METHOD AND APPARATUS FOR PROCESSING A PRODUCT

A method of processing a product by subjecting the product to elevated hydrostatic pressure and simultaneously contacting the product with carbon dioxide, and apparatus for use in the method.

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METHOD AND APPARATUS FOR PROCESSING A PRODUCT

FIELD OF THE DISCLOSURE

[0001] Disclosed is a method of processing a product such as a food product or a pharmaceutical product using a combination of elevated hydrostatic pressure and carbon dioxide, including liquid, near critical or supercritical (dense phase) carbon dioxide, and to apparatus for use in the method.

BACKGROUND

[0002] Enzymes and microorganisms in foods cause quality deterioration and spoilage during storage and distribution. In the food industry, non-thermal processing alternatives have been developed in response to an increasing consumer demand for fresh-like and high quality food products. These technologies aim to economically produce safe, nutritious, and tasty foods using less severe processing conditions.

[0003] The application of high hydrostatic pressure (HHP) (also known as ultra high pressure (UHP) processing or high pressure processing (HPP)) allows the inactivation of undesirable microorganisms and enzymes in liquid and solid foods, and related products such as supplements and pharmaceuticals, without altering their quality to the same extent as thermal treatments and with a comparable preservation effect. Use of HHP in the food industry for the inactivation of undesirable microorganisms is reported by, for example, Daryaei and Balasubramaniam, 2012 (see H. Daryaei, M. Balasubramaniam, Microbial decontamination of food by high pressure processing, in: A. Demirci, M.O. Ngadi (Eds.), Microbial Decontamination in the Food Industry, Woodhead Publishing, Cambridge, UK, 2012, pp.370–406).

[0004] However, undesirable enzymes, such as PPO (polyphenol oxidase) and some isoenzymes of PME (pectin methylesterase), are reported to be highly pressure resistant (see M.J. Eisenmenger, J.I. Reyes-De-Corcuera, High pressure enhancement of enzymes: a review, Enzyme and Microbial Technology 45 (2009) 331–347).

[0005] It is an object of the invention to provide an improved or at least alternative method and apparatus for processing products, particularly food products including beverages.

SUMMARY

[0006] In broad terms, disclosed in one aspect is a method of processing a product, the method comprising, consisting essentially of, or consisting of, subjecting one or more
products to elevated hydrostatic pressure and simultaneously contacting the one or more products with carbon dioxide. In various embodiments the carbon dioxide may be liquid, near-critical or supercritical carbon dioxide. In various embodiments the one or more products may be a liquid, and sufficient carbon dioxide may be provided to substantially saturate the liquid with carbon dioxide at the elevated hydrostatic pressure.

[0007] In various embodiments, the one or more products may be enclosed in a sealed container comprising

a flexible portion comprising an internal volume, the one or more products being in the internal volume, and

a removable reservoir of variable volume that is fluidly communicable with the internal volume of the flexible portion, the volume of the removable reservoir being variable on the application of hydrostatic pressure to the exterior of the removable reservoir, the reservoir comprising at least about 0.1% w/w carbon dioxide relative to the weight of the one or more products.

[0008] In another aspect, disclosed is a method of processing a product, the method comprising

providing a sealed container comprising

a flexible portion comprising an internal volume, with one or more products in the internal volume, and

a removable reservoir of variable volume that is fluidly communicable with the internal volume of the flexible portion, the volume of the removable reservoir being variable on the application of hydrostatic pressure to the exterior of the removable reservoir, the reservoir comprising at least about 0.1% w/w carbon dioxide relative to the weight of the one or more products,

subjecting the sealed container to elevated hydrostatic pressure, the pressure varying the volume of the removable reservoir and transferring at least a portion of the carbon dioxide from the reservoir into the internal volume.

[0009] In another aspect, disclosed is a method of processing a product, the method comprising

placing one or more products in a container, the container comprising

a flexible portion comprising an internal volume, the internal volume enclosing the one or more products, and
a removable reservoir of variable volume that is fluidly communicable with
the internal volume of the flexible portion, the volume of the reservoir being
variable on the application of hydrostatic pressure to the exterior of the reservoir,
the reservoir comprising at least about 0.1% w/w carbon dioxide relative to the
weight of the product or products,

subjecting the container to elevated hydrostatic pressure, the pressure varying
the volume of the reservoir and transferring at least a portion of the carbon dioxide from
the reservoir into the internal volume.

[0010] In another aspect, disclosed is apparatus for use, or when used, in a
disclosed method comprising

a fitting that is removably attachable to a flexible container, the flexible
container comprising an internal volume, and

a reservoir of variable volume that is fluidly communicable with the
internal volume of the flexible container, the volume of the reservoir being
variable on the application of hydrostatic pressure to the exterior of the
removable reservoir.

[0011] In another aspect, disclosed is apparatus for use, or when used, in a
disclosed method comprising

a flexible portion comprising an internal volume, and

a removable reservoir of variable volume that is fluidly communicable with
the internal volume of the flexible portion, the volume of the removable reservoir
being variable on the application of hydrostatic pressure to the exterior of the
removable reservoir.

[0012] In another aspect, disclosed are one or more products or a container
processed according to a method of the invention.

[0013] Any of the following embodiments may relate to any of the above aspects, in
any combination.

[0014] In various embodiments, the carbon dioxide in the reservoir may be a gas or
a liquid. In various embodiments, the step of subjecting the container to elevated
hydrostatic pressure may convert the carbon dioxide from a gas to a liquid, a near-
critical liquid, or a supercritical fluid. In various embodiments, the step of subjecting the
container to elevated hydrostatic pressure may convert the carbon dioxide from a liquid
to a near-critical liquid or a supercritical fluid. It should be understood that the pressure
treatment may result in temperature fluctuations in the product and conversion to a supercritical fluid will be in part due to an increase in temperature due to the increase in hydrostatic pressure.

[0015] In various embodiments the one or more products may be a liquid, and sufficient carbon dioxide may be provided to substantially saturate the liquid with carbon dioxide at the elevated hydrostatic pressure.

[0016] In various embodiments, depending on the nature of the one or more products, the method may result in an increase in keeping quality of the one or more products, a decrease in the viability of plant material, a decrease in the viability and/or pathogenicity of one or more unwanted microorganisms (as measured by aerobic plate count, for example), a decrease in the activity and/or pathogenicity of one or more viruses, a decrease in the activity of one or more enzymes (as determined by a suitable assay, as discussed below), or any combination of any two or more thereof.

[0017] In various embodiments, the one or more products may substantially fill the internal volume.

[0018] In various embodiments, the ratio of volume of the headspace (the ullage) in the flexible container to the volume of one or more products (i.e. the ratio of the volume of headspace:product) may be at least about 1:10, 1:11, 1:12, 1:13, 1:14, 1:15, 1:16, 1:17, 1:18, 1:19, or 1:20, or more, and useful ranges may be selected between any of these values (for example about 1:10 to about 1:20 headspace:product).

[0019] In various embodiments, the one or more products may comprise a liquid product, a semi-liquid product, one or more solid products, or any combination of any two or more thereof.

[0020] In various embodiments, the one or more products as described above may comprise any product requiring such treatment, including but not limited to foods, food ingredients, beverages, beverage ingredients, supplements, laboratory reagents, laboratory products, pharmaceuticals, medical devices, medical products, biological material, industrial chemicals, and hydrocarbons, or any other product benefiting from such treatment.

[0021] In various embodiments, the liquid or beverage may comprise a solution, a suspension, an emulsion, a dispersion, a carbonated liquid, juice, milk, yoghurt, soup, or honey, for example.
[0022] In various embodiments, the semi-liquid product or beverage may comprise a slurry, a gel, a paste, or a pulpy product, such as high pulp content juice, concentrated milk, or yoghurt.

[0023] In various embodiments, the solid product or food may comprise any one or more solid foods, including but not limited to a powder, granules, pastry, pasta, cheese, meat, fruit, or vegetables, for example, or any combination of any two or more thereof.

[0024] In various embodiments, the one or more products may comprise one or more of one or more powders, one or more pills, one or more capsules, one or more tablets, one or more solid food items, or any combination of any two or more thereof.

[0025] In various embodiments, the pH of the one or more products before being subjected to the hydrostatic pressure may comprise a pH of at least about 1.0, 2.0, 3.0, 3.1, 3.2, 3.3, 3.4, 3.5, 3.6, 3.7, 3.8, 3.9, 4.0, 4.1, 4.2, 4.3, 4.4, 4.5, 4.6, 4.7, 4.8, 4.9, 5.0, 5.1, 5.2, 5.3, 5.4, 5.5, 5.6, 5.7, 5.8, 5.9, 6.0, 6.1, 6.2, 6.3, 6.4, 6.5, 6.6, 6.7, 6.8, 6.9, 7.0, 7.1, 7.2, 7.3, 7.4, 7.5, 7.6, 7.7, 7.8, 7.9, 8.0, 8.25, 8.5, 8.75, 9.0, 9.5 or 10.0, or greater, and useful ranges may be selected between any of these values (for example, a pH of about 1 to about 7, about 3 to about 8, or about 7 to about 10).

[0026] In various embodiments, the elevated hydrostatic pressure may comprise a hydrostatic pressure ("treatment pressure") of at least about 33, 40, 45, 50, 55, 60, 65, 70, 75, 80, 85, 90, 95, 100, 125, 150, 175, 200, 225, 250, 275, 300, 325, 350, 375, 400, 425, 450, 475, 500, 525, 550, 575, 600, 625, 650, 675, 700, 750, 800, 850, 900, 950 or 1000 MPa, or greater, and useful ranges may be selected between any of these values (for example, about 33 to about 1000, about 100 to about 400, about 100 to about 500, about 100 to about 600, about 100 to about 700, about 100 to about 800, about 100 to about 900, about 100 to about 1000, about 200 to about 400, about 200 to about 500, about 200 to about 600, about 200 to about 700, about 200 to about 800, about 200 to about 900, about 200 to about 1000, about 300 to about 400, about 300 to about 500, about 300 to about 600, about 300 to about 700, about 300 to about 800, about 300 to about 900, about 300 to about 1000, about 400 to about 1000, about 450 to about 1000, about 500 to about 1000, about 550 to about 1000, about 600 to about 1000, about 650 to about 1000, and about 700 to about 1000 MPa).

