

Recovery of natural colouring in plant-based beverages after thermal treatment

Introduction

Current methods of creating red colour in plant-based beverages mainly involve the use of artificial red colours such as carmine, allura red AC, carmosine or the use of oil soluble carotenoids, such as beta-carotene and lycopene. Artificial red colours can create stable red colour in products but are disliked by consumers because of concerns about side effects such as hyperactivity and mild allergy development. The use of carotenoids for red colour creation is also limited by the fact that they turn orange upon heat treatment.

Natural extracts from beetroot have been proposed as natural red colourants for plant-based meat alternatives. However, it is well known that red beetroot colour degrades upon heating. The thermal degradation of red beetroot occurs at elevated temperatures that are typical of the pre-heater and holding tube of UHT processors which are required to render a plant based ready to drink beverage microbiologically safe for consumption. The thermal degradation of red beetroot changes the colour of a product by decreasing the intensity of positive a-value (red hue) and increasing the intensity of positive b-value (yellow hue) on the lab colour scale. This has the net result of turning the product from a natural red colour to an orange/brown colour.

A possible explanation for the colour loss and regeneration is that betalain, the molecule responsible of the red color of beetroot, undergoes hydrolysis due to the pH of the milk above 6 and heat treatment. The hydrolysis of betalain is reversible after short heat treatment and the regeneration rate is improved when stored at temperatures less than 10°C. Betanin regeneration after hydrolysis is pH dependent, the reaction being favored at pH 6 in the absence of oxygen, while at pH 7 no pigment re-condensation was found.

Chinese patent CN111067009 outlines a method for improving the thermal stability of beet red pigment via a complex combination of hydrocolloids, anti-oxidants and cyclodextrin. This complex combination has several drawbacks including its high cost, low consumer familiarity and increasing product viscosity.

New solutions are therefore needed to prevent the colour degradation of red beetroot in pasteurised and UHT treated plant-based ready to drink beverages.

Summary of the invention

The present invention uses a combination of less refined proteins, anti-oxidants and chelation agents to recover the red colour of red beet based RTD plant based beverages. In particular, the colour recovery is focused on the decrease in positive b-value during the first two weeks of shelf life which effectively eliminates orange colour. This loss of orange hue after UHT treatment is what is key in the recovery of the red colour. It also seems that the addition of sodium ascorbate improves the regeneration rate of the red color.

Embodiments of the invention

The invention relates in general to a method of making a plant-based liquid, said method comprising dissolving a plant protein source in water to form a plant protein mixture; adding a red beet extract to the plant protein mixture; dispersing oil in the plant protein mixture; homogenizing the plant protein mixture to form an emulsion; applying a thermal treatment to the emulsion; and applying a shear treatment to the thermal treated emulsion to form a plant-based liquid.

The invention further relates to a method of making a plant-based liquid, said method comprising dissolving a plant protein source in water to form a plant protein mixture; adding a powdered or liquid red beet extract to the plant protein mixture; dispersing oil in the plant protein mixture; homogenizing the plant protein mixture to form an emulsion; applying a thermal treatment to the emulsion; and applying a shear treatment to the thermal treated emulsion to form a plant-based liquid.

The invention further relates to a method of making a plant-based liquid, said method comprising

- a. Dissolving a plant protein source in water to form a plant protein mixture;
- b. Adding a powdered or liquid red beet extract to the plant protein mixture;
- c. Optionally incubating the plant protein mixture with enzymes;
- d. Optionally adding a hydrocolloid(s) to the plant protein mixture;
- e. Dispersing oil in the plant protein mixture;
- f. Homogenizing the plant protein mixture to form an emulsion;
- g. Applying a thermal treatment to the emulsion; and
- h. Applying a shear treatment to the thermal treated emulsion to form a plant-based liquid.

