding 200 where 2-40 wt% of the seed is removed and a seed coat fraction 50 comprising surface layer of the seeds and a sanded seed fraction 40 comprising the sanded seeds are obtained, the seed coat fraction 50 and an aqueous medium 60 are subjected to mixing 300 to obtain a mixture 70 comprising bioactive compounds with antimicrobial activity. The mixture 70 is subjected to separation 400 to obtain hydrothermal extract 80 and hydrothermal residue 82. The hydrothermal extract 80 is subjected to drying 800 to obtain dry fraction 81 comprising bioactive compounds.

The seed fraction comprises seeds. Occasionally it may comprise small amounts of any of skin, peel and pulp.

The skin fraction comprises skin, peel and pulp. Occasionally it may comprise small amounts of any of leaves, arbors and conifer needles.
In a preferable embodiment the berry and/or fruit material has water content of 0.1-10 wt%, particularly preferably 0.1-8 wt%.

In the process sieving (separation of seeds) is carried out, where the berry material or fruit material having water content not more than 15 wt% is sieved, whereby the seeds remain on the sieve and the skin, peels, pulp etc. pass through the sieve.

Suitably a sieving device, air classification device, air jet sieve device, screening device or rotary screen is used, preferably a vibratory or a shaking sieving/screening device is used, where the sieve size is selected according to the seed size of the berry or fruit.

For example, when sieving berry material obtained from the genus *Rubus,* suitably a sieve is used where the sieve has a mesh opening of 0.5-2.0 mm, preferably 0.6-1.6 mm.

The sanding is carried out using a sanding or polishing apparatus selected from grain polishing machines (e.g. barley), rice etc. polishing machines, pearling machines, dehullers and polishers/hullers.

In the sanding from 2 to 40 wt%, preferably 3-35 wt%, particularly preferably from 3 to 30 wt% of the seed surface layer is removed as finely divided powder fraction (seed coat fraction or seed peel fraction) from the seeds and a sanded seed fraction is also obtained.

Pretreatment of berry material or fruit material

In an embodiment of the invention the berry material or fruit material is pretreated prior to sieving. The pretreatment may be carried by subjecting the berry material or fruit material to methods selected from heat treatment, fermentation, enzymatic treatment, pressing, squeezing, drying, crushing and combinations thereof.

According to one embodiment of the invention the berry material or fruit material is pressed, separated, decanted or centrifuged to separate the juice from solid matter (peels and seeds).

According to one embodiment of the invention the berry material or fruit material is heat treated for the removal of harmful microbes. Suitably the heat treatment is carried out at 80°C for 5 min.

According to one embodiment of the invention the berry material or fruit material is fermented using lactic acid bacteria to modify the phenolic compounds and the carbohydrate components of the seed. Preferably the starter culture is selected from the genera *Lactococcus, Lactobacillus, Pediococcus* and *Oenococcus*.

Figure 2 illustrates an embodiment where the berry material or fruit material is pretreated prior to sieving. Berry material or fruit material 90 is subjected to heat treatment and fermentation 500 whereby fermented berry or fruit material 91 is obtained, which is then subjected to pressing 600, whereby juice fraction 92 is separated from press cake 93. The press cake 93 is subjected to drying 700 to obtain berry material or fruit material 10 having water content not more than 15 wt%, which is subjected to sieving 100, whereby a seed fraction 30 is separated from a skin fraction 20, the seed fraction 30 is subjected to sanding 200 where 2-40 wt% of the seed is removed and a seed coat fraction 50 comprising surface layer of the seeds and a sanded seed fraction 40 comprising the sanded seeds are obtained. The seed coat fraction 50 and an aqueous medium 60 are subjected to mixing 300 to obtain a mixture 70 comprising bioactive compounds with antimicrobial activity. The mixture 70 is subjected to separation 400 to obtain hydrothermal extract 80 and hydrothermal residue 82. The hydrothermal extract 80 is subjected to drying 800 to obtain dry fraction 81 comprising bioactive compounds with antimicrobial activity. The hydrothermal residue may be subjected to drying at mild conditions, such as freeze-drying or drying in a fluid-bed drier at a temperature from 35 to 70°C preferably 35-45°C. (Not shown in the figure)

In the fermentation typically frozen berry or fruit material and water, suitably ultra-pure water, are mixed together (1:1) and heated, suitably at 80°C for 5 min. The mixture is cooled, suitably in an ice bath and if needed berry or fruit material is crushed. The pH of the mixture is adjusted to approx. pH 5.0, suitably with 5 N sodium hydroxide. The microbes are pre-grown in food-grade media. The fermentation is carried out in a bioreactor (a vessel) etc., for example for 3 days at 30°C under constant mixing. Lactic acid bacteria fermentations are purged with sterile nitrogen gas to create anaerobic conditions.

According to one embodiment of the invention the berry material or fruit material is treated with carbohydrate hydrolyzing enzymes. The berry material or fruit material is preferably pressed or squeezed after the enzyme incubation. Suitably the enzyme is selected from cellulose, pectinase, xylanase and combinations thereof. The juice yield is increased and the press cake or berry cake contains decreased amounts of sugars and water.

In the enzyme treatment the enzymes are dosed based on their main activity (e.g. 100-200 nkat/g or 0.01-1 % by weight). Thawed, mashed and heated (40-45 °C) berry or fruit material is incubated at 40-45 °C for 2-4 hours. Enzyme is diluted in water before mixing with the mashed and heated berry and fruit materials. The treatments are carried out at the intrinsic pH of the used material (about pH 3). After enzyme incubations, the berry or fruit juice is extracted by a juice pressing device. Enzyme treatment typically increases the yields of the hydrothermal extract and compounds therein.

According to one embodiment of the invention the berry material or fruit material is dried prior to introducing to the process for removing excess water, until it has water content of not more than 15 wt%, preferably 0.1-10 wt%. The drying may be carried out as convective drying, such as hot-air drying, vacuum drying or steam drying, microwave drying with or without vacuum drying, or freeze-drying. The drying may be carried out using a fluid-bed drier at a temperature from 35 to 70°C preferably 35-45°C. Suitably the freeze drying is carried out at a temperature from -40 to 0°C, and convective drying at a temperature from 40 to 70°C, preferably from 40 to 50°C. Any conventional drying devices suitable for the drying can be used.

According to one embodiment the berry material or fruit material is crushed prior to sieving, suitably using compression crushing to break lumps of skin, peels, pulp etc from the seeds and to cause minimum damage to the seeds. The crusher may be selected from roll crusher, a ball crusher, manual type crusher, a kneader grinder or a combination thereof. A kneader grinder may be used as the crusher, by which the seeds of the berry are detached from the dry pulp and skin portion without breaking the seeds. A suitable grinder is a falling number mill containing a rotating rotor and a stationary stator. This mill provides a kneading and slightly cutting and striking effect, wherein the grinding energy is, however, not sufficient to break the seeds, but they are detached from the matrix. A disc mill or an impact mill with a guided impact is preferably used, whereby a gentler grinding process is achieved.

The sanded seeds comprising the seed core and the seed oil may be used as such in food and animal feed applications as healthy nutritional components. Alternatively the sanded seeds may be treated with an enzyme.

The sanded seed fraction may be further treated with an enzyme selected from cellulase, pectinase, xylanase and combinations thereof to soften the still existing seed coat, preferably combinations are used. As the seed coat is thinner and further softer after the

enzyme treatment, the healthy fatty acids can be delivered more easily from the seeds during consumption and the mouth-feel is also better as the seed coat is softer. These sanded and enzyme treated seeds are particularly useful in various food applications, for example in snacks, cereals, muesli, bakery products, etc, and is animal feeds.

The enzyme treated sanded seeds may also be subjected to extraction of phenolics or seed oil using suitable extraction technique, such as conventional super critical or solvent extraction methods or combinations thereof. The enzyme treatment improves the extraction of fatty acids from the sanded seeds, whereby valuable seed oil may be obtained.

Alternatively the sanded seeds may be milled, followed by optional dry fractionation.