[0027] In various embodiments, the method may be conducted at a temperature ("treatment temperature") of at least about -10, -5, 0, 5, 10, 15, 20, 25, 30, 35, 40, 45, 50, 55 or 60 degrees Celsius, or more, and useful ranges may be selected between any of these values (for example, about -10 to about 60, about 0 to about 50, about 5 to about 50, about 10 to about 50, or about 20 to about 50 degrees Celsius). It should be
understood that the pressure treatment may result in temperature fluctuations in the product and so reference to a treatment temperature is a reference to the temperature of the product before the pressure is raised.

[0028] In various embodiments, the treatment pressure may be applied for a treatment time of about, or up to about, 0.1, 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 20, 30, 60, 90, 120, 150, 180, 210, 240, 270, or 300 seconds or about 0.5, 1, 1.5, 2, 2.5, 3, 3.5, 4, 4.5, 5, 6, 7, 8, 9, 10, 15, 20, 25, 30, 35, 40, 45, 50, 55, or 60 minutes, and useful ranges may be selected between any of these values (for example, about 0.1 seconds to about 60 minutes, or about 1 to about 10, about 1 to about 9, about 1 to about 8, about 1 to about 7, about 1 to about 6, about 1 to about 5, about 1 to about 4, about 2 to about 10, about 2 to about 9, about 2 to about 8, about 2 to about 7, about 2 to about 6, about 2 to about 5, or about 2 to about 4 minutes).

[0029] In various embodiments, the pressure may be raised to the treatment pressure and then held substantially constant for the treatment time before the pressure is reduced to ambient pressure (i.e. the pressure is released), or the pressure may be raised from ambient pressure to the treatment pressure and back to ambient pressure within the treatment time, or may be cycled between two or more pressures within the treatment time.

[0030] In various embodiments, a treatment time of a period listed above may be the time that the pressure is held at the treatment pressure (the "hold time") such that a treatment time of 1 minute means that the pressure is held at the treatment pressure for 1 minute. Therefore, a treatment time of 0 (or "no hold") in this embodiment means that the pressure is raised to the treatment pressure but not held, and the pressure is then returned to ambient pressure (typically atmospheric pressure).

[0031] In various embodiments, the treatment pressure may be changed from one treatment pressure to another, without first returning to ambient pressure. Each pressure treatment may be conducted for a separate treatment time. Accordingly, in various embodiments the pressure may be increased from ambient pressure to a first treatment pressure for a first treatment time and then the pressure may be changed to a second treatment pressure for a second treatment time. Two, three, four or more different pressure treatments are contemplated.

[0032] In various embodiments, the elevated hydrostatic pressure may be held at a pressure of at least about 33 MPa for at least about 1 second. In various embodiments, once the elevated hydrostatic pressure comprises a pressure of at least about 33 MPa, the pressure is released.
[0033] In various embodiments the one or more products may be subjected to an
elevated hydrostatic pressure of at least about 150 MPa (or other treatment pressure or
range as described herein) for at least about 1 minute (or other treatment time or range
as described herein).

[0034] In various embodiments, the one or more products may be subjected to
and/or the reservoir may comprise at least about 0.1, 0.2, 0.3, 0.4, 0.5, 0.6, 0.7, 0.8,
0.9, 1, 1.25, 1.5, 1.75, 2, 2.25, 2.5, 2.75, 3, 3.25, 3.5, 3.75, 4, 4.25, 4.5, 4.75, 5, 5.25,
5.5, 5.75, 6, 6.5, 7, 7.5, 8, 8.5, 9, 9.5, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20, 25, or
30 % w/w carbon dioxide, or more, relative to the weight of the one or more products,
and useful ranges may be selected between any of these values (for example, about 0.1
to about 10, about 0.1 to about 20, about 0.1 to about 30, about 0.2 to about 10, about
0.2 to about 20, about 0.2 to about 30, about 0.3 to about 10, about 0.3 to about 20,
about 0.3 to about 30, about 1 to about 10, about 1 to about 20, 1 to about 30, about 2
to about 10, about 2 to about 20, about 2 to about 30, about 3 to about 10, about 3 to
about 20, or about 3 to about 30%). In other words, a product is subjected to at least
about 0.1 g of carbon dioxide per 100 g of product, and so on. Accordingly, the above
percentages may be alternatively expressed as grams of carbon dioxide per 100 g of
product. A person of ordinary skill in the art will be able to select an appropriate carbon
dioxide concentration and/or loading for the reservoir for an intended application of the
method, in view of that skill and the teaching of this specification.

[0035] In various embodiments, where carbon dioxide is to be loaded into the
reservoir at a temperature of about 20 °C and pressure of about 1 atmosphere, the
capacity of the reservoir may comprise at a capacity of least about 1.5, 5, 10, 15, 20,
25, 30, 35, 40, 45, 50, 55, 60, 65, 70, 75, 80, 85, 90, 95, 100, or 105 millilitres per
millilitre of internal volume of the flexible portion, and useful ranges may be selected
between any of these values (for example, about 1.5 to about 105 millilitres per millilitre
of internal volume). It should be understood that increasing the pressure of the carbon
dioxide in the reservoir will reduce the volume needed to contain it and, typically,
doubling the pressure will halve the volume required. Thus, in various embodiments,
where carbon dioxide is to be loaded into the reservoir at a temperature of about 20 °C
and pressure of about 10 atmospheres, the capacity of the reservoir may comprise at a
capacity of least about 0.15, 0.5, 1, 1.5, 2.0, 2.5, 3.0, 3.5, 4.0, 4.5, 5.0, 5.5, 6.0, 6.5,
7.0, 7.5, 8.0, 8.5, 9.0, 9.5, 10, or 10.5 millilitres per millilitre of internal volume, and so
on, and useful ranges may be selected between any of these values (for example, about
0.15 to about 105, about 0.15 to about 90, about 0.15 to about 80, about 0.15 to about
70, about 0.15 to about 60, about 0.15 to about 50, about 0.15 to about 40, and about
0.15 to about 10.5 millilitres per millilitre of internal volume). A person of ordinary skill
in the art will be able to select an appropriate size for the reservoir for an intended application of the method, in view of that skill and the teaching of this specification.

[0036] In various embodiments the one or more products may be substantially free of (comprise less than about 1, 0.1, or 0.01% by weight) carbon dioxide following application of the disclosed method.

[0037] In various embodiments, the aerobic plate count of the one or more products may be less than about 10, 100, 1,000, 10,000, 20,000, 30,000, 40,000, 50,000, 60,000, 70,000, 80,000, 90,000 or 100,000 colony forming units per millilitre (cfu/ml) after the step of subjecting the one or more products or the sealed container to the elevated hydrostatic pressure, and useful ranges may be selected between any of these values (for example, about 10 to about 100,000, or about 50,000 to about 100,000 cfu/ml).

[0038] In various embodiments, the one or more products may comprise one or more enzymes and after the step of subjecting the one or more products or the sealed container to the elevated hydrostatic pressure the activity of one or more enzymes is at least about 5, 10, 15, 20, 25, 30, 35, 40, 45, 50, 55, 60, 65, 70, 75, 80, 85, 90, 95, 98, 99 or 100% less than the activity of an untreated control, or a control treated with HHP only, or a control treated with carbon dioxide (including supercritical carbon dioxide) only, and useful ranges may be selected between any of these values (for example, about 5 to about 100, about 10 to about 100, about 25 to about 100, about 50 to about 100, about 75 to about 100, and about 90 to about 100% less). In various embodiments, the one or more enzymes may be selected from enzymes that may adversely affect the one or more products. Such enzymes may include one or more oxidoreductases (such as an oxidase, peroxidase and/or polyphenol oxidase), one or more transferases, one or more hydrolases (such as a protease, lipase, carboxydrase, nuclease, esterase or pectinesterase), one or more lyases, one or more isomerases, one or more ligases, or any combination of any two or more thereof. The activity of enzymes may be assessed using known methods, such as those discussed in the examples below.

[0039] In various embodiments, the one or more products may comprise one or more enzymes selected from pectin methylesterase (PME), peroxidase (POD), polyphenol oxidase (PPO), or any combination of any two or more thereof.

[0040] In various embodiments, the visual appearance of the one or more products is not substantially different after the step of subjecting the one or more products or the sealed container to the elevated hydrostatic pressure.
In various embodiments, the organoleptic properties of the one or more products are not substantially different after the step of subjecting the one or more products or the sealed container to the elevated hydrostatic pressure. Organoleptic properties such as taste and texture may be assessed by a sensory panel.

In various embodiments, the removable reservoir may comprise opposing one-way valves that are fluidly communicable with the internal volume of the flexible portion.

In various embodiments, the volume of the removable reservoir may be varied by the movement of a piston.

In various embodiments, the volume of the removable reservoir may be varied by the deformation of a bladder.

In various embodiments, the method may further comprise one or more steps selected from removing the removable reservoir, recovering the carbon dioxide from the reservoir, fixing a consumer closure to the container, and aggregating a plurality of containers into a package.

It is intended that reference to a range of numbers disclosed herein (for example, 1 to 10) also incorporates reference to all rational numbers within that range (for example, 1, 1.1, 2, 3, 3.9, 4, 5, 6, 6.5, 7, 8, 9 and 10) and also any range of rational numbers within that range (for example, 2 to 8, 1.5 to 5.5 and 3.1 to 4.7) and, therefore, all sub-ranges of all ranges expressly disclosed herein are hereby expressly disclosed. These are only examples of what is specifically intended and all possible combinations of numerical values between the lowest value and the highest value enumerated are to be considered to be expressly stated in this application in a similar manner.