The invention further relates to a method of making a plant-based liquid, said method comprising

- a. Dissolving a plant protein source in water to form a plant protein mixture with a pH of 6.5 or greater;
- 5 b. Adding a powdered or liquid red beet extract to the plant protein mixture at a pH greater than 6 and stirring to dissolve;
- c. Optionally incubating the plant protein mixture with enzymes;
- d. Optionally adding a hydrocolloid(s) to the plant protein mixture;
- e. Dispersing oil in the plant protein mixture;
- 10 f. Homogenizing the plant protein mixture to form an emulsion;
- g. Applying a thermal treatment to the emulsion; and
- h. Applying a shear treatment to the thermal treated emulsion to form a plant-based liquid.

In some embodiments, sodium ascorbate is dissolved in the plant protein mixture before or during step e).

In some embodiments, sodium ascorbate and citric acid are dissolved in the plant protein mixture before or during step e).

In some embodiments, the plant protein source is derived from a legume source such as red bean, adzuki bean (*Vigna angularis*), red kidney bean (*Phaseolus vulgaris*), pea (*Pisum sativum*), faba bean (*Vicia faba*), chickpea (*Cicer arietinum*) or lentil (*Lens culinaris*).

In some embodiments, a calcium source is dissolved in the plant protein mixture.

In some embodiments, a sugar is dissolved in the plant protein mixture.

In some embodiments, the calcium source comprises tricalcium phosphate, calcium citrate or calcium carbonate.

25 In some embodiments, the plant protein mixture is adjusted to a pH of between 6.5 to 8 before incubating with enzymes.

In some embodiments, the enzymes are amylase and glycoside hydrolase.

In some embodiments, the emulsion is formed using a two-stage high pressure homogenizer.

In some embodiments, the emulsion average particle size is less than 1 μm , as measured by laser diffraction.

In some embodiments, the viscosity of the plant based liquid is less than 75 mPa.s at 12.7s⁻¹.

In some embodiments, the plant based liquid comprises less than 0.5% starch.

In some embodiments, the plant based liquid has an A value > than 10 and a hue angle between 330 and 30°, preferably 345 and 15° more preferably 350 and 10°.

35 The invention further relates to a plant based milk analogue made by a method according to the invention.

Detailed description of the invention

In some embodiments, the red bean concentrate has a protein content of at least 45 wt% on a dry basis, or at least 50 wt% on a dry basis, or between 45 to 70 wt% on a dry basis, or between 50 to 65 wt% on a dry basis.

- 5 Further ingredients that act as a buffer, as well as sugar, can be added. In some embodiments, a phosphate source is dissolved in the plant protein mixture. Preferably, the phosphate source comprises tricalcium phosphate and dipotassium phosphate. Preferably, the sugar is sucrose. In some embodiments, the sugar is a sucrose alternative.

- The plant protein mixture is optionally incubated with enzymes to reduce liquid viscosity. In
10 some embodiments, the plant protein mixture is adjusted to a pH of between 7 to 8 followed by incubating with enzymes. In some embodiments, the enzymes are amylase and glycoside hydrolase.

- The plant protein mixture is emulsified. In some embodiments, the emulsion is formed using a two-stage high pressure homogenizer. In some embodiments, the fat droplet emulsion
15 average particle size is between 0.1 and 1µm for d[3,2] and between 0.3 and 2 µm for d[4,3]. Preferably the emulsion average particle size is between 0.1 and 0.7 for d[3,2] and between 0.3 and 1.5 µm for d[4,3], as measured by laser diffraction.

- The red beet (*Beta vulgaris* Conditiva Group) extract is a liquid or a powder made of concentrated juice coming from red beet (also known as beetroot, table beet, garden beet,
20 dinner beet or golden beet), maltodextrin or other carrier and ascorbic acid or other antioxidant.

A thermal treatment is applied to the emulsion to render it microbiologically stable as well as to reduce its viscosity. In some embodiments, the thermal treatment is ultra high temperature treatment (UHT).

