

Spent coffee grounds-derived beverageTechnical Field

The present invention relates to an alcoholic beverage derived from spent coffee grounds.

Background

Spent coffee grounds (SCG) are the residue remaining after coffee brewing and instant coffee manufacturing. Huge amounts of SCG are generated every year. SCG is discarded as food waste and this causes serious environmental hazards. The rising concerns about the negative effects of SCG on environment have resulted in the development of processing SCG for use in the food industry. However, fermentable sugars present in SCG cannot support sufficiently the growth of bacteria or yeasts.

There is therefore a need for an improved method and use of SCG.

Summary of the invention

The present invention seeks to address these problems, and/or provides a novelty spent coffee grounds-derived beverage.

According to a first aspect, there is provided an alcoholic beverage comprising esters and having an ethanol content of $\leq 10\%$ by volume, wherein the esters comprise at least one of: ethyl 2,4-hexadienoate and ethyl decanoate; and wherein the alcoholic beverage is an undistilled alcoholic beverage