The invention may also be said broadly to consist in the parts, elements and features referred to or indicated in the specification of the application, individually or collectively, in any or all combinations of two or more of said parts, elements or features, and where specific integers are mentioned herein which have known equivalents in the art to which the invention relates, such known equivalents are deemed to be incorporated herein as if individually set forth.

**DETAILED DESCRIPTION OF THE DRAWINGS**

The disclosed methods and apparatus will now be described with reference to the following Figures.
[0049] Figure 1 is a process flow of various implementations of the disclosed methods.

[0050] Figure 2 is a perspective view of one embodiment of a disclose apparatus.

[0051] Figure 3 is an exploded perspective view of one embodiment of a disclosed apparatus depicting two alternative components.

[0052] Figure 4 is a cross-sectional view of one embodiment of a disclosed apparatus.

[0053] Figure 5 is (A) a perspective view of a disclosed embodiment, (B) a cross-sectional view of a disclosed embodiment, and (C) a cross-sectional view of another disclosed embodiment.

[0054] Figure 6 is a cross-sectional view of one embodiment of a disclosed apparatus, (A) with a cap 420, and (B) without.

[0055] Figure 7 is a cross-sectional view of one embodiment of a disclosed apparatus.

[0056] Figure 8 is a cross-sectional view of a disclosed embodiment.

[0057] Figure 9 is a cross-sectional view of a disclosed embodiment in use in an HHP unit (A) before and (B) during application of pressure.

[0058] Figure 10 is a graph showing residual POD activity in feijoa puree after HHP, HHPcarb and HHPcarb + CO2 treatments at 300, 450 and 600 MPa (initially at room temperature, 5 min). Data is shown as the mean ± SD.

[0059] Figure 11 is a graph showing residual PPO activity in feijoa puree after HHP, HHPcarb and HHPcarb + CO2 treatments at 300, 450 and 600 MPa (initially at room temperature, 5 min). Data is shown as the mean ± SD.

[0060] Figure 12 is a graph showing residual PME activity in feijoa puree after HHP, HHPcarb and HHPcarb + CO2 treatments at 300, 450 and 600 MPa (initially at room temperature, 5 min). Data is shown as the mean ± SD.

[0061] Figure 13 is a graph showing total colour difference of feijoa puree after HHP, HHPcarb and HHPcarb + CO2 treatments at 300, 450 and 600 MPa. Data is shown as the mean ± SD.
[0062] Figure 14 is a graph showing inactivation of *E. coli* in broth after HHP, HHPcarb and HHPcarb + CO₂ at 100, 400 and 550 MPa (35°C for 10 min). Data is shown as the mean ± SD.

[0063] Figure 15 is a graph showing inactivation of *E. coli* in broth after HHP, HHPcarb and HHPcarb + CO₂ at 100, 300 and 600 MPa (25°C for 5 min). Data is shown as the mean ± SD.

[0064] Figure 16 is a graph showing inactivation of *E. coli* in Ayran yogurt after HHP, HHPcarb and HHPcarb + CO₂ at 300, 450 and 600 MPa (25°C for 5 min). Data is shown as the mean ± SD.

[0065] Figure 17 is a graph showing inactivation of *E. coli* in Ayran yogurt after HHP, HHPcarb and HHPcarb + CO₂ at 300, 450 and 600 MPa (25°C for 10 min). Data is shown as the mean ± SD.

[0066] Figure 18 is a graph showing inactivation of *B. subtilis* in broth after HHP, HHPcarb and HHPcarb + CO₂ at 200, 250 and 300 MPa (25 °C for 2, 4 and 6 min). Data is shown as the mean ± SD.

[0067] Figure 19 is a graph showing inactivation of *S. cerevisiae* in broth after HHP, HHPcarb and HHPcarb + CO₂ at 200, 250 and 300 MPa (25 °C for 2, 4 and 6 min). Data is shown as the mean ± SD.

[0068] Figure 20 is a graph of POD activity in feijoa samples treated with different CO₂ levels, at 600 MPa, 20 °C for 5 min, before and after storage of 28 days, where an asterisk represents a significant difference at p< 0.05.

[0069] Figure 21 is a graph of PPO activity in feijoa samples treated with different CO₂ levels, at 600 MPa, 20 °C for 5 min, before and after storage of 28 days, where an asterisk represents a significant difference at p< 0.05.

[0070] Figure 22 is a graph of PME activity in feijoa samples treated with different CO₂ levels, at 600 MPa, 20 °C for 5 min, before and after storage of 28 days, where an asterisk represents a significant difference at p< 0.05.

**DETAILED DESCRIPTION**

[0071] The inventors have determined that use of elevated hydrostatic pressure simultaneously with carbon dioxide deactivates microorganisms and certain enzymes while having limited effects on flavours, and colours. As herein described, the carbon
dioxide used in the method may be converted to a liquid, a near-critical liquid, or a supercritical fluid by the application of elevated hydrostatic pressure, or in the case of a liquid, the product may be substantially saturated with carbon dioxide. The described method may be used to maintain or improve the keeping quality of a product without unacceptable adverse effects on the visual appearance, organoleptic properties, or efficacy of the product. In some embodiments, the method may be useful as an alternative to pasteurising and/or sterilising a product. Accordingly, disclosed are methods for maintaining or increasing the keeping quality of one or more products, including reducing the viability of one or more microorganisms, including unwanted microorganisms, and/or reducing the activity of one or more enzymes, including but not limited to carrying out such methods without substantially altering the organoleptic or other desirable properties of the one or more products.

1. Definitions

[0072] As used herein the term "and/or" means "and" or "or", or both.

[0073] The term "comprising" as used in this specification means "consisting at least in part of". When interpreting statements in this specification which include the term "comprising", the features prefaced by that term in each statement or claim all need to be present but other features can also be present. Related terms such as "comprise" and "comprised" are to be interpreted in the same manner.

[0074] The term "keeping quality" as used herein is intended to mean the ability of a product to resist the growth of unwanted microorganisms, and/or the effects of enzyme activity, over time. Products that are not treated with heat or an acceptable alternative such as reported herein are unlikely to have a commercially acceptable keeping quality. Reference to maintaining keeping quality is intended to mean that a disclosed method is at least as effective as a heat treated control at extending the shelf-life of a product. Reference to increasing or increased, or improving or improved keeping quality is intended to mean that the ability of a product to resist the growth of unwanted microorganisms, and/or the effects of enzyme activity, over time is enhanced compared to an untreated product.

[0075] The keeping quality of product or container treated according to the described methods may be assessed in relation to the growth of unwanted microorganisms with reference to the Aerobic Plate Count (APC). APC is an enumeration procedure used to estimate microorganism density in a sample and is otherwise known as Total Plate Count, Standard Plate Count or Total Viable Count. Samples are collected, blended, diluted, and plated in an agar medium suitable for detecting the microorganism to be
studied (for example, food contaminants such as *Escherichia coli*, *Staphylococcus aureus*, *Salmonellae*, *Shigellae*, coliforms, yeasts and moulds, mesophilic spores, thermophilic spores). The APC result is the number of colony forming units in one millilitre (cfu/ml) of sample that is plated and incubated for 72 hrs at 32 °C.

5 [0076] The keeping quality of product or container treated according to the described methods may be assessed in relation to the effects of enzyme activity using assays such as those described herein, and others as are known in the art. The lower the residual enzyme activity following treatment according to a disclosed method, the greater the keeping quality.

10 [0077] As used herein the term "pasteurising" means inactivation of pathogens, often in the form of vegetative bacteria.

[0078] As used herein the term "sterilising" means substantially complete inactivation of thermophilic spores.

[0079] The term "supercritical" as used herein refers to the pressure-temperature region above the critical point of a substance, such as carbon dioxide. The term "subcritical" as used herein refers to the pressure-temperature region equal to or above the vapour pressure for the liquid, but below the critical temperature. The term "near critical" as used herein encompasses both "supercritical" and "subcritical" regions, and refers to pressures and temperatures near the critical point. The critical point of carbon dioxide is about 7.4 MPa and about 31 degrees Celsius. As used herein, "near critical carbon dioxide" is intended to mean carbon dioxide within about 10, 20, 30, or 40 degrees Celsius of its critical temperature and/or within about 1, 2, 3, or 4 MPa of its critical pressure, and any combination thereof.

[0080] The term "unwanted microorganisms" refers to all microorganisms that may be present in a product before processing with a disclosed method, including bacteria such as *Escherichia coli*, *Staphylococcus aureus*, *Salmonellae*, *Shigellae*, coliforms, yeasts and moulds, mesophilic spores, and thermophilic spores.

2. Processing methods

[0081] Referring to Figure 1, a product 1 is prepared according to standard methods for the particular product type and placed in a flexible container 2. The product 1 may comprise a liquid product, a semi-liquid product, a solid product, or a combination, as described above. The flexible container 2 may comprise any suitable polymer known in the art and comprises an internal volume for holding and substantially enclosing the product and an opening through which a consumer may access the product. Such
containers are discussed below. The container may be made of food grade, laboratory
grade or pharmaceutical grade materials, as are known the art. The composition of the
container should be chosen such that it remains intact after processing by a disclosed
method. The flexible container 2 is sealed by attachment of a carbon dioxide reservoir 3
to produce a sealed container 4. In alternative embodiments, the container opening is
sealed with a temporary closure or a consumer closure and the reservoir is subsequently
attached. The carbon dioxide reservoir 3 is described above and below. Before, during
or after being attached to the flexible container 2, the carbon dioxide reservoir 3 is
charged with carbon dioxide gas or liquid 5 to a suitable concentration, as described
above. A suitable concentration may be determined by the nature of the product to be
treated, the nature of the flexible container and the nature of the reservoir, as discussed
below.