- 25 A shear treatment is applied to the thermal treated emulsion. In some embodiments, the shear treatment is applied using a high shear homogenizer. In some embodiments, the viscosity of the plant based liquid after shear treatment is between 0.1 and 75mPa.s, preferably less between 0.5 and 50 mPa.s, more preferably between 15 and 40 mPa.s at a shear rate of 12.7s⁻¹ at 25°C.

- 30 The plant-based liquid is typically low in starch. In some embodiments, the plant-based liquid comprises less than 2 wt% starch, preferably less than 1 wt% starch, more preferably less than 0.5 wt% starch.

The plant based liquid can take several forms. In some embodiments, the plant based liquid is a milk analogue.

- 35 The invention also provides a plant based milk analogue made by a method as described herein.

The invention also provides a plant based liquid comprising a plant protein source; optionally a hydrocolloid(s); and triglyceride.

In some embodiments, the plant-based liquid has a viscosity between 0.1 and 100mPa.s, preferably less between 0.5 and 30 mPa.s, more preferably between 0.5 and 15 mPa.s at a
5 shear rate of 10s⁻¹ at 25°C.

The plant-based liquid is typically low in starch. In some embodiments, the plant based liquid comprises less than 2 wt% starch, preferably less than 1 wt% starch, more preferably less than 0.5 wt% starch.

10 When a composition is described herein in terms of wt%, this means wt% of the total recipe, unless indicated otherwise.

As used herein, "about" is understood to refer to numbers in a range of numerals, for example the range of -30% to +30% of the referenced number, or -20% to +20% of the referenced number, or -10% to +10% of the referenced number, or -5% to +5% of the referenced number,
15 or -1% to +1% of the referenced number. All numerical ranges herein should be understood to include all integers, whole or fractions, within the range. Moreover, these numerical ranges should be construed as providing support for a claim directed to any number or subset of numbers in that range. For example, a disclosure of from 45 to 55 should be construed as supporting a range of from 46 to 54, from 48 to 52, from 49 to 51, from 49.5 to 50.5, and so
20 forth.

As used herein, an "analogue" of a substance is considered to be a parallel of that substance in regard to one or more of its major characteristics. A "milk analogue" as used herein will parallel milk in the major characteristics of purpose, usage, and nutrition. It has similar levels of energy, protein, carbohydrates, vitamins and minerals. Preferably, the milk analogue is an
25 analogue of cow's milk.

The term "vegan" refers to an edible composition which is entirely devoid of animal products, or animal derived products.

Plant protein sources based on faba, pea, chickpea, lentil, cowpea, pinto bean, mung bean, adzuki bean, common bean, kidney beans, navy beans or similar high carbohydrate (greater
30 than 30 wt%) - low fat (less than 15%) crops or the like may be used.

Starch degrading enzymes such as amylase, α -amylase (from any species, from *Bacillus amyloliquefaciens*, *Bacillus licheniformis*, *Aspergillus oryzae*, *Aspergillus niger*), more preferably a Saccharifying α -amylase (e.g. from *Bacillus subtilis* (*amyloliquefaciens*), or most
35 preferably an amylase in combination with glucoamylase (also known as amyloglucosidase or glycoside hydrolase for example AMG 1100 BG from novozymes) or the like may be used.

Fat sources may include vegetable oils, animal fats, milk fats, fish oil, algal oil, sunflower oil, olive oil, canola oil, cotton seed oil, palm fat, palm stearin, palm kernel oil, corn oil, coconut oil, and/or high oleic acid sunflower oil, any solid fat stock such as refined coconut oil, anhydrous milk fat, hydrogenated vegetable oil, tallow, lard, any nut butter/oil such as almond butter, peanut butter, walnut butter, cashew butter and/or hydrogenated or partially hydrogenated fats. Preferably, the fat source is a plant based fat source, for example vegetable oils, algal oil, sunflower oil, olive oil, canola oil, cotton seed oil, palm fat, palm stearin, palm kernel oil, corn oil, coconut oil, and/or high oleic acid sunflower oil, any solid fat stock such as refined coconut oil, anhydrous milk fat, hydrogenated vegetable oil, any nut butter/oil such as almond butter, peanut butter, walnut butter, cashew butter and/or hydrogenated or partially hydrogenated fats.