The fiber rich berry skin or fruit skin fraction separated from the seeds may be used as such or it may be further ground finer by a suitable mill, which is preferably a pin crusher. The amount of the skin fraction is typically 2-10 wt% of the berry material or fruit material, calculated on dry basis. As an example the skin fraction of cloudberry contains typically ellagic acid 20 mg/g dry weight. It may be used as an ingredient in cosmetics, food products and animal feeds.

The hydrothermal extracts (aqueous mixtures or dry fractions) comprising bioactive compounds, obtained with process of the invention are rich in phenolic compounds, such as ellagic acid, ellagitannins and their derivatives and other bioactive compounds, and also C18 fatty acids. Said hydrothermal extracts can be used as natural preservatives in cosmetics, hygiene products, nutraceuticals, food products, food supplements, animal feeds, packages and in pharmaceutical products. The hydrothermal extracts comprising bioactive compounds with antimicrobial activity, particularly ellagitannins, obtained with process of the invention have particularly good effect against *Staphylococcus aureus*, as can be seen from the examples, also effect against *Pseudomonas aeruginosa* and *Escherichia coli* was shown.

In skin care and hygiene products the invention could also be utilized to convert berry and fruit materials to fractions that stabilize, balance and protect healthy skin microbiome, as these fractions inhibit the growth of many pathogenic microbes without effecting the growth of beneficial microbes.

The hydrothermal extracts comprising bioactive compounds with antimicrobial activity, obtained with process of the invention are suitably incorporated in food products, in cosmetic products, in hygiene products, in pharmaceutical products, in animal feeds, in packaging materials, particularly in packaging materials of products, such as food which is easily spoiled, and in pharmaceuticals, such as topical products like creams, ointments, etc.

Examples of said easily spoiled food products are poultry products, such as marinades, milk based products, such as yoghurts, drinks, sour cream products, fermented milk based products; berry or fruit containing products, such as jams, beverages, berry soups, conserves, pastes, purees, babyfood; nutritional food products, particularly for special use, such as hospital use and hose administration; grain products, such as bread, cereals, snack products, muesli, precooked porridge, fermented grain based products and gluten-free products.

The hydrothermal residues contain high amounts of ellagic acid (approx. 80 g/kg), C18 fatty acids, fibers and lignin, and thus they provide an excellent additive particularly for nutraceuticals, food products, food supplements and feeds.

The present invention provides several advantages. Particularly the by-products, side streams and waste materials originating from berry and fruit processing industry can be effectively utilized in the simple and economic process of the invention, for obtaining mixtures and dry fractions comprising bioactive compounds, particularly ellagic acid and/or derivatives thereof, obtained with process of the invention, as well as skin fractions and sanded seed fractions, which also find several valuable uses. With the process of the invention practically all the waste and by-product material can be utilized effectively.

Said bioactive mixtures and fractions may be used as effective antioxidants, antimicrobial agents and preservatives, particularly in the field of cosmetics, hygiene products, food products and animal feeds, as well as in packages and in pharmaceutical products.

The process of the invention provides enrichment of phenolic compounds, such as of ellagic acid and ellagitannin and their derivatives, whereby said compounds are concentrated in specific fractions for further use. The specific fractions can be added as such to various products. Dumping of the waste materials to the landfills can be avoided or at least significantly reduced. This is also a clear environmental and ecological benefit. Nutritionally rich and valuable waste materials and by-products from the berry industry and fruit

industry can be utilized in a simple and efficient way in food products and animal feeds, as well as in packages and in pharmaceutical products.

The antimicrobial effect of the hydrothermal extract is dose dependent.

The invention provides improved storage and microbiological safety to the products, as these fractions comprising the bioactive compounds act as preservatives. It is possible to decrease the amount of synthetic preservatives in the products and replace them by these natural compounds. In addition, in cosmetic products these natural compounds also balance skin microbiota, as they effectively inhibit the growth of skin pathogens, such as *Staphylococcus*.

The invention provides improved microbiological preservation, improved inhibition of oxidation reactions and increased antioxidant status to the products where they are incorporated. In general this means improved stability and microbiological safety. The hydrothermal extracts are readily soluble in water, which is an important technological benefit. The hydrothermal extracts contain no or only very limited amounts of free sugars, which improves stability of the products. Thus the hydrothermal extracts are not sticky and easily stored in frozen form. Whole procedure to prepare hydrothermal extracts is food grade, no toxic or harmful reagents or process steps are used. Thus the extracts are particularly suitable also for food purposes.

EXAMPLES

The following examples are illustrative of embodiments of the present invention, as described above, and they are not meant to limit the invention in any way.

Example 1: **Sanding of cloudberry seeds separated from press cake**

A dried pressed cake of cloudberry from a commercial juice pressing process was sieved. The seeds were separated from the skin fraction by using a vibratory sieve shaker with a 1.6 mm screen at settings of 10 minutes sieving time and 1.5 mm amplitude. The seeds were sanded by using an abrasive machine (barley pearling machine) for 15 minutes sanding time. Table 1 shows yields obtained by the different process steps and distribution of fractions obtained by sieving and sanding.

Table 1

Process:	Sample:	Yields:
-	Dried cloudberry press cake	100%
Separation of seeds from press cake by sieving	Sieved seeds	93%
	Skin fraction	7%
Sanding of sieved seeds (sanding time 15 min)	Sanded seeds	69%
	Seed coat powder	11%
	Loss	13%

Example 2: Sanding of cloudberry seeds separated from press cake

A dried pressed cake of cloudberry from a commercial juice pressing process was sieved. The seeds were separated from the skin fraction by using a vibratory sieve shaker with a 1.60 mm screen at settings of 5 minutes sieving time and 1.5 mm amplitude. The seeds were sanded by using an abrasive machine (barley pearling machine) for 15 minutes sanding time. The yields of the sieving and sanding fractions and distribution of fractions obtained by sieving and sanding are shown in Table 2.

Table 2

Process:	Sample:	Yields:
-	Dried cloudberry press cake	100%
Separation of seeds from press cake by sieving	Sieved seeds	96%
	Skin fraction /Dry fraction A	4%
Sanding of sieved seeds (sanding time 15 min)	Sanded seeds	74%
	Seed coat powder/Dry fraction B	18%
	Loss	4%

Example 3: Sanding of raspberry seeds separated from press cake

Frozen raspberries were thawed and crushed with a pestle. The crushed raspberries were warmed up to 45°C and iopectinase Super 8X enzyme was added. The enzyme dosage was 100 nkat/g berries (i.e. 1.98 ml/1 kg berries, active 51000 nkat/ml). After the

incubation time (4 hours), juice pressing was performed by a High Pressure Tincture Press H P5 presser. The amount of press cake was 16% by weight and the dry matter of the press cake was 48% by weight. The press cake was dried by a quick drying machine with an air flow at 45°C to a dry matter of 89%.

Seeds were separated from the dried press cake by using a vibratory sieve shaker with a 0.63 mm screen at settings of 5 minutes sieving time and 1.0 mm amplitude. The seeds were sanded by using an abrasive machine (barley pearling machine) for 15 minutes sanding time. The yields obtained by the different process steps and distribution of fractions obtained by the sieving and the sanding machines are shown in Table 3.

Table 3

Process:	Sample:	Yields:
-	Dried raspberry press cake	100%
Separation of seeds from raspberry press cake by sieving	Sieved seeds	96%
	Skin fraction/Dry fraction A	4%
Sanding trial I:		
Sanding of sieved seeds (sanding time 1 min)	Sanded seeds	73%
	Seed coat powder/Dry fraction B	4%
	Loss	19%
Sanding trial II:		
Sanding of sieved seeds (sanding time 2 min)	Sanded seeds	72%
	Seed coat powder/Dry fraction B	7%
	Loss	17%

Example 4: Sanding of seeds separated from cloudberries

Cloudberries were frozen and freeze-dried. The dried cloudberries (less than 15 wt% of water) were crushed by hand to separate the skin, fruit flesh and seed portion from the whole berries. The seeds were separated from the skin and fruit flesh portion by using a vibratory sieve shaker with a 1.6 mm screen. The cloudberry material was first sieved using the 5 minutes sieving time and 1.0 mm amplitude and after that sieved by using the same sieve settings with ten glass balls. The glass balls assisted to separate the fruits flesh

and skin from the seeds. After that the seeds were sanded using an abrasive machine (barley pearling machine) for 15 and 30 minutes sanding time. The yields obtained by the different process steps and distribution of fractions obtained by sieving and sanding are shown in Table 4.