[0082] The sealed container 4 is placed in the chamber 6 of a high pressure
processing unit. Water is the typical working fluid of high pressure processing units.
The sealed container 4 may be processed alone or in batches, dictated by the size and
capability of the high pressure processing unit. Various high pressure processing units
are known in the art and are available commercially. The chamber 6 is then sealed and
the pressure in the chamber is raised to a predetermined set pressure 7 (the "treatment
pressure"). The duration and temperature of the pressure treatment 7 is dictated by the
intended application, with suitable treatment times, temperatures and other conditions
discussed above. The treatment pressure subjects the product 1 to hydrostatic pressure
and also changes the volume of the reservoir 3 as discussed below to transfer at least a
portion of the carbon dioxide from the reservoir to the internal volume of the flexible
container 2. The carbon dioxide contacts at least a portion if not substantially all of the
product, depending on the nature of the product. The treatment pressure may also
convert the carbon dioxide from a gas to a liquid, near critical gas or liquid, or a
supercritical fluid, or from a liquid to a near critical liquid, or a supercritical fluid, or in
the case of a liquid product, substantially saturate the product with carbon dioxide that
may or may not dissolve in the liquid product. Depending on the nature of the product,
the carbon dioxide may contribute to a decrease in the viability of plant material, a
decrease in the viability and/or pathogenicity of one or more unwanted microorganisms,
a decrease in the activity and/or pathogenicity of one or more viruses, and/or a decrease
in the activity of one or more enzymes, that may be present. The pressure in the
chamber may be immediately released once the treatment pressure 7 is reached, or the
treatment pressure 7 may be held for a predetermined time (the "hold time").
Immediately on reaching the treatment pressure, or once the hold time has passed, the
pressure may be released released and the sealed container 4 is removed from the
chamber 6. At the point of pressure release substantially all of the carbon dioxide is released from the product and the container and returned to the reservoir 3. The reservoir 3 is then removed from the container 8 and, if necessary, a consumer closure 9 such as a cap or lid is affixed to the container opening. The carbon dioxide in the reservoir 3 may be recovered or reused. Optionally, with or without further processing to remove surface contaminants, two or more containers may then enter a packing station 10 where they are packed into a suitable format for shipping and sale. At one or more points in the process, containers may be selected for quality control.

3. Apparatus

[0083] With reference to Figure 2 to Figure 8, and as described above, apparatus 100 for use in a disclosed method comprises a removable carbon dioxide reservoir 110 and a flexible container 120, which may be sold separately. The flexible container 120 is made of standard polymer material, as described above, and may be of any suitable shape. The flexible container 120 comprises an internal volume 130 which holds a product 135 and an opening 140 through which a consumer may access the product. Generally, the opening 140 in the container 120 will also comprise a headspace (ullage) above the product 135, as described above.

[0084] The removable carbon dioxide reservoir 110 comprises a fitting 150 that is removably attachable to the opening 140 of the flexible container 120. The fitting 150 may be a threaded section or a push-fit or click-fit section, for example, or a clamp or similar fitting may be used to hold the reservoir 110 in place over the opening 140 of the flexible container 120. Alternatively, the opening 140 may be sealed and the reservoir 110 attached separately.

[0085] The reservoir 110 is of variable volume and is fluidly communicable with the internal volume 130 of the flexible container 120. That is, the reservoir 110 is in fluid communication with the internal volume 130 during the processing method but the design and operation of the apparatus, as discussed below, may in some embodiments resist the passage of fluid at ambient temperatures and pressures. Generally, reservoir 110 is fluidly communicable with the internal volume 130 of the flexible container 120 through a pair of one-way valves 160, 170 but embodiments without valves are also contemplated. Valve 160 allows transfer of carbon dioxide from the reservoir 110 to the internal volume 130. Valve 170 allows transfer of carbon dioxide from the internal volume 130 to the reservoir 110. The carbon dioxide may then be recovered or reused. It should be appreciated that the volume of the reservoir may be adapted to the particular application, as discussed above.
With reference to Figure 2 to Figure 6 and Figure 8, reservoir 110 comprises reservoir body 200, valve plate 210, piston 220 and top cap 230. Reservoir body 200 is made of a suitably resilient material or rigid material, including plastic or metal, such that it can resist the force of the pressure treatment and not impede the passage of piston 220 through the interior of the reservoir body 200. Valve plate 210 may be integral with body 200, may be a separate component that is retained within the fitting 150 of the reservoir 110 as shown, or may be removably affixed to the opening 140 of the flexible container 120 before reservoir 110 is attached to the container 120. Valve plate 210 supports the pair of one-way valves 160, 170. Valve plate 210 may be of any suitable material able to resist the forces involved and carry a system of one-way valves. Piston 220 may comprise suitable material and may comprise sealant o-rings (not shown) such that it makes a substantially gas-tight and/or water-tight fit within the body 200 of the reservoir. Top cap 230 may be integral with the body 200 (not shown) or removable through a suitable fitting system such as opposing threads (as shown) or a press-fit connection (not shown) that engages the top of the body 200. Top cap 230 comprises one or more openings 240 to allow water to enter the top of the reservoir body 200 and apply force on the piston 220. Figure 3 shows two alternative top caps 203a comprising a plurality of holes 240 and 230b comprising a mesh 250 with a plurality of holes 240. Reservoir body 200 comprises a carbon dioxide inlet port 260 that allows the reservoir 110 to be charged with carbon dioxide.

In use, water is able to pass through the holes 240 into the body 200 of the reservoir and apply force to the piston 220, moving piston 220 downwards through the body 200, changing the volume of the reservoir 110 and transferring carbon dioxide into the internal volume 230 of the container 120.

Referring to Figure 5, valve plate 210 is shown with two one-way valves 160 and 170. Referring to (A) and (B) of Figure 5, the valves comprise poppet valves or the like, comprising discs or plugs 165 and 175 that are biased closed by springs 166 and 176. In use, on the application of the treatment pressure and thus hydrostatic force to the piston 220, pressurised carbon dioxide overcomes the biasing force of the spring 166 and passes through valve 160 into the internal volume 130 of the container 120. On the release of the treatment pressure, carbon dioxide overcomes the biasing force of the spring 176 and passes through valve 170 to return to the reservoir. The carbon dioxide may then be recovered or reused. Referring to (C) of Figure 5, an alternative embodiment is shown where the valves 160, 170 comprise spheres such as beads or ball bearings 168, 178 that are biased into a closed position by springs 169, 179.
With reference to Figure 7, reservoir 110 comprises reservoir body 300 and valve plate 310. Valve plate 310 is identical to valve plate 210. Body 300 comprises a flexible material such as rubber or the like that is deformable on the application of hydrostatic force. In one embodiment body 300 comprises a bladder. In use, on the application of the treatment pressure, hydrostatic force deforms body 300 to pressurise the carbon dioxide in the reservoir, which in turn overcomes the biasing force of the spring 166 and passes through valve 160 into the internal volume 130 of the container 120. On the release of the treatment pressure, carbon dioxide overcomes the biasing force of the spring 176 and passes through valve 170 to return to the reservoir.

With reference to Figure 6, and as an alternative to the valve plate 210, reservoir body 200 comprises an elongate outlet needle 400 that extends into the internal volume 130 of the container 120 and, preferably, extends into the product 135, and a shorter elongate inlet needle, or return port, 410. Like with the valve plate 210, the outlet 400 and inlet 410 are optionally provided with one-way valves 160, 170 as described above. This inlet/outlet configuration is preferably integral with reservoir body 200 but multi-part components are contemplated (not shown), connected with suitable fittings such as opposing threads or a press-fit connection (not shown). Configuration (A) of Figure 6 includes cap 420 whereas configuration (B) does not. Cap 420 is applied to the opening 140 of the container 120 before the reservoir is fitted, and may either comprise holes or perforated sections for the passage of needles 400, 410 or be made of a suitable penetrable material.

It should be appreciated that the inlet/outlet configuration of Figure 6 may be used with the reservoir body 300 of Figure 7.

With reference to Figure 8, a further embodiment is shown that does not employ a valve plate. Instead, reservoir body 200 may comprise a tapered neck 500 between the main portion of the body 200 and the fitting 150. Additionally or alternatively, valves 160, 170 are held within the wall of the reservoir body 200 which may either sit flush with the opening 140 (not shown) or which may extend into the opening 140.

With reference to Figure 9, apparatus 100 is placed in the chamber 600 of a high pressure processing device, as described above and exemplified below (A). Operating fluid 610 such as water is pumped into the chamber until the treatment pressure is reached. Hydrostatic pressure 620 is applied to the apparatus, including piston 220 (or deformable body 300 – not shown), which varies the volume of the reservoir 110 and transfers carbon dioxide 630 into the internal volume 130 of the
container 120. On release of the treatment pressure, carbon dioxide is transferred back into the reservoir 110.

[0094] The invention is further illustrated by the following examples.

EXAMPLES

EXAMPLE 1

1. Introduction

[0095] The example investigates the effect of the disclosed methods on the physical-chemical properties and residual enzyme levels of treated fruit puree samples.

2. Materials and methods

2.1. Raw material

[0096] 15 kg ripened feijoa (A. sellowiana) was cleaned, peeled, chopped, sealed in plastic bags and stored at −20°C.

2.2. Chemical-physical analysis

[0097] 30 feijoa pieces were randomly selected, and assessed for colour, pH and firmness. The feijoa pieces were processed to form a puree, and the moisture, °Brix and water activity of the puree were determined.

Colour determination

[0098] Assessment of fruit and fruit puree colour was conducted at 25°C using a CR400-ChromaMeter™ Colorimeter (Konica Minolta, USA) in CIE L*a*b*™ colour space system. Colour was measured at three sites around each end of fruit and three sites at the equator to obtain a mean value per fruit.