Sodium ascorbate alternatives include vitamin C, sodium ascorbate, calcium ascorbate, vitamin C palmitate, fruit juices rich in vitamin C (≥ 500 mg vitamin C per 100 mL), acerola extract, sodium bisulfite, iodine, potassium iodide, sorbic acid, potassium sorbate, sulfite derivatives such as sodium sulfite, sodium hydrogen sulfite, sodium metabisulfite, potassium metabisulfite, calcium sulfite, calcium hydrogen sulfite.

Buffer alternatives include dipotassium phosphate, trisodium citrate, tripotassium citrate, tripotassium phosphate, sodium bicarbonate, baking soda, bicarbonate of soda, disodium phosphate, trisodium phosphate, monopotassium phosphate, citric acid, lemon juice.

Calcium sources include tricalcium phosphate, calcium carbonate, calcium glycerolphosphate, calcium citrate.

Sucrose alternatives include cane sugar, beet sugar, glucose syrup, maltodextrin, honey and other natural sugar syrups such as agave. Preferably, the sucrose alternative is glucose syrup. A hydrocolloid means a stabiliser based on (high or low acetyl) gellan, guar gum, (high or low methoxy) pectin, locust bean gum, alginate, carrageenan, carboxymethylcellulose, microcrystalline cellulose, curdlan, xanthan gum or the like may be used. In one embodiment, the hydrocolloid is gellan.

The fat in the plant protein mixture is emulsified. In some embodiments, the emulsion is formed using a two-stage high pressure homogenizer. In some embodiments, the fat emulsion average particle size is between 0.1 and 1 μm for $d[3,2]$ and 0.3 and 2 μm for $d[4,3]$, preferably the fat emulsion average particle size is between 0.1 and 0.7 μm for $d[3,2]$ and 0.3 and 1.5 μm for $d[4,3]$, as measured by laser diffraction.

Pasteurization heat treatment can be within a range from 60°C – 100°C for 1 second to 300 seconds.

Thermal treatment, for example direct and indirect UHT heat treatment can be within a range of 110°C to 150°C for 3 seconds to 60 seconds, preferably the thermal heat treatment is indirect heat treatment.

Retort heat treatment the thermal process delivered to this product shall be designed to deliver a lethality (F_0) in the range 5.0 – 15 minutes (or higher) but in no case shall be lower than 3.0 minutes. Upon establishing the come-up time (CUT) to reach the pre-determined minimum temperature in the retort, a time and temperature range that may satisfy the lethality range is 7 – 25 minutes at 119 – 125 deg C in the sterilization step. The CUT and sterilization time are to be established by a competent thermal process authority.

The plant protein mixture typically contains up to 20 wt% starch, preferably between 2 and 14 wt% and up to 20 wt% fibre, preferably between 6 to 18 wt% on a dry weight basis.

A legume is a plant in the family Fabaceae (or Leguminosae), the seed of such a plant (also called pulse). Legumes are grown agriculturally, primarily for human consumption, for livestock forage and silage, and as soil-enhancing green manure. As used herein, the term "legume" may include: pea, faba bean, chickpea, lentils, kidney beans, navy beans, pinto beans, haricot beans, lima beans, butter beans, red beans, azuki beans, mung beans, golden gram, green gram, black gram, urad, scarlet runner beans, rice beans, garbanzo beans, cranberry beans, lima beans, green peas, snow peas, snap peas, split peas and black-eyed peas. Preferably, the legume is selected from red beans, adzuki beans, pea, faba bean, chickpea, cannellini beans, navy beans and lentils.