Table 4

Process:	Sample:	Yields:
-	Freeze-dried cloudberries	100%
Seeds separating from berries by sieving twice (without and with 10 glass balls)	Sieved seeds	48%
	Skin and fruit flesh fraction	52%
Sanding trial I:		
Sanding of sieved seeds (sanding time 15 min)	Sanded seeds	38%
	Seed coat powder/Dry fraction B	4%
	Loss	6%
Sanding trial II:		
Sanding of sieved seeds (sanding time 30 min)	Sanded seeds	39%
	Seed coat powder/Dry fraction B	6%
	Loss	3%

EXAMPLE 5: Fermentation of cloudberries, pressing and sieving

Fermentation

Frozen, ripe cloudberries (*Rubus chamaemorus)* were used as the berry material. The berry material was first heat treated and then inoculated with approximately 10^6 cfu g^{-1} of washed LAB cells. *Pedicoccus pentosaceus* VTT E-072742 from VTT Culture Collection was used as a starter culture in the fermentation of cloudberries (http://culturecollection.vtt.fi/). Prior to fermentations, the strain was refreshed in de Man Rogosa Sharpe broth for 1 day in a 100% carbon dioxide atmosphere which was created using anaerobic jars and Anaerocult C strips. The cells were collected from refreshed cultures by centrifugation and washed once in Ringer's solution. The fermentations were performed in a 6 kg scale in a 15-l capacity bioreactor for 3 days at 30 °C under constant mixing (130 rpm). The bioreactor was purged with sterile-filtered nitrogen gas in order to create anaerobic conditions. The viable counts of lactic acid bacteria and yeasts were

determined before and after the fermentations using plate count technique. The results were expressed as colony-forming units (CFU) per gram of wet weight. The fermented berry mash was stored frozen.

Pressing and drying

After fermentation the berry mash was treated with a hydraulically operated high-pressure tincture press using 5 litres filling material to separate juice and insoluble press cake.

The press cake from juice pressing was dried in a fluid bed dryer using +45°C air flow, until the water content of the berry press cake was below 15 % by weight. After that, the dried berry press cake was dry sieved using different sieve sizes or using a suction apparatus. A skin fraction having average particle less than 1250 µm was separated and seed fraction having average particle size of more than 750 µm was collected.

The mass yields of cloudberry press cake (fermented and non-fermented samples) were 8-10 %. About 5 % of the press cake consisted of peels and pulp, and remaining 95% were seeds.

EXAMPLE 6: Hydrothermal extraction of cloudberry and sea buckthorn seed coat material

Cloudberry and sea buckthorn seed coat materials, obtained with the method as described in example 1, were processed, each as follows:

A.

50 g of the seed coat material was extracted with 1 l of water for 1 hour at 80°C (with 500 rpm mixing). The mixture was filtered using Miracloth® filter material with 22-25 µm pore size. The filtrate was freeze dried.

B.

50 g of the seed coat material was mixed with 1 l of water and 0.1 wt% of ascorbic acid agitated for 1 hour at 80°C (with 500rpm mixing). The mixture was filtered using Miracloth® filter material with 22-25 µm pore size. The filtrate was freeze dried. The extraction was carried out in acidic conditions in order to stabilize anthocyanins.

The antimicrobial activity of the cloudberry and sea buckthorn freeze dried materials was tested against selected microbes including *Staphylococcus aureus* and *Pseudomonas aeruginosa*.

Antimicrobial activities were measured in liquid cultures. Freeze-dried materials of 1 – 7.5 mg ml^{-1} were suspended into microbial cultures. Microbial culture without berry material was used as positive control, and culture with antibiotic (chloramphenicol for bacteria or hygromycin B for *C. albicans*) was used for negative growth control. The microbial cultures were incubated in their optimal growth conditions, and growth by cell counts was followed by plate count during 24 h of cultivation.

The results show clear bactericidal activity of the cloudberry and sea buckthorn fractions against *Staphylococcus aureus* compared to positive control culture with no antimicrobial agents. Cloudberry freeze dried material prepared by hydrothermal extraction (1 hour extraction in 80°C water) was the best inhibitor. Moderate growth inhibition was detected with *Pseudomonas aeruginosa* and *Escherichia coli* too.

Figure 3A shows the antimicrobial effect of hydrothermal extracts and water extracts without heating of cloudberry seed coat fractions against *Staphylococcus aureus* in liquid culture during cultivation for 24 hours. Culture with antibiotic chloramphenicol was used as negative control. Two different batches of sanded cloudberry seed coat powder were compared (A and B). Effect of extract was tested in two concentrations in the culture: 1 mg/ml and 5 mg/ml.

Figure 3B shows antimicrobial effect of hydrothermal extracts of cloudberry seed coat fraction (B) against *Staphylococcus aureus*. Concentration of extract tested was 5 mg/ml microbial culture. Abbreviations:

- cloudberry seed coat/ cold = extraction 1 h room temperature, freeze-drying
- cloudberry seed coat = extraction 1h, 80°C, freeze-drying
- cloudberry seed coat/ +enzyme = 2 h 45°C + enzymes followed by extraction 1h 80°C, freeze-drying
- cloudberry seed coat/ no enzyme = 2 h 45°C, followed by extraction 1h, 80°C, freeze-drying
- cloudberry seed coat/ oven = extraction 1h 80°C, oven drying (85°C)

Figure 3C shows antimicrobial effect of hydrothermal extracts of sea buckthorn seed coat fraction against *Staphylococcus aureus*. Concentrations of extracts tested were 2.5 mg/ml, 5 mg/ml and 7.5 mg/ml microbial culture.

Figure 3D shows antimicrobial activity of hydrothermal extracts of cloudberry seed coat fraction (B) against *Pseudomonas aeruginosa*. Concentration of extracts tested were 5 mg/ml and 7.5 mg/ml microbial culture. Abbreviations:

- cloudberry seed coat/ cold = extraction 1 h room temperature, freeze-drying
- cloudberry seed coat = extraction 1h, 80°C, freeze-drying
- cloudberry seed coat/ +enzyme = 2 h 45°C + enzymes followed by extraction 1h 80°C, freeze-drying
- cloudberry seed coat/ no enzyme = 2 h 45°C, followed by extraction 1h, 80°C, freeze-drying
- cloudberry seed coat/ oven = extraction 1h 80°C, oven drying (85°C)

Figure 3E shows antimicrobial activity of hydrothermal extracts of cloudberry seed coat fraction B and buckthorn seed materials against *Escherichia coli.* Concentrations of extracts tested were 5.0 mg/ml microbial culture.

Abbreviations:

- cloudberry seed coat/ cold = extraction 1 h room temperature, freeze-drying
- cloudberry seed coat = extraction 1h, 80°C, freeze-drying
- cloudberry seed coat/ +enzyme = 2 h 45°C + enzymes followed by extraction 1h 80°C, freeze-drying
- cloudberry seed coat/ no enzyme = 2 h 45°C, followed by extraction 1h, 80°C, freeze-drying
- cloudberry seed coat/ oven = extraction 1h 80°C, oven drying (85°C)

EXAMPLE 7: Utilization of hydrothermal extraction residue of cloudberry as main ingredient in cosmetic pearling cream

Hydrothermal extraction was carried out for cloudberry seeds as in Example 6. The remaining extraction residue was frozen and stored in freezer. Before further processing, the extraction residue was thawn and glycerol and water was added to give a thick slurry (1,24 kg extraction residue + 530 g water + 230 g glycerol w/w). The slurry was subjected to wet grinding in a Masuko Supermasscolloider stone mill. After one grinding pass the paste still contained some pieces. 760 g of water was added to give the final dry weight content of 10 % and the paste was exposed to another grinding pass.

- The grinding was performed at 1500 rpm. The grinding stone was a modified stone type MKGA10-80 made of aluminum oxide and resins with a diameter of 10". The quality of the material was controlled by moving the lower stone to set the clearance

between the grinding stones. In the first pass the clearance was set to 0.25mm and in the second pass it was 0.22mm. The average operating power during the second pass was 2.4kW.