[0099] The colour of puree was measured after thawing and before enzyme analysis. Chroma (C*), hue angle (H°) and total colour difference (ΔE) with respect to control sample were calculated.

pH

[00100] The pH inside fruit was measured at 25°C using a digital pH meter (PerpHec LogR™ meter, model 320, Orionresearch Inc., USA). The pH of puree was measured before enzyme analysis. For samples containing CO₂ (HHPCarb and HHPCarb + CO₂), the puree was decarbonated prior to pH measurement by agitation under vacuum (10 mmHg, 25°C).

Texture analysis
The firmness of fresh feijoa (Table 1) was measured using a universal texture analyzer (TA.XT Plus Texture Analyser, Stable Micro Systems Ltd., UK) linked to a computer for data acquisition and processing (Exponent software, Stable Micro System Ltd., UK), using a small cylindrical probe (10 mm diameter). The maximum force (firmness, N) was measured and computed with a test speed of 0.03 mm/s and travel distance of 5 mm down the fruit surface, at the centre of its equator and at each side of the fruit (2 punctures per side).

Moisture content

The moisture content of fresh feijoa puree was determined using an official vacuum oven method. 5 g puree was accurately weighed and then dried at -70°C and 10 mmHg vacuum for 24 h in a vacuum oven. The dried samples were weighed using an analytical balance. Moisture content was calculated using the equation: Moisture content (%) = (Total moisture loss after drying (g) / Initial weight (g)) × 100.

Brix

The °Brix of fresh feijoa puree (Table 1) was measured at 25°C using an E-Line ATC range 0–18°Brix refractometer (Bellingham + Stanley Ltd., UK).

Water activity

The water activity of fresh feijoa puree was measured at 25°C using a digital water activity meter (Aqua Lab 4TE, Decagon Devices, USA). The water activity of fresh feijoa puree was 0.9901 ± 0.0018.

2.3. Sample preparation and storage

Frozen fruit was thawed at 4°C for 12 to 14 hours, blended until well mashed and mixed into a puree. 30 g puree was poured into plastic bags (155 mm × 180 mm × 30 mm, SURT155180, Cas-Pak Products Ltd., New Zealand), vacuum sealed and stored at −20°C until required.

2.4. Sample treatment

CO₂ treatment

Frozen puree was thawed in the bag at 4°C for 12 to 14 hours. Three CO₂ levels were considered in this study: puree without CO₂ (HHP); carbonation at 1 atm (HHPcarb); carbonation and addition of 8.5 mL CO₂/g puree into the headspace of the package (HHPcarb + CO₂). Carbonation of samples was achieved by bubbling CO₂ at atmospheric pressure at 1.28 L/min from the bottom of the puree for 5 min at 0–3°C in an ice water bath with vigorous agitation of the bags. The bags were immediately sealed without gas loss and placed on ice until HHP treatment.
High pressure processing

[00107] The Avure 2L Food Processor (Avure Technologies, Columbus, OH, USA) was used for HPP processing. The equipment consists of a cylindrical pressure treatment chamber, a pumping system, water circulation and a control system operated by manufacturer's software. Water was used as the working fluid in the pressure chamber. For each treatment, three bags (1 HHP sample, 1 HHPcarb sample and 1 HHPcarb + CO₂ sample) were treated together at a pressure of 300, 450 or 600 MPa for 5 min. Pressure come up times were approximately 0.5 min and 1.5 min to reach 300 MPa and 600 MPa, respectively. Depressurization occurred in less than 2 s. The temperature history of the water in the chamber was recorded by two thermocouples during processing. The starting temperature of samples was 25°C. The maximum temperature reached for a 600 MPa treatment was 42°C. Two replicates were conducted per sample at each pressure. The plastic bags were frozen after treatment at -70°C.

2.5. Analysis of treated samples

[00108] Treated and untreated frozen puree was thawed at 4°C for 12 to 14 hours before analysis. The colour and pH of thawed puree was measured as described above.

Polyphenol oxidase (PPO) and peroxidase (POD) assays

[00109] 10 g feijoa puree was homogenised with 30 mL 0.05 M potassium phosphate buffer solution at 13,000 rpm for 2 min. The slurries were centrifuged and filtered to obtain a supernatant for analysis.

[00110] PPO and POD activities of the supernatant were assayed using the method described by Chen et al. (2010) Innovative Food Science and Emerging Technologies, 11: 623–329. The following modifications were made to the method. PPO assay medium contained 0.4 mL sample and 2.6 mL substrate solution (1.3 mL 0.05 M sodium phosphate buffer pH 6.8, added to 1.3 mL 0.02 M catechol solution). POD assay medium contained 0.2 mL sample and 3 mL substrate solution (3 mL 30% hydrogen peroxide added to 1.9 mL of liquid guaiacol, made up to 300 mL with 0.2 M sodium phosphate buffer pH 6). For the blank, 0.2 mL of distilled water was added in place of sample.

[00111] The increase in absorbance at 420 nm (PPO) or 470 nm (POD) was monitored at intervals of 5 s immediately after the addition of sample to the corresponding substrate solution at ambient temperature. One unit of specific PPO or POD activity was defined as the change per min and millilitre of sample in the absorbance measured at 420 nm or 470 nm, respectively.
[00112] The residual activity of each enzyme was obtained using the following equation:

\[
PPO \ (POD) \text{ residual activity} = \frac{\text{Specific activity PPO (POD) after treatment} \times 100}{\text{Specific activity PPO (POD) control sample}}
\]

5 Pectin methyl esterase (PME) activity measurement

[00113] Thawed puree was de Carbonated by agitation under vacuum \((10 \text{ mmHg, 25}^\circ\text{C})\). PME activity was determined as described by Castaldo et al. (1997) LWT – Food Science and Technology 30: 479–484. The following modifications were made to the method. The substrate solution was prepared by heating \(1 \text{ L} 0.15 \text{ M NaCl to 50–55}^\circ\text{C, adding to a blender and sprinkling 10 g pectin powder was sprinkled on the dissolving 10 g pectin powder in surface and blended. 4 mL feijoa puree was added to 12 mL pectin solution of pH 7. The pH was quickly adjusted to 7, and PME activity was measured by recording the decrease in pH every 5 s until pH dropped to 6.5.}

[00114] One unit of specific PME activity was defined as the slope of pH vs. time in min. The residual activity of PME was calculated using the following equation:

\[
PME \text{ residual activity} = \frac{\text{Specific activity PME after treatment} \times 100}{\text{Specific activity PME control sample}}
\]

2.6. Statistical analysis

[00115] All treatment conditions were duplicated and analyses were conducted in triplicate. Simple ANOVA and a two-way ANOVA analyses were conducted and LSD (least significant differences) was determined to evaluate the effect of pressure, CO\(_2\) level and the possible interaction between factors, on the residual PPO, POD and PME activity of treated samples. A two-way ANOVA was conducted in order to evaluate the effect of pressure and CO\(_2\) level on the pH, and colour parameters of the treated samples, compared with the control sample.

3. Results

[00116] The results of a chemical-physical analysis of fresh feijoa fruit is summarised in Table 1.

<table>
<thead>
<tr>
<th></th>
<th>% Moisture</th>
<th>°Brix</th>
<th>pH</th>
<th>Firmness (N)</th>
<th>L*</th>
<th>a*</th>
<th>b*</th>
</tr>
</thead>
<tbody>
<tr>
<td>Fresh feijoa</td>
<td>83.33±0.30</td>
<td>11.8±0.8</td>
<td>3.30±0.02</td>
<td>20.67±3.87</td>
<td>54.56±2.56</td>
<td>-8.32±2.07</td>
<td>14.01±3.26</td>
</tr>
</tbody>
</table>

All data shown are means ± SD of 30 fruit.
3.1. POD activity

[00117] Figure 10 shows the effect of HHP, HHPcarb and HHPcarb + CO₂ treatments on POD activity in feijoa puree. The addition of CO₂ had a significant effect (p < 0.05) on residual POD activity in puree treated at 300, 450 and 600 MPa.

[00118] At 300 MPa, the residual POD activity following HHPcarb + CO₂ treatment decreased to 13 ± 8%, compared to 140 ± 5% and 32 ± 7% for HHP alone and HHPcarb treatment, respectively. The residual POD activity in HHPcarb + CO₂ samples was 60 and 45% lower at 300 and 600 MPa, respectively, compared with HHPcarb samples.

[00119] Moreover, the residual POD activity in HHPcarb + CO₂ samples at 300 MPa (13 ± 8%) could only be achieved at 600 MPa using high pressure alone (HHP) (22 ± 13%).

[00120] These results indicate that simultaneous HHP and carbon dioxide processing of feijoa puree reduces residual POD activity more effectively than HHP treatment alone, and at least as effectively as HHPcarb treatment but at lower treatment pressure.

3.2. PPO activity

[00121] Figure 11 shows the effect of HHP, HHPcarb and HHPcarb + CO₂ treatments on PPO activity in feijoa puree. The residual PPO activity for HHP, HHPcarb and HHPcarb + CO₂ samples treated at 300 MPa was 102 ± 8%, 85 ± 2% and 56 ± 5%, respectively; at 450 MPa was 47 ± 4%, 42 ± 6% and 42 ± 1%, respectively; and at 600 MPa was 38 ± 5%, 44 ± 4% and 26 ± 3%, respectively.

[00122] Therefore, similar to POD activity, the addition of CO₂ into the headspace of the package resulted in greater inactivation of PPO when HHP is applied at all the pressures studied.

3.3. PME activity

[00123] Figure 12 shows the effect of HHP, HHPcarb and HHPcarb + CO₂ treatments on PME activity in feijoa puree. For both HHPcarb and HHPcarb + CO₂ treatments, the remaining PME activity at 600 MPa was significantly lower than at 300 and 450 MPa.