Red bean, also known as Adzuki bean, azuki bean, aduki bean, or red mung bean, is a species of flowering plant in the bean family *Vigna angularis*.

By way of example and not limitation, the following examples are illustrative of various embodiments of the present invention.

Examples

Example 1

Dairy milk with red beet extract

A dairy pink milk was prepared by dissolving 1 g of beetroot extract (Naturex beetroot extract HC PWS) in 2 L of whole milk. The product was rendered microbiologically stable by thermal treatment of 138°C 15 s through heat exchangers.

5 Example 2

Red bean milk plus red beet extract with no additives

A red bean pink milk was prepared by dissolving 1.2 g of sodium bicarbonate in 1.8 L water at 65°C. Once the salt is dissolved, 39 g of air classified red bean concentrate (52% protein dry mass) were added and dispersed under stirring with a rotor stator for 15 minutes to ensure
10 good dissolution of the proteins. 88 g of sugar and 2.4 g of gellan (High acyl 103B) were added to the red bean dispersion and mixed for 5 minutes. 70 g of high oleic sunflower oil were then added and mixed at high shear to obtain a pre-emulsion. Finally, 1 g of beetroot extract (Naturex beetroot extract HC PWS) is added to the pre-emulsion. A fine emulsion was then created by passing through a two-stage high pressure homogenizer (200 bar / 50 bar
15 first/second stage homogenization pressures). The product was rendered microbiologically stable by thermal treatment of 138°C 15 s through heat exchangers.

Example 3

Red bean milk plus red beet extract with ascorbic acid

A red bean pink milk was prepared by dissolving 1.2 g of sodium bicarbonate in 1.8 L water at 65°C. Once the salt is dissolved, 39 g of red bean concentrate and 1.4 g of sodium ascorbate were added and dispersed under stirring with a rotor stator for 15 minutes to ensure good dissolution of the proteins. 88 g of sugar and 2.4 g of gellan (High acyl 103B) were added to the red bean dispersion and mixed for 5 minutes. 70 g of high oleic sunflower oil were then
25 added and mixed at high shear to obtain a pre-emulsion. Finally, 1 g of beetroot extract (Naturex beetroot extract HC PWS) is added to the pre-emulsion. A fine emulsion was then created by passing through a two-stage high pressure homogenizer (200 bar / 50 bar first/second stage homogenization pressures). The product was rendered microbiologically stable by thermal treatment of 138°C 15 s through heat exchangers.

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Example 4

Red bean milk plus red beet extract with tripotassium citrate

A red bean pink milk was prepared by dissolving 1.2 g of sodium bicarbonate and 10 g tripotassium citrate in 1.8 L water at 65°C. Once the salt is dissolved, 39 g of red bean concentrate were added and dispersed under stirring with a rotor stator for 15 minutes to ensure good dissolution of the proteins. 88 g of sugar and 2.4 g of gellan (High acyl 103B) were added to the red bean dispersion and mixed for 5 minutes. 70 g of high oleic sunflower
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oil were then added and mixed at high shear to obtain a pre-emulsion. Finally, 1 g of beetroot extract (Naturex beetroot extract HC PWS) is added to the pre-emulsion. A fine emulsion was then created by passing through a two-stage high pressure homogenizer (200 bar / 50 bar first/second stage homogenization pressures). The product was rendered microbiologically stable by thermal treatment of 138°C 15 s through heat exchangers.

Example 5

Red bean milk plus red beet extract with ascorbic acid plus tripotassium citrate

A red bean pink milk was prepared by dissolving 1.2 g of sodium bicarbonate and 10 g tripotassium citrate in 1.8 L water at 65°C. Once the salt is dissolved, 39 g of red bean concentrate and 1.4 g of sodium ascorbate were added and dispersed under stirring with a rotor stator for 15 minutes to ensure good dissolution of the proteins. 88 g of sugar and 2.4 g of gellan (High acyl 103B) were added to the red bean dispersion and mixed for 5 minutes. 70 g of high oleic sunflower oil were then added and mixed at high shear to obtain a pre-emulsion. Finally, 1 g of beetroot extract (Naturex beetroot extract HC PWS) is added to the pre-emulsion. A fine emulsion was then created by passing through a two-stage high pressure homogenizer (200 bar / 50 bar first/second stage homogenization pressures). The product was rendered microbiologically stable by thermal treatment of 138°C 15 s through heat exchangers.