- The resulting smoot paste was used as such as cosmetic skin pearling cream. The extraction residue is rich in fibre, and the pearling effect is related to fibre particles, which act as natural pearling particles in the cream. Fresh cloudberry flavor, velvety consistency and golden yellow color were typical for the cream.

Sensory properties of the cream were evaluated in SLUSH 2017 event in Helsinki in small-scale consumer research. Eleven volunteers tested the cream and answered the following questionnaire:

1. Do you use cosmetic products containing natural ingredients?

- *Never*
- *Seldom*
- *Sometimes*
- *Always*

2. You just tested cream containing cloudberry seed material. What is your message to us?

3. Is color/flavor an important factor when you choose cosmetic products?

4. Do you prefer natural or synthetic preservatives, antioxidant and colorants in cosmetic products?

5. Is Nordic or Finnish raw-material important for you in cosmetic products?

Answers of this study are in Table 5 in Figure 4 as consumer feed-back to cloudberry pearling cream. They showed that consistency, fragrance/smell and touch with skin appealed the consumer. In addition, natural ingredients, preservatives and colorants and Nordic raw-materials were highly valued by the consumers.

Phenolic profiling and fatty acid analyses were also carried out for the extraction residue as such after air-drying and also after using enzymes in the extraction process. Figure 5 shows HPLC profiling and fatty acid content of hydrothermal extraction residue of cloudberry seed coat powder. The results showed that extraction residue is very rich in beneficial ellagic acid and it also contained healthy fatty acids. These findings support its use as excellent ingredient for cosmetic applications, and also its potential as healthy food ingredient.

While the invention has been described with respect to specific examples including presently preferred modes of carrying out the invention, those skilled in the art will appreciate that there are numerous variations and permutations of the above described embodiments that fall within the spirit and scope of the invention. It should be understood that the invention is not limited in its application to the details of construction and arrangements of the components set forth herein. Variations and modifications of the foregoing are within the scope of the present invention.

CLAIMS

1. A process for converting berry and fruit materials to fractions comprising bioactive compounds, **characterized** in that the process comprises the steps, where
in the first step at least one berry material or fruit material selected from berries, fruits, by-products, side streams and waste materials originating from berries or fruits selected from the genus *Rubus, Sorbus, Rosa, Empetrum, Aronia* and *Hippophae* and from combinations thereof, and the fruit are selected from the genus *Vitis, Punica, Pyrus, Malus, Citrullus, Benincasa, Cucumis, Momordica, Olea* and from *species Argania spinosa*, and combinations thereof, said berry material or fruit material having water content not more than 15 wt%, is sieved, whereby a seed fraction is separated from a skin fraction,
in the second step the seed fraction is subjected to sanding where 2-40 wt% of the seed is removed and a seed coat fraction comprising surface layer of the seeds and a sanded seed fraction comprising the sanded seeds are obtained,
in the third step the seed coat fraction is mixed with an aqueous medium in a ratio from 1:3 to 1:80 by weight, at a temperature from 15 to 95°C for 20 min to 10 hours to obtain a mixture comprising bioactive compounds, and the mixture is subjected to separation whereby a hydrothermal extract is separated from a hydrothermal residue.

2. The process according to claim 1, **characterized** in that the bioactive compounds comprise ellagic acid and/or derivatives thereof, preferably ellagic acid and/or ellagitannins.

3. The process according to claim 1 or 2, **characterized** in that the hydrothermal extract is dried.

4. The process according to any one of claims 1-3, **characterized** in that in the third step the ratio of the seed coat fraction to the aqueous medium is from 1:5 to 1:50, respectively.

5. The process according to any one of claims 1-4, **characterized** in that the aqueous medium is selected from water or a mixture of water with ascorbic acid.

6. The process according to any one of claims 1-5 **characterized** in that in the third step at least one enzyme selected from carbohydrate hydrolyzing enzymes, suitably from cellulose, pectinase, xylanase and combinations thereof is added to the aqueous medium.

7. The process according to any one of claims 1-6, **characterized** in that the berry material or fruit material is pretreated prior to sieving and the pretreatment may be carried by subjecting the berry material or fruit material to a method selected from heat treatment, fermentation, enzymatic treatment, pressing, squeezing, drying, crushing and combinations thereof.

8. The process according to any one of claims 1-7, **characterized** in that the berry material or fruit material is fermented using lactic acid bacteria, preferably said lactic acid bacteria is selected from the genera *Lactococcus, Lactobacillus, Pediococcus* and *Oenococcus.*

9. The process according to any one of claims 1-8, **characterized** in that the berry material or fruit material is treated with carbohydrate hydrolyzing enzymes, preferably said enzymes are selected from cellulose, pectinase, xylanase and combinations thereof.

10. The process according to any one of claims 1 - 9, **characterized** in that the sieving is carried out using a sieving device, air classification device, air jet sieve device, screening device, rotary screen or screening device, preferably a vibratory sieving device.

11. The process according to any one of claims 1 - 10, **characterized** in that the sanding is carried out using a sanding or polishing apparatus selected from grain polishing machines from grain polishing machines, rice polishing machines, pearling machines, dehullers and polishers.

12. The process according to any one of claims 1 - 11, **characterized** in that the berry material or fruit material is dried until it has water content of 0.1-10 wt%, preferably 0.1-8 wt%.

13. The process according to any one of claims 1 - 12, **characterized** in that the berry material or fruit material is crushed prior to sieving, preferably using a crusher selected from roll crusher, a ball crusher, manual type crusher, a kneader grinder or a combination thereof.

14. Use of the hydrothermal extract or the hydrothermal residue obtained with the process of any one of claims 1-12 in in cosmetics, hygiene products, nutraceuticals, food products, food supplements, animal feeds, packages and pharmaceutical products.

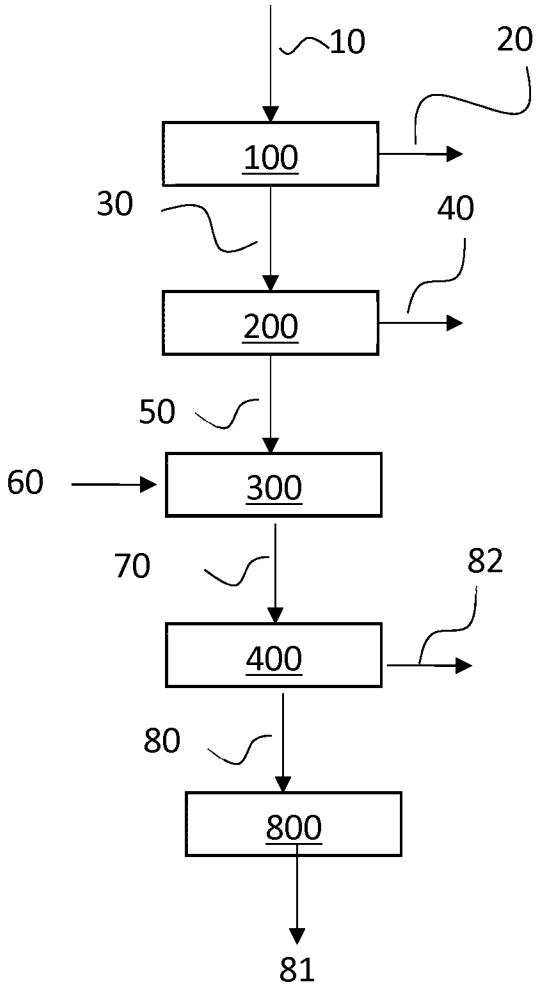
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Fig. 1