[00124] No significant differences (p > 0.05) on the level of residual PME activity were observed between the different levels of CO₂ studied. The enhancing effect of CO₂ addition to the HHP inactivation process of PME in feijoa puree was only observed at 600 MPa.
[00125] The results show that simultaneous HHP and carbon dioxide processing is at least as effective as HHP only or HHPcarb treatment for inactivating PME in feijoa puree.

3.4. pH

[00126] The mean pH of feijoa fruit was pH 3.30 as shown in Table 1. The mean pH of the untreated puree sample after the freeze-thaw process was 3.45 (Table 2), indicating that processing of the fruit had a significant (p < 0.05) effect on pH, even before HHP or CO₂ treatment.

[00127] The pH of the treated samples was compared with the pH of control puree subjected to the same temperature changes as shown in Table 2. Overall, the pH of all treated samples at different pressures was significantly increased (p < 0.05) compared to the control puree. However, pressure did not significantly affect pH. pH significantly decreased (p < 0.05) at all pressures with increasing CO₂. This effect is not due to residual CO₂ in the samples as CO₂ was removed from the samples by vacuum pulling before pH measurement.

3.5. Colour

[00128] Increasing pressure significantly affected ΔE, L*, a* and b*, Chroma and Hue angle values as shown in Figure 13 and Table 2. The lightness and yellowness, and Chroma and Hue angle values of the samples significantly decreased, while the redness significantly increased as pressure increased.

[00129] On average, the ΔE value was significantly higher for HHPcarb samples than for HHP or HHPcarb + CO₂ samples, between which no significant differences were found. Two-way ANOVA showed that the lightness and redness of samples treated with CO₂ was significantly lower (p < 0.05) than samples treated only with HHP. However, the yellowness did not change with the addition of CO₂ into the headspace compared with HHP only. On the other hand, increased CO₂ level had a significant (p < 0.05) effect on Chroma but not Hue angle values.

[00130] Generally, a ΔE value of <1.6 is considered an imperceptible difference to the human eye. Therefore, all the treatments caused a perceptible colour change except HHPcarb at 450 MPa as shown in Figure 13. The feijoa puree changed from bright yellow to shades of brown with lower brightness after all treatments. However, the addition of CO₂ into the headspace of the package did not further increase the colour change of the puree compared with puree treated only with HHP.
Table 2: Values of pH and colour of feijoa puree for control and treated samples.

<table>
<thead>
<tr>
<th></th>
<th>pH</th>
<th>L*</th>
<th>a*</th>
<th>b*</th>
<th>Chroma</th>
<th>Hue angle</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>3.45 ± 0.03</td>
<td>55.13 ± 0.86</td>
<td>3.16 ± 0.55</td>
<td>20.76 ± 1.69</td>
<td>21.08 ± 0.76</td>
<td>1.42 ± 0.02</td>
</tr>
<tr>
<td>HHP</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>300</td>
<td>3.63 ± 0.02</td>
<td>55.78 ± 0.53</td>
<td>3.93 ± 0.20</td>
<td>18.6 ± 1.07</td>
<td>19.08 ± 0.72</td>
<td>1.36 ± 1.09</td>
</tr>
<tr>
<td>450</td>
<td>3.68 ± 0.01</td>
<td>54.24 ± 0.92</td>
<td>4.43 ± 0.10</td>
<td>18.57 ± 0.17</td>
<td>19.09 ± 0.48</td>
<td>1.34 ± 0.14</td>
</tr>
<tr>
<td>600</td>
<td>3.64 ± 0.01</td>
<td>54.63 ± 0.16</td>
<td>3.97 ± 0.22</td>
<td>17.84 ± 0.65</td>
<td>18.28 ± 0.58</td>
<td>1.35 ± 0.68</td>
</tr>
<tr>
<td>HHPcarb</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>300</td>
<td>3.61 ± 0.02</td>
<td>53.76 ± 0.60</td>
<td>4.30 ± 0.78</td>
<td>18.83 ± 0.82</td>
<td>19.33 ± 0.62</td>
<td>1.35 ± 0.31</td>
</tr>
<tr>
<td>450</td>
<td>3.50 ± 0.01</td>
<td>54.88 ± 0.65</td>
<td>3.61 ± 0.12</td>
<td>21.04 ± 0.09</td>
<td>21.35 ± 0.18</td>
<td>1.40 ± 0.07</td>
</tr>
<tr>
<td>600</td>
<td>3.55 ± 0.03</td>
<td>52.30 ± 0.18</td>
<td>4.58 ± 0.30</td>
<td>17.90 ± 0.16</td>
<td>18.48 ± 0.09</td>
<td>1.32 ± 0.08</td>
</tr>
<tr>
<td>HHPcarb+CO₂</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>300</td>
<td>3.46 ± 0.04</td>
<td>54.84 ± 0.38</td>
<td>2.57 ± 0.80</td>
<td>19.06 ± 0.53</td>
<td>19.25 ± 0.28</td>
<td>1.44 ± 0.42</td>
</tr>
<tr>
<td>450</td>
<td>3.56 ± 0.03</td>
<td>54.58 ± 0.85</td>
<td>3.93 ± 0.20</td>
<td>18.09 ± 0.31</td>
<td>18.52 ± 0.85</td>
<td>1.36 ± 1.85</td>
</tr>
<tr>
<td>600</td>
<td>3.51 ± 0.01</td>
<td>53.68 ± 0.02</td>
<td>4.20 ± 0.09</td>
<td>16.93 ± 0.23</td>
<td>17.44 ± 0.20</td>
<td>1.33 ± 0.25</td>
</tr>
</tbody>
</table>

All data shown are means ± SD.

5 **EXAMPLE 2**

1. Introduction

[00131] This example investigates the effect of the disclosed methods on microbial inactivation.

2. Materials and methods

10 2.1 Micro-organism broth preparation

[00132] *E. coli* DH1 was grown in Bacto - Tryptic Soy Broth, (Becton, Dickinson and Company, USA) at 37 °C, using an incubation chamber and an orbital shaker at 120rpm.

[00133] *B. subtilis* was grown in Nutrient Broth (OXOID, England), using an incubation chamber and an orbital shaker at 140 rpm.

15 [00134] *S. cerevisiae* was grown in YPD Broth (Becton, Dickinson and Company, USA) at 30 °C, using an incubation chamber and an orbital shaker at 140 rpm.

2.2 Enumeration of microorganisms

[00135] Viability of *E. coli, B. subtilis* and *S. cerevisiae* was determined by the plate count method. Samples were serially diluted with sterilized water, and 100μl of the appropriate dilution were plated in triplicate onto the appropriate growth plate. Tryptic Soy Broth, Nutrient Agar and YPD were used as the agar plates for *E.coli, B. subtilis*, and *S. cerevisiae* respectively. The plates were incubated at 37 °C for 24 h for *E. coli* and *B.*
*subtilis* and at 30 °C for 48 h for *S. cerevisiae* after which time cells were counted and the arithmetic mean of every three plates calculated.

2.3 **HHP processing, carbonation and CO\(_2\) addition for *E. coli***

[00136] Three CO\(_2\) treatments were used for this study as for Example 1: no added CO\(_2\) (HHP); carbonation at 1 atm (HHPcarb); carbonation and addition of 8.5 mL CO\(_2\)/g puree into the headspace of the package (HHPcarb + CO\(_2\)).

[00137] HHP treatment, carbonation and addition of CO\(_2\) to the headspace of the sample packages was performed as described for Example 1. All treatments were performed in triplicate.

[00138] For Treatment 1, HHP treatment was conducted at 100, 400 or 550 MPa at 35°C for 10 min. For the HHPcarb + CO\(_2\) samples, CO\(_2\) was injected into the headspace of the sample packages at the supercritical stage (pressure and temperature above the critical point of 7.28 MPa, 31.1°C) during HHP.

[00139] For the Treatment 2, HHP treatment was conducted at 100, 300 or 600 MPa at 25°C for 5 min. For the HHPcarb + CO\(_2\) samples, CO\(_2\) was injected into the headspace of the sample packages at a subcritical stage as the temperature during processing was below 31°C.

2.4 **HHP processing, carbonation and CO\(_2\) addition for *B. subtilis* and *S. cerevisiae***

[00140] Three CO\(_2\) treatments were used for this study, with the first two treatments (HHP and HHP carb) the same as in Example 1. The third treatment was carbonation and addition of CO\(_2\) into the bottle headspace for a further 1 minute to achieve a volume ratio of 2:1 (CO\(_2\); sample) equal to 0.4 % w/w CO\(_2\) (HHP + CO\(_2\)). In each case the bottles were capped and sealed.

[00141] HHP treatment was carried out using the same machinery as for Example 1. For each treatment, two bags (1 HHP sample and 1 HHPcarb) were treated together at a pressure of 200, 250 or 300 MPa for 2, 4 or 6 min at 25 °C. Each treatment was carried out in duplicate.

2.5 **Treatment of Ayran (salted) yoghurt samples***

[00142] Ayran yoghurt samples were prepared by mixing yogurt and salted distilled water (salt concentration of 0.01 g/g) at a ratio of 1:1 w/w.
[00143]  *E. coli* was first adapted to the acidic growth conditions of Ayran yogurt, and then inoculated into the samples. The inoculated samples were subjected to the HHP, HHPcarb and HHPcarb + CO₂ treatments described above. All treatments were performed in triplicate.

5 [00144]  For Treatment 1, HHP pressures of 300, 450 and 600 MPa were tested at a temperature of 25°C for 5 min.

[00145]  For Treatment 2, HHP pressures of 300, 450 and 600 MPa were tested at a temperature of 25°C for 10 min.

[00146]  For Treatment 3, HHP pressures of 300, 450 and 600 MPa were tested at a temperature of 25°C for 15 min.

3. Results

3.1.  *E. coli* broth medium

[00147]  Samples of *E. coli* broth medium were subjected to HHP, HHPcarb and HHPcarb + CO₂ treatments, and the effect of the different treatments on microbial inactivation was determined.