Example 6

Red color results

In all the milks the heat treatment alters the colour of all the milks decreasing the red hue and increasing the orange one leading to more orange than pink milks. The dairy milk is the less affected by the heat treatment and shows no colour recovery over time. The four plant-based red bean milks are more affected by the heat treatment than the dairy one, being more orange just after heat treatment (lower a and higher b values), but recovering the pink colour over storage time at 4°C.

The addition of ascorbic acid in the milk improves the recovery rate of the pink colour leading to milks with higher a and lower b values after 10 weeks, than samples without. The combination of ascorbic acid and citrate leads to the highest removal of orange hue and thus the more pink milk over time, reaching a and b value close to the ones before heat treatment.

	a value before heat treatment	b value before heat treatment
Dairy milk	24.0	-6.6
No additives	22.9	-1.9

Ascorbic acid	22.2	-3.3
Citrate	23.2	-2.8
Ascorbic acid + citrate	23.2	-2.3

Claims

1. A method of making a plant based liquid, said method comprising
 - a. Dissolving a plant protein source in water to form a plant protein mixture with a pH of 6.5 or greater;
 - 5 b. Adding a powdered or liquid red beet extract to the plant protein mixture at a pH greater than 6 and stirring to dissolve;
 - c. Optionally incubating the plant protein mixture with enzymes;
 - d. Optionally adding a hydrocolloid(s) to the plant protein mixture;
 - e. Dispersing oil in the plant protein mixture;
 - 10 f. Homogenizing the plant protein mixture to form an emulsion;
 - g. Applying a thermal treatment to the emulsion; and
 - h. Applying a shear treatment to the thermal treated emulsion to form a plant based liquid.
2. The method according to claim 1, wherein sodium ascorbate is dissolved in the plant protein mixture before or during step e).
- 15 3. The method according to claim 1, wherein sodium ascorbate and citric acid are dissolved in the plant protein mixture before or during step e).
4. The method according to claims 1 and 2, wherein the plant protein source is derived from a legume source such as red bean, adzuki bean (*Vigna angularis*), red kidney bean (*Phaseolus vulgaris*), pea (*Pisum sativum*), faba bean (*Vicia faba*), chickpea (*Cicer arietinum*) or lentil (*Lens culinaris*).
- 20 5. The method according to any preceding claim, wherein a sugar is dissolved in the plant protein mixture.
6. The method according to claim 4, wherein the calcium source comprises tricalcium phosphate, calcium citrate or calcium carbonate.
- 25 7. The method according to any preceding claim, wherein the plant protein mixture is adjusted to a pH of between 6.5 to 8 before incubating with enzymes.
8. The method according to any preceding claim, wherein the enzymes are amylase and glycoside hydrolase.
- 30 9. The method according to any preceding claim, wherein the emulsion is formed using a two-stage high pressure homogenizer.
10. The method according to any preceding claim, wherein the emulsion average particle size is less than 1 μm , as measured by laser diffraction.
11. The method according to any preceding claim, wherein the viscosity of the plant based liquid is less than 75 mPa.s at 12.7s⁻¹.
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12. The method according to any preceding claim, wherein the plant based liquid comprises less than 0.5% starch.
13. The method according to any preceding claim, wherein the plant based liquid has an A value > than 10 and a hue angle between 330 and 30°, preferably 345 and 15° more preferably 350 and 10°.
- 5 14. A plant based milk analogue made by a method according to claims 1 to 13.