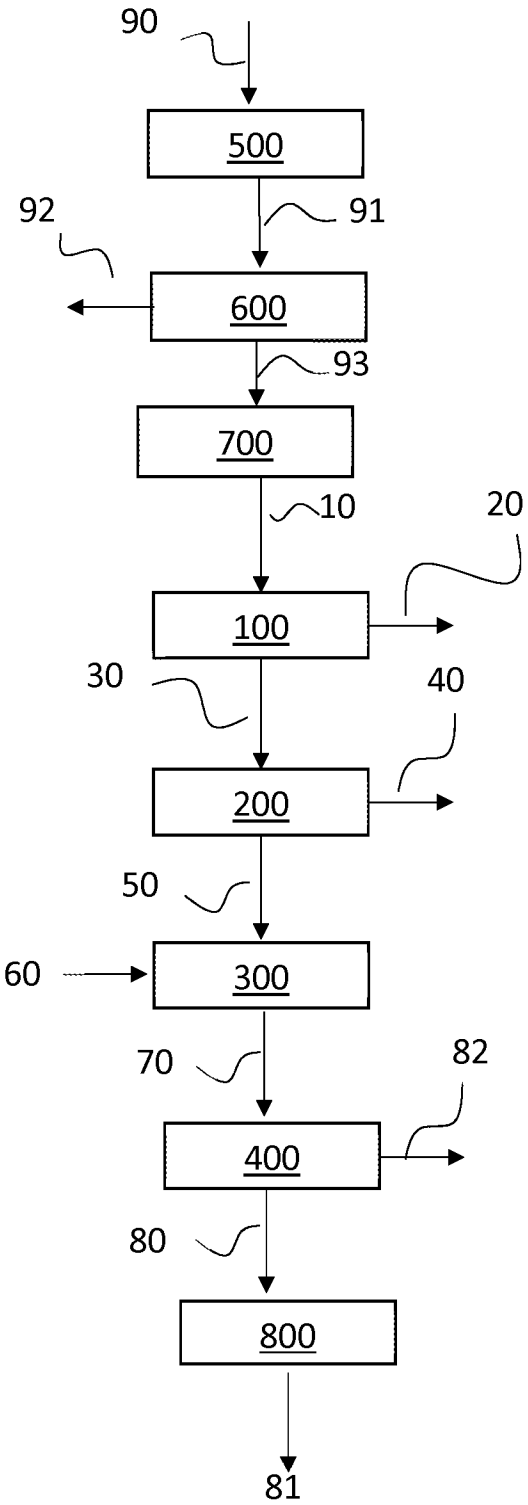
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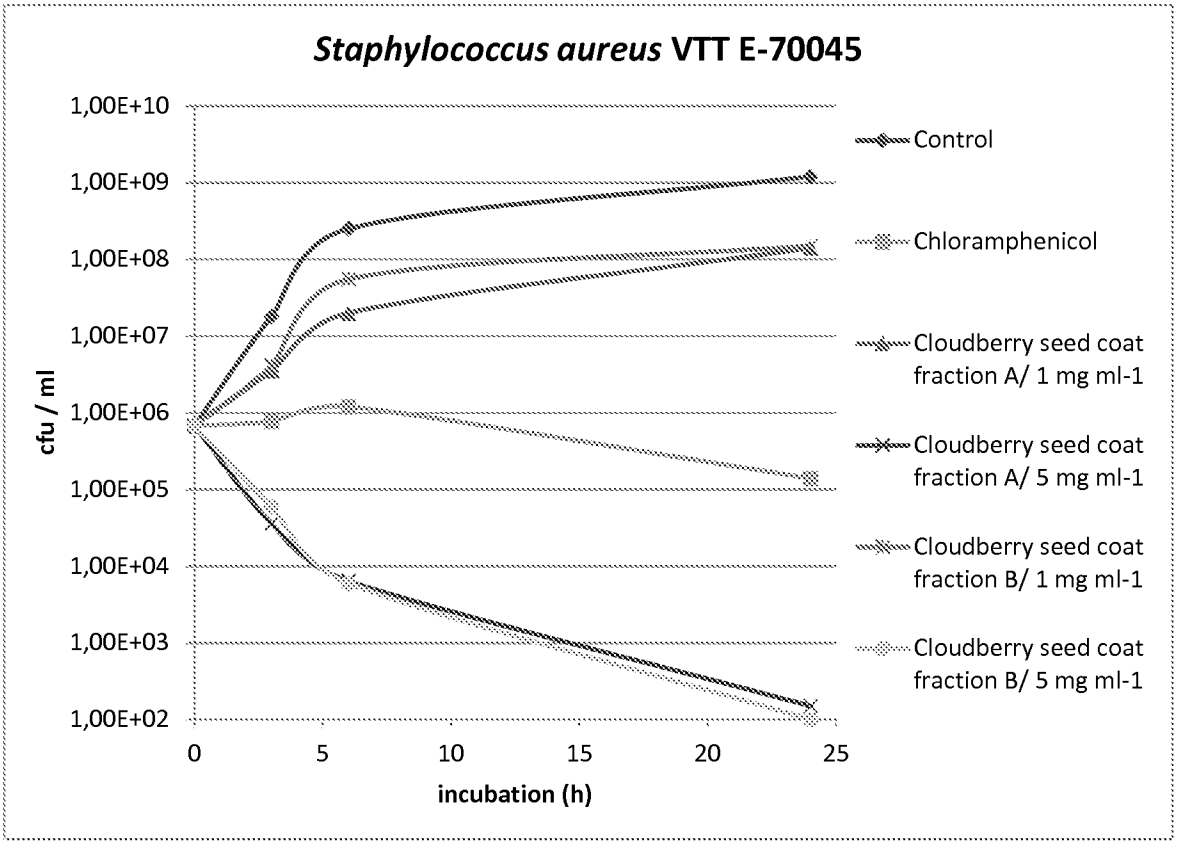
Fig. 2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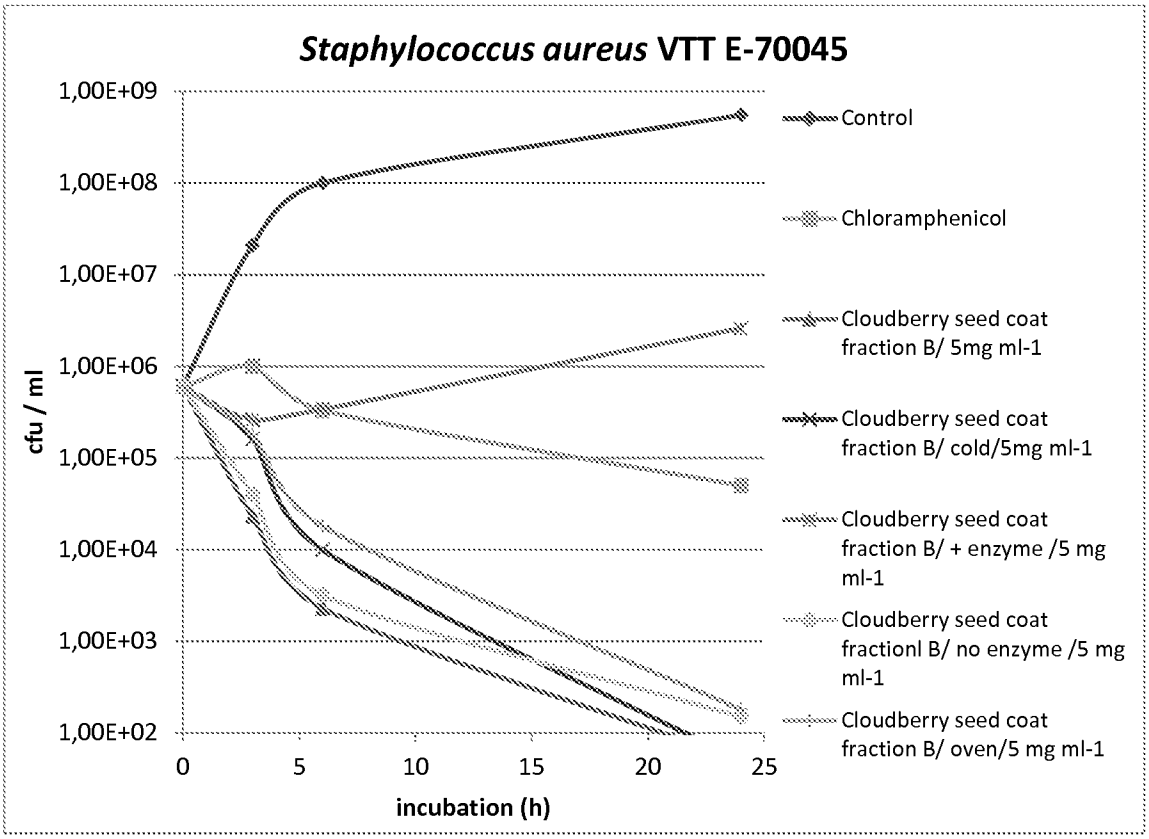
1,00E+07
1,00E+06
1,00E+05
1,00E+04
1,00E+03
1,00E+02
cfu / ml
0
5
10
15
20
25
incubation (h)
Control
Chloramphenicol
Cloudberry seed coat fraction A/ 1 mg ml-1
Cloudberry seed coat fraction A/ 5 mg ml-1
Cloudberry seed coat fraction B/ 1 mg ml-1
Cloudberry seed coat fraction B/ 5 mg ml-1