*Treatment 1*

[00148]  The addition of CO₂ at 400 MPa increased the rate of reduction of *E. coli* from a 4-log reduction with HHP treatment alone to an 8 log reduction in samples having CO₂ injected into the package headspace (HHPcarb + CO₂) as shown in Figure 14. At 550 MPa, an 8-8.5 log reduction in *E. coli* was observed with all treatments. No detectable difference in the appearance or viscosity of the samples was observed.

*Treatment 2*

[00149]  No significant difference in microbial inactivation was observed between the HHP only and HHPcarb + CO₂ samples at 100 and 600 MPa as shown in Figure 15.

[00150]  At 300 MPa, the addition of CO₂ reduced the microbial inactivation effect of HHP treatment from a 4 log reduction to a log reduction of less than 1 for HHPcarb and HHPcarb + CO₂ samples.

[00151]  All *E. coli* were eliminated with all treatments at 600 MPa. At this pressure, the temperature inside the HHP chamber climbed to 39–42 °C. At 100 and 300 MPa, the temperature did not reach 32°C.
3.2. Ayran (salted) yogurt samples

[00152] Microbial inactivation of *E. coli* was measured in salted yoghurt samples subjected to HHP, HHPcarb or HHPcarb + CO₂ treatments.

**Five minute HHP treatment**

[00153] No significant difference in microbial inactivation was observed between HHP, HHPcarb or HHPcarb + CO₂ treatments conducted for five minutes at all pressures tested as shown in Figure 16.

**Ten minute HHP treatment**

[00154] The addition of CO₂ during a 10 minute HHP treatment was inclined to enhance the microbial inactivation effect of high pressure treatment as shown in Figure 17. The HHPcarb + CO₂ treated samples had a greater log reduction of *E. coli* than samples treated with HHP alone at 300 and 450 MPa, by approximately 1.5 log. All *E. coli* were killed at 600Mpa in all treated samples.

[00155] At 450 MPa, a 6-log reduction in *E. coli* levels was achieved with HHPcarb + CO₂ treatment, which meets the Food Safety regulation “5-log reduction” requirement for an appropriate pasteurisation process.

[00156] This example shows that by adding CO₂ into the headspace of packaging during HHP treatment, the process can be conducted at a reduced pressure in order to meet the appropriate food safety requirement for microbial load.

**Visual observations**

[00157] There were no visual differences between Ayran salted yoghurt samples treated with HHP alone compared to samples treated with HHP with added CO₂ headspace.

3.3. *B. subtilis* broth medium

[00158] This example shows that the combined HHP and CO₂ treatment had a significant effect on the log reduction of *B. subtilis* (Figure 18).

[00159] The increase in CO₂ level had a significant correlation with the inactivation of *B. subtilis*. Thr addition of *in-situ* CO₂ resulted in an additional reduction of 6.5-7.5 log units in *B. subtilis* inactivation at low pressure (200 MPa) after a short treatment time of 4 min.
3.4. *S. cerevisiae* broth medium

[00160] This example shows that at 250 MPa and a treatment time of 4 min the inactivation of *S. cerevisiae* is significantly higher with the combined HHP and CO₂ treatment than with HHP treatment alone.

EXAMPLE 3

1. Introduction

[00161] This example investigates the use of flexible and durable plastic bottles in the disclosed methods.

2. Methods and results

[00162] Varying amounts of fruit puree were added to plastic bottles as shown in Table 3 below, resulting in specific ratios of sample volume to bottle headspace. CO₂ was injected into the bottles, and the volume and amount of CO₂ in the headspace and the weight ratio of CO₂ to sample were measured.

Table 3:

<table>
<thead>
<tr>
<th>Sample</th>
<th>Ratio by volume (sample: headspace)</th>
<th>Vol of sample (mL)</th>
<th>Weight of sample (g)</th>
<th>Vol CO₂ in headspace (mL)</th>
<th>Amount of CO₂ in headspace (g)</th>
<th>Ratio in weight (g/g)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>0</td>
<td>To do in a bag</td>
<td>30</td>
<td>Sat. with CO₂</td>
<td>0</td>
<td>0</td>
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<tr>
<td>2</td>
<td>5:1</td>
<td>325</td>
<td>300</td>
<td>65</td>
<td>0.1277</td>
<td>0.0004</td>
</tr>
<tr>
<td>3</td>
<td>4:2</td>
<td>260</td>
<td>240</td>
<td>130</td>
<td>0.2554</td>
<td>0.0011</td>
</tr>
<tr>
<td>4</td>
<td>3:3</td>
<td>195</td>
<td>180</td>
<td>195</td>
<td>0.3830</td>
<td>0.0021</td>
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<tr>
<td>5</td>
<td>2:4</td>
<td>130</td>
<td>120</td>
<td>260</td>
<td>0.5107</td>
<td>0.0043</td>
</tr>
<tr>
<td>6</td>
<td>1:5</td>
<td>65</td>
<td>60</td>
<td>325</td>
<td>0.6384</td>
<td>0.0106</td>
</tr>
<tr>
<td>7</td>
<td>1:11</td>
<td>33</td>
<td>30</td>
<td>357</td>
<td>0.7013</td>
<td>0.0234</td>
</tr>
</tbody>
</table>

[00163] The concentration of CO₂ in the samples that can be achieved is limited by the volume of the bottle. The maximum amount of CO₂ that could be achieved in the headspace of the bottle was 0.0234 g CO₂ per g sample (1:11 v/v sample: CO₂), that is, 2.5% w/w.

[00164] The plastic bottles containing the samples and CO₂ were subjected to high pressure processing. The bottles remained intact and retained their contents but were deformed following high pressure processing.

EXAMPLE 4
This example demonstrates the sensory attributes associated with fruit products processed using a combination of HHP and CO₂ treatments.

1. Sample preparation and storage

Frozen peeled feijos were thawed 16-18 h prior to processing. Thawed fruit was cut into 3-4cm cubes and blended with water (1:1 w/w). The blended feijoa puree was then divided into bottles and treated at three levels. The first two treatments (HHP and HHP carb) were the same as in Example 1. The third treatment was carbonation and addition of CO₂ into the bottle headspace for a further 1 minute to achieve a volume ratio of 2:1 (CO₂: sample) equal to 0.4 % w/w CO₂ (HHP + CO₂). In each case the bottles were capped and sealed.

2. Experimental procedure

Two sensory experiments were conducted in which 38 participants evaluated the organoleptic properties of HHP, HHPcarb and HHPcarb + CO₂ treated feijoa samples. Participants were asked to evaluate the feijoa products on appearance, colour, flavour and texture. In the first experiment, the participants evaluated unsweetened feijoa samples. In the second experiment, the participants evaluated feijoa samples sweetened with 10 % (w/w) sucrose. This added sucrose represented the amount of sugar in other commercially available fruit drinks. Participants were also asked to state whether they preferred the control sample or one of the treated samples.

3. Results

Participants found that the organoleptic properties of the unsweetened feijoa samples were only moderately different to the control samples (fresh feijoa juice). The sweetened samples had comparable properties to the control samples. There was no significant difference in preference for the sweetened HHPcarb + CO₂ treated samples as compared to the control.

EXAMPLE 5

This example demonstrates the enhanced shelf life of products treated with a combination of HHP and CO₂. The samples prepared and treated in Example 3 were stored at -20 °C for 28 days and residual enzyme activity was measured.

The results showed that samples treated with the highest level of CO₂ showed a significant reduction in POD, PPO and PME activity (Figures 20, 21 and 22 respectively) as compared to the untreated control sample.
[00171] The foregoing describes the invention including specific implementations thereof. Alterations and modifications as will be obvious to those skilled in the art are intended to be incorporated within the scope thereof.
WHAT IS CLAIMED IS

1. A method of processing a product, the method comprising subjecting one or more products to elevated hydrostatic pressure and simultaneously contacting the one or more products with carbon dioxide.

2. A method of claim 1 comprising contacting the one or more products with at least about 1% w/w carbon dioxide, relative to the weight of the one or more products.

3. A method of claim 1 or 2 wherein the one or more products are enclosed in a sealed container comprising
   a flexible portion comprising an internal volume, the one or more products being
   in the internal volume, and
   a removable reservoir of variable volume that is fluidly communicable with the internal volume of the flexible portion, the volume of the removable reservoir being variable on the application of hydrostatic pressure to the exterior of the removable reservoir, the reservoir comprising at least about 0.1% w/w carbon dioxide relative to the weight of the one or more products.

4. A method of processing a product, the method comprising
   providing a sealed container comprising
   a flexible portion comprising an internal volume, with one or more products in the internal volume, and
   a removable reservoir of variable volume that is fluidly communicable with the internal volume of the flexible portion, the volume of the removable reservoir being variable on the application of hydrostatic pressure to the exterior of the removable reservoir, the reservoir comprising at least about 0.1% w/w carbon dioxide relative to the weight of the one or more products,
   subjecting the sealed container to elevated hydrostatic pressure, the pressure varying the volume of the removable reservoir and transferring at least a portion of the carbon dioxide from the reservoir into the internal volume.

5. A method of claim 4 comprising
   placing one or more products in a container, the container comprising
   a flexible portion comprising an internal volume, the internal volume enclosing the one or more products, and
a removable reservoir of variable volume that is fluidly communicable with
the internal volume of the flexible portion, the volume of the reservoir being
variable on the application of hydrostatic pressure to the exterior of the reservoir,
the reservoir comprising at least about 0.1% w/w carbon dioxide relative to the
weight of the product or products,

subjecting the container to elevated hydrostatic pressure, the pressure varying
the volume of the reservoir and transferring at least a portion of the carbon dioxide from
the reservoir into the internal volume.

6. A method of any one of claims 3 to 5 wherein the one or more products
substantially fill the internal volume.

7. A method of any one of claims 1 to 6 wherein the one or more products
comprises a liquid product, a semi-liquid product, one or more solid products, or any
combination of any two or more thereof.

8. A method of any one of claims 1 to 6 wherein the pH of the one or more products
is about 1 to about 7.

9. A method of any one of claims 1 to 8 wherein the one or more products comprise
a carbonated liquid.

10. A method of any one of claims 1 to 9 wherein the one or more products comprise
a juice.

11. A method of any one of claims 1 to 6 wherein the one or more products
comprises one or more of one or more powders, one or more pills, one or more capsules,
one or more tablets, one or more solid food items, or any combination of any two of
more thereof.

12. A method of any one of claims 1 to 6 wherein the pH of the one or more products
is about 7 to about 10.

13. A method of any one of claims 1 to 12 wherein the elevated hydrostatic pressure
comprises a hydrostatic pressure of at least about 33 MPa.

14. A method of any one of claims 1 to 12 wherein the elevated hydrostatic pressure
is held at a pressure of at least about 33 MPa for at least about 1 second, or once the
elevated hydrostatic pressure comprises a pressure of at least about 33 MPa, the
pressure is released.
15. A method of any one of claims 1 to 12 wherein the one or more products are subjected to an elevated hydrostatic pressure of at least about 150 MPa for at least about 1 minute.

16. A method of any one of claims 1 to 15 wherein the one or more products is subjected to and/or the reservoir comprises at least about 2% w/w carbon dioxide relative to the weight of the one or more products.

17. A method of any one of claims 1 to 15 wherein the one or more products is subjected to and/or the reservoir comprises at least about 5% w/w carbon dioxide relative to the weight of the one or more products.

18. A method of any one of claims 1 to 17 wherein the aerobic plate count of the one or more products is less than about 100,000 cfu/ml after the step of subjecting the one or more products or the sealed container to the elevated hydrostatic pressure.

19. A method of any one of claims 1 to 17 wherein the aerobic plate count of the one or more products is less than about 50,000 cfu/ml after the step of subjecting the one or more products or the sealed container to the elevated hydrostatic pressure.

20. A method of any one of claims 1 to 19 wherein the one or more products comprise one or more enzymes and after the step of subjecting the one or more products or the sealed container to the elevated hydrostatic pressure the activity of one or more enzymes is at least about 5% less than the activity of activity of the one or more enzymes in an untreated control.

21. A method of any one of claims 1 to 20 wherein the visual appearance and/or organoleptic properties of the one or more products are not substantially different after the step of subjecting the one or more products or the sealed container to the elevated hydrostatic pressure.

22. A method of any one of claims 2 to 21 wherein the removable reservoir comprises opposing one-way valves that are fluidly communicable with the internal volume of the flexible portion.

23. A method of any one of claims 2 to 22 wherein the volume of the removable reservoir is varied by the movement of a piston or the deformation of a bladder.

24. A method of any one of claims 2 to 23 further comprising removing the removable reservoir and fixing a consumer closure to the container.

25. Apparatus for use in a method of any of claims 1 to 24 comprising
a fitting that is removably attachable to a flexible container, the flexible container comprising an internal volume, and

a reservoir of variable volume that is fluidly communicable with the internal volume of the flexible container, the volume of the reservoir being variable on the application of hydrostatic pressure to the exterior of the removable reservoir.

26. Apparatus for use in a method of any of claims 1 to 24 comprising

a flexible portion comprising an internal volume, and

a removable reservoir of variable volume that is fluidly communicable with the internal volume of the flexible portion, the volume of the removable reservoir being variable on the application of hydrostatic pressure to the exterior of the removable reservoir.
FIGURE 10

FIGURE 11
FIGURE 22
## A. CLASSIFICATION OF SUBJECT MATTER

A23L 3/00 (2006.01)  A23L 2/52 (2006.01)  A23L 2/42 (2006.01)  A23L 3/3418 (2006.01)  B65D 47/12 (2006.01)

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)

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)


## C. DOCUMENTS CONSIDERED TO BE RELEVANT

<table>
<thead>
<tr>
<th>Category*</th>
<th>Citation of document, with indication, where appropriate, of the relevant passages</th>
<th>Relevant to claim No.</th>
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</table>

Documents are listed in the continuation of Box C

[X] Further documents are listed in the continuation of Box C  
[X] 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 3 August 2015

Date of mailing of the international search report 03 August 2015

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Telephone No. 0262832927

Form PCT/ISA/210 (fifth sheet) (July 2009)
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<td>ORTUÑO, C. et al., ‘Combined high hydrostatic pressure and carbon dioxide inactivation of pectin methylsterase, polyphenol oxidase and peroxidase in feijoa puree’, Journal of Supercritical Fluids, 82, 2013, pages 56-62. Page 58, sections 2.4.1-2.4.2; page 60, Section 3.4; Fig. 1-4</td>
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<td>PARK, S.J. et al., ‘Inactivation Kinetics of Food Poisoning Microorganisms by Carbon Dioxide and High Hydrostatic Pressure’, Journal of Food Science, Vol. 68, Nr. 3, 2003, Pages 976-981. Abstract; Figures 1-2; Section: Serial treatment of CO2 and HHP on pages 976-977; Figure 1</td>
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<td>US 2011/0070341 A1 (RICHTER) 24 March 2011 Para 0011, 0029, 0045, 0062; Fig. 1</td>
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<td>US 2014/0072678 A1 (JENKINS) 13 March 2014 Fig. 1-2</td>
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<td>US 2013/0175304 A1 (PEIRSMAN et al.) 11 July 2013 Para 0047; Figure 1</td>
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<td>EP 1 392 567 B1 (THE COCA-COLA COMPANY) 18 January 2006 Para 0015-0020; Fig. 2-4</td>
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</tbody>
</table>
Box No. II  Observations where certain claims were found unsearchable (Continuation of item 2 of first sheet)

This international search report has not been established in respect of certain claims under Article 17(2)(a) for the following reasons:

1. ☐ Claims Nos.:
   because they relate to subject matter not required to be searched by this Authority, namely:
   the subject matter listed in Rule 39 on which, under Article 17(2)(a)(i), an international search is not required to be
carried out, including

2. ☐ Claims Nos.:
   because they relate to parts of the international application that do not comply with the prescribed requirements to such
an extent that no meaningful international search can be carried out, specifically:

3. ☐ Claims Nos.:
   because they are dependent claims and are not drafted in accordance with the second and third sentences of Rule 6.4(a)

Box No. III  Observations where unity of invention is lacking (Continuation of item 3 of first sheet)

This International Searching Authority found multiple inventions in this international application, as follows:

See Supplemental Box for Details

1. ☐ As all required additional search fees were timely paid by the applicant, this international search report covers all
searchable claims.

2. ☒ As all searchable claims could be searched without effort justifying additional fees, this Authority did not invite
payment of additional fees.

3. ☐ As only some of the required additional search fees were timely paid by the applicant, this international search report
covers only those claims for which fees were paid, specifically claims Nos.:

4. ☐ No required additional search fees were timely paid by the applicant. Consequently, this international search report is
restricted to the invention first mentioned in the claims; it is covered by claims Nos.:

Remark on Protest

☐ The additional search fees were accompanied by the applicant's protest and, where applicable, the payment of a protest fee.

☐ The additional search fees were accompanied by the applicant's protest but the applicable protest fee was not paid within the time limit specified in the invitation.

☐ No protest accompanied the payment of additional search fees.
Continuation of: Box III

This International Application does not comply with the requirements of unity of invention because it does not relate to one invention or to a group of inventions so linked as to form a single general inventive concept.

This Authority has found that there are different inventions based on the following features that separate the claims into distinct groups:

Group I.

The claims 1-24 are directed to a method of treating to products comprising the step of simultaneously treating the said products with carbon dioxide and elevated hydrostatic pressure.

Group II.

The claims 25-26 are directed to an apparatus comprising a flexible container in fluid communication with a reservoir, volume of which is variable on application of hydrostatic pressure. Note that the claimed apparatus merely has to be suitable for use in the claimed method and not limited to the features of the said method.

PCT Rule 13.2, first sentence, states that unity of invention is only fulfilled when there is a technical relationship among the claimed inventions involving one or more of the same or corresponding special technical features. PCT Rule 13.2, second sentence, defines a special technical feature as a feature which makes a contribution over the prior art.

When there is no special technical feature common to all the claimed inventions there is no unity of invention.

In the above groups of claims, the identified features may have the potential to make a contribution over the prior art but are not common to all the claimed inventions and therefore cannot provide the required technical relationship. Therefore there is no special technical feature common to all the claimed inventions and the requirements for unity of invention are consequently not satisfied a priori.

Note that the use of the wording "Apparatus for use in a method" in claims 25-26 defines a capability only and apparatus is not considered to be limited to the use of apparatus in the claimed method.

While the PCT rules for determining unity of invention allow an independent claim for a given method plus an independent claim for an apparatus or means specifically designed for carrying out said method, the apparatus or means is only considered specifically designed for carrying out a method if the contribution over the prior art of the apparatus or means corresponds to the contribution the process makes over the prior art. In the present case, the apparatus of claims 25-26 appear to be indistinguishable from other prior art apparatus (See D9-D11 in ISR). The apparatus of claims 25-26 do not have any special adaptation to carry out the method of claim 1.
This Annex lists known patent family members relating to the patent documents cited in the above-mentioned international search report. The Australian Patent Office is in no way liable for these particulars which are merely given for the purpose of information.

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Due to data integration issues this family listing may not include 10 digit Australian applications filed since May 2001.
Form PCT/ISA/210 (Family Annex)(July 2009)
This Annex lists known patent family members relating to the patent documents cited in the above-mentioned international search report. The Australian Patent Office is in no way liable for these particulars which are merely given for the purpose of information.

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End of Annex