Fig 3A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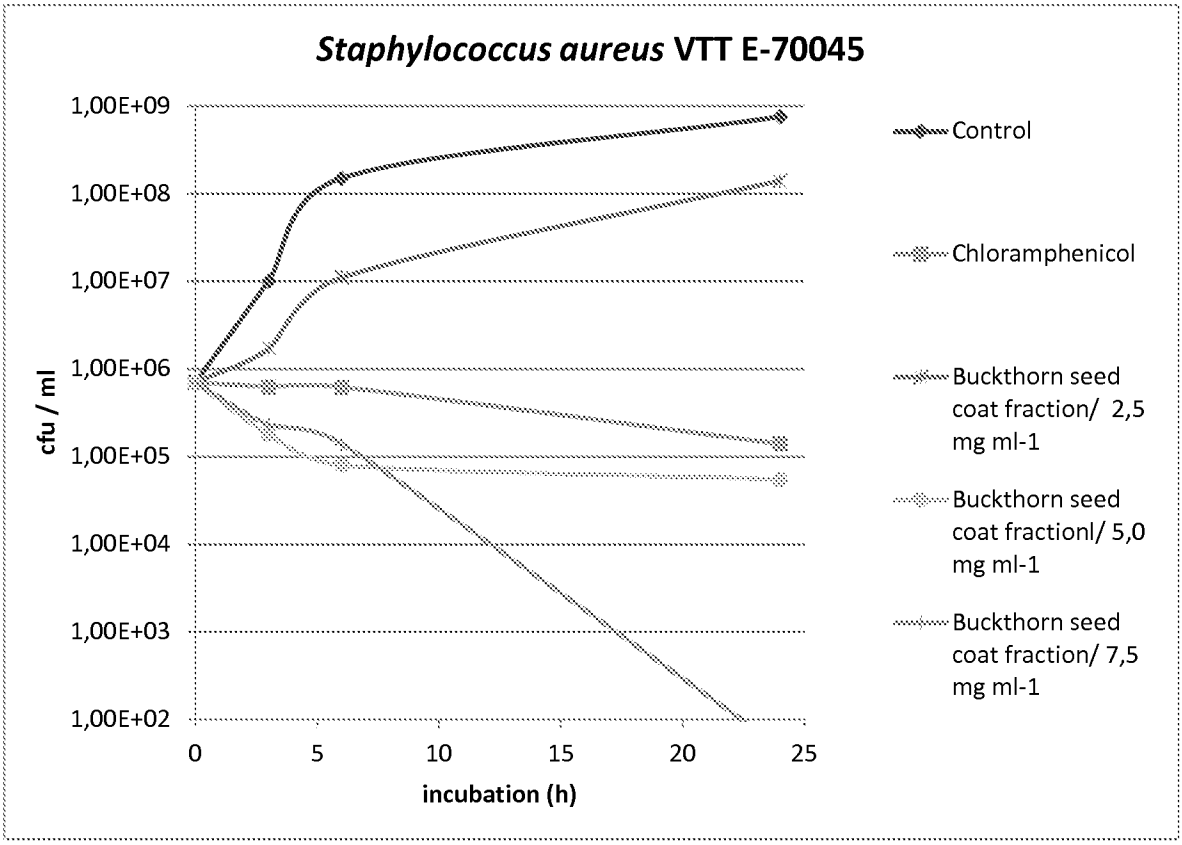
1,00E+05
1,00E+04
1,00E+03
1,00E+02
cfu / ml
0
5
10
15
20
25
incubation (h)
Control
Chloramphenicol
Cloudberry seed coat fraction B/ 5mg ml-1
Cloudberry seed coat fraction B/ cold/5mg ml-1
Cloudberry seed coat fraction B/ + enzyme /5 mg ml-1
Cloudberry seed coat fractionl B/ no enzyme /5 mg ml-1
Cloudberry seed coat fraction B/ oven/5 mg ml-1

Fig 3B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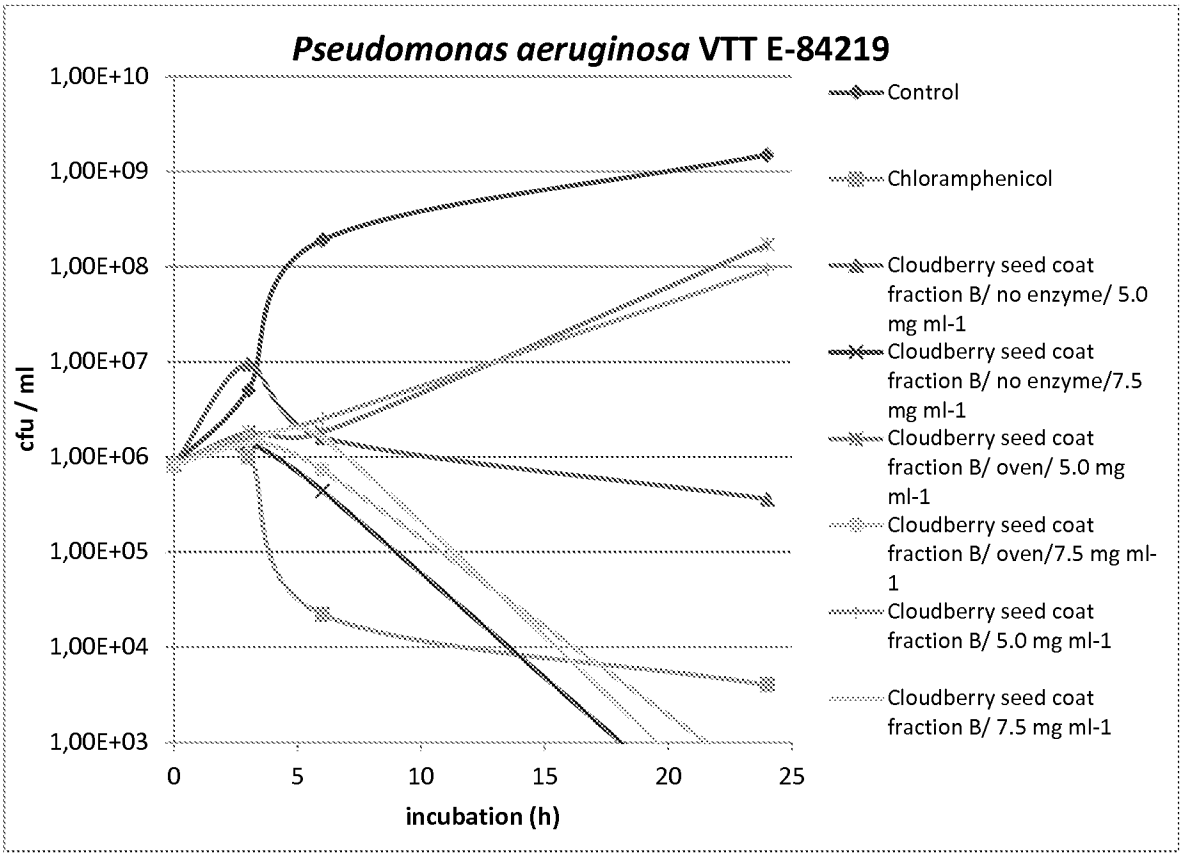
1,00E+07
1,00E+06
1,00E+05
1,00E+04
1,00E+03
1,00E+02
cfu / ml
0
5
10
15
20
25
incubation (h)
Control
Chloramphenicol
Buckthorn seed coat fraction/ 2,5 mg ml-1
Buckthorn seed coat fractionl/ 5,0 mg ml-1
Buckthorn seed coat fraction/ 7,5 mg ml-1

Fig 3C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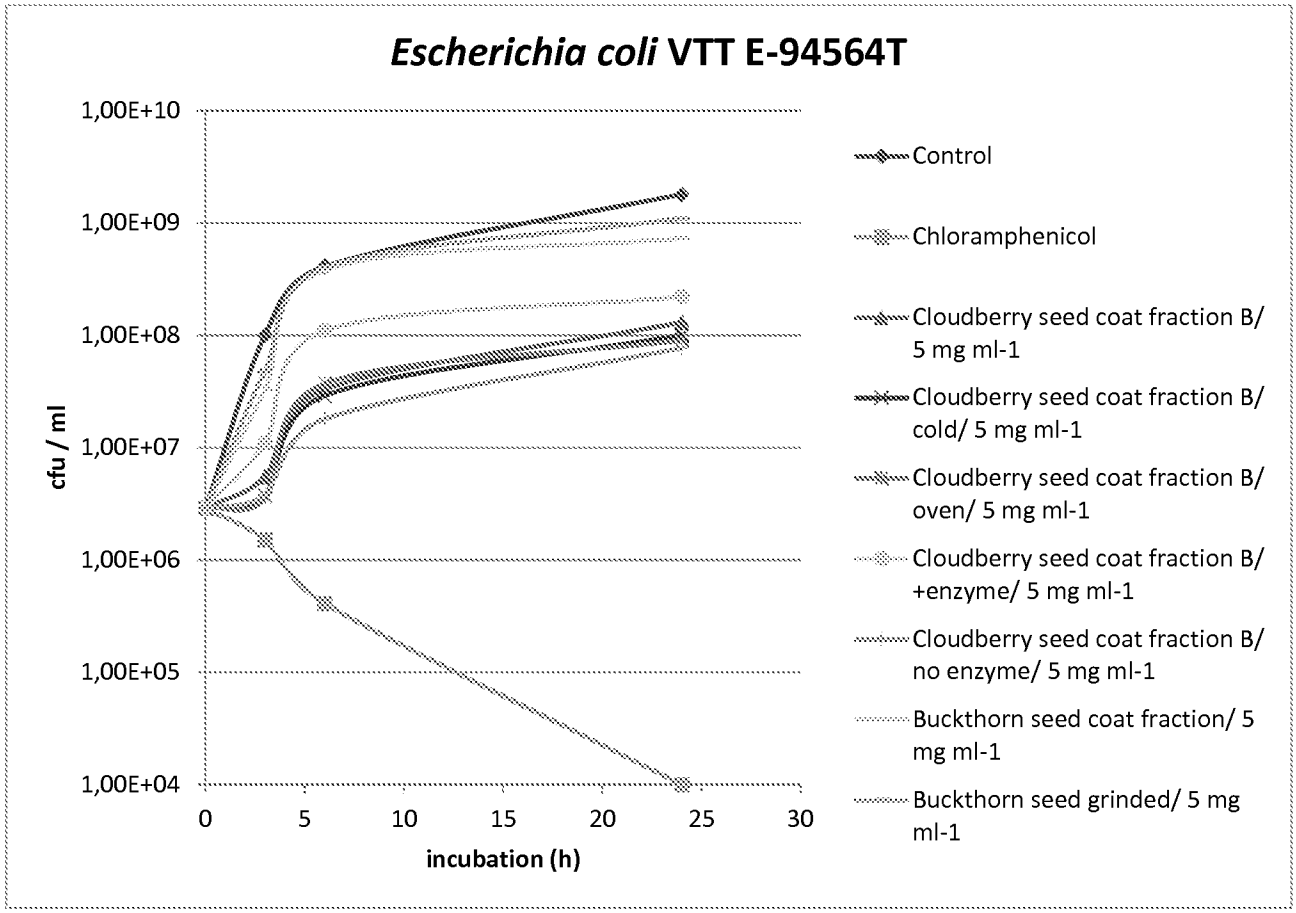
1,00E+07
1,00E+06
1,00E+05
1,00E+04
1,00E+03
cfu / ml
0
5
10
15
20
25
incubation (h)
Control
Chloramphenicol
Cloudberry seed coat fraction B/ no enzyme/ 5.0 mg ml-1
Cloudberry seed coat fraction B/ no enzyme/7.5 mg ml-1
Cloudberry seed coat fraction B/ oven/ 5.0 mg ml-1
Cloudberry seed coat fraction B/ oven/7.5 mg ml-1
Cloudberry seed coat fraction B/ 5.0 mg ml-1
Cloudberry seed coat fraction B/ 7.5 mg ml-1

Fig 3D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1,00E+08
1,00E+07
1,00E+06
1,00E+05
1,00E+04
cfu / ml
0
5
10
15
20
25
30
incubation (h)
Control
Chloramphenicol
Cloudberry seed coat fraction B/ 5 mg ml-1
Cloudberry seed coat fraction B/ cold/ 5 mg ml-1
Cloudberry seed coat fraction B/ oven/ 5 mg ml-1
Cloudberry seed coat fraction B/ +enzyme/ 5 mg ml-1
Cloudberry seed coat fraction B/ no enzyme/ 5 mg ml-1
Buckthorn seed coat fraction/ 5 mg ml-1
Buckthorn seed grinded/ 5 mg ml-1

Fig 3E

Do you use cosmetic products containing natural ingredients?	You just tested VTT cream containing cloudberry seed material. What is your message to us?	Is colour/flavour important factor when you choose cosmetic products?	Do you prefer natural or synthetic preservatives, antioxidant and colourants in cosmetic products?	Is Nordic or Finnish raw material important for you in cosmetic products?
Sometimes	It's great, but how long does it last?	The smell is important	No but preservation time is important	Yes!
Sometimes	Interesting. Why is VTT doing this, not Lumene?	Yes.	No opinion	Yes
Sometimes	Great work - go further on developing	Yes!	Yes	Yes
Sometimes	Nice texture, smell ok, could be better smell	Smell is more important. Natural looking is better than full of synthetic colour.	Natural if available and ~ same prize	Yes!
Sometimes	I like it	Colour: Yes. Flavour: no	Natural	Yes
Sometimes	I like it, but would make it little bit less liquid.	Colour and smell are important.	Natural	Yes
Seldom	It's very fresh, remain cloudberry smell. Makes me feel it is good.	Function, body effect	80% in natural, but synthetic is also ok.	Definately Finish! Their own products!
Sometimes	Consistency is great, colour is not the most appealing :)	Colour is!	Yes	Definitely
Sometimes	Smooth skin feeling, nice aroma	No	Natural cosmetics	
Seldom	Nice and veelvety feeling. It aggregated in unpleasant way when removing.	Yes	Yes	Yes
Sometimes	Smells nice and light on the skin :) Leaves the skin hydrated.		Colour yes but flavor not	Natural (when available) but effectiveness is most important to me.

Fig. 4

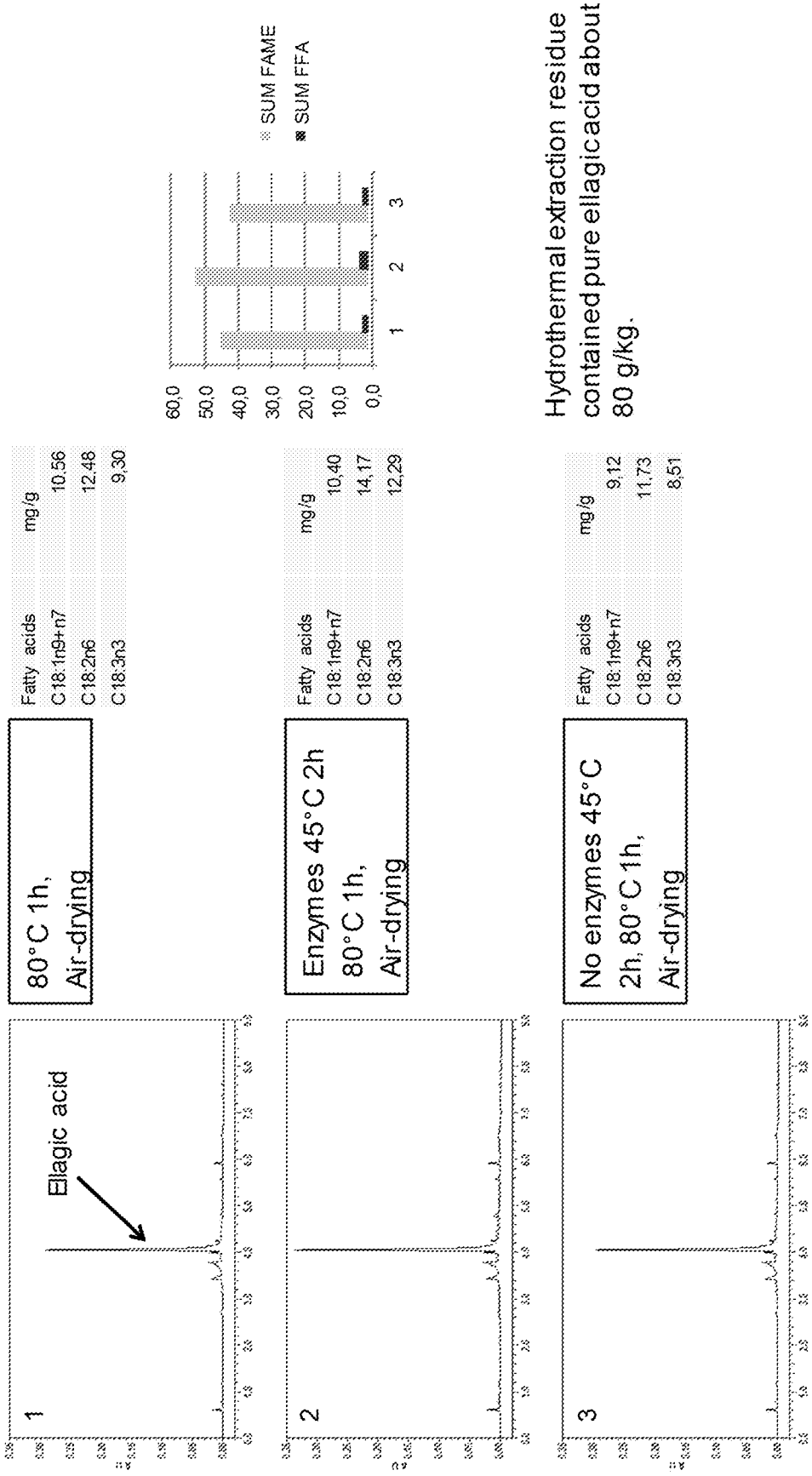
1
Ellagic acid
80°C 1h,
Air-drying
Fatty acids mg/g
C18:1n9+n7 10,56
C18:2n6 12,48
C18:3n3 9,30
2
Enzymes 45°C 2h
80°C 1h,
Air-drying
Fatty acids mg/g
C18:1n9+n7 10,40
C18:2n6 14,17
C18:3n3 12,29
3
No enzymes 45°C
2h, 80°C 1h,
Air-drying
Fatty acids mg/g
C18:1n9+n7 9,12
C18:2n6 11,73
C18:3n3 8,51
60,0
50,0
40,0
30,0
20,0
10,0
0,0
1
2
3
SUM FAME
SUM FFA
Hydrothermal extraction residue contained pure ellagic acid about 80 g/kg.

Fig. 5

INTERNATIONAL SEARCH REPORT

International application No.
PCT/FI2017/050450

A. **CLASSIFICATION OF SUBJECT MATTER**

See extra sheet

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

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)

IPC: A61K, A23L, C12P

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

FI, SE, NO, DK

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

EPODOC, EPO-Internal full-text databases, Full-text translation databases from Asian languages, WPIAP, PRH-Internal, BIOSIS, COMPDX, EMBASE, MEDLINE, XPESP, XPOAC, XPRD

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	WO 2007026101 A1 (COOPERATIVE AGRICOLE D ELLE ET [FR]) 08 March 2007 (08.03.2007)	14
A	abstract; page 6, line 20 – page 7, line 11; page 10, lines 4-12; page 14, line 12 – page 15, line 16; page 17, line 27 – page 19, line 4; page 20, line 34 – page 21, line 21; page 27, lines 23-25; page 44, lines 1-28; page 47, lines 20-25; pages 66-68, examples 3-5, 7; pages 70-71, example 10	1-13
A	US 2013040005 A1 (HIRAYAMA MASAO [JP] et al.) 14 February 2013 (14.02.2013) paragraphs [0003], [0033]-[0041], [0078], [0082], [0083], [0086]	1-14

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

* Special categories of cited documents:

"A" document defining the general state of the art which is not considered to be of particular relevance

"E" earlier application or patent but published on or after the international filing date

"L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)

"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

"T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention

"X" document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone

"Y" document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art

"&" document member of the same patent family

Date of the actual completion of the international search	Date of mailing of the international search report
18 September 2017 (18.09.2017)	20 September 2017 (20.09.2017)

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INTERNATIONAL SEARCH REPORT

International application No.
PCT/FI2017/050450

C (Continuation). DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
A	Puupponen-Pimiä, R. et al. Fermentation and dry fractionation increase bioactivity of cloudberry (Rubus chamaemorus). Food Chemistry, April 2016, Vol. 197, Part A, p. 950-958 abstract; page 951, Section 2. "Materials and methods", subsections 2.1.-2.3.; pages 951–952, subsection 2.5.; page 952, Figure 1; pages 956-967, Section 4. "Conclusion"	1-14
A	Xu, C. et al. Extraction, distribution and characterization of phenolic compounds and oil in grapeseeds. Food Chemistry, October 2010, Vol. 122, No. 3, p. 688-694 the whole document	1-14
A	Arimboor R. et al. Simultaneous estimation of phenolic acids in sea buckthorn (Hippophae rhamnoides) using RP-HPLC with DAD. Journal of Pharmaceutical and Biomedical Analysis, 2008, Vol. 47, pages 31-38 abstract; page 32, section 2. Materials and methods, subsection 2.2. Optimization of extraction; pages 32-33, subsection 2.3. Preparation of crude phenolic extracts; pages 33 and 35, section 3. Results and discussion, subsection 3.1 Optimization of phenolic extraction; pages 37-38, section 3.4.2. Seeds	1-14
A	Guerrero, M. et al. Extraction of polyphenols from white distilled grape pomace: optimization and modelling. Bioresource Technology. 2008, Vol. 99, p. 1311-1318 abstract; page 1312, section 2.1. "Raw material"; page 1314, Table 1; pages 1314 - 1315, Section 3.3. "Aqueous extraction"	1-14
A	Su-Jung K. et al. Antioxidant activity of a hydrothermal extract from watermelons. LWT - Food Science and Technology, 2014, Vol. 59, pages 361-368 abstract; page 362, section 2.2. Preparation of hydrothermal extracts from watermelons	1-14
P, X	WO 2016097488 A1 (TEKNOLOGIAN TUTKIMUSKESKUS VTT OY [FI]) 23 June 2016 (23.06.2016) the whole document	1-14

INTERNATIONAL SEARCH REPORT
Information on Patent Family Members

International application No.
PCT/FI2017/050450

Patent document cited in search report	Publication date	Patent family members(s)	Publication date
WO 2007026101 A1	08/03/2007	EP 1945046 A1	23/07/2008
		FR 2889911 A1	02/03/2007
		FR 2889955 A1	02/03/2007
		FR 2889955 B1	19/12/2008
US 2013040005 A1	14/02/2013	US 8859022 B2	14/10/2014
		CA 2755715 A1	10/02/2013
		JP 2013035810 A	21/02/2013
		JP 6000521 B2	28/09/2016
		NZ 595951 A	30/08/2013
WO 2016097488 A1	23/06/2016	FI 20146128 A	20/06/2016
		FI 126681 B	31/03/2017

INTERNATIONAL SEARCH REPORT

International application No.
PCT/FI2017/050450

CLASSIFICATION OF SUBJECT MATTER

IPC
A61K 8/97 (2017.01)
A23L 19/00 (2016.01)
C12P 1/04 (2006.01)
A61K 36/00 (2006.01)
A61K 31/00 (2006.01)
A23L 3/3472 (2006.01)
C07D 311/00 (2006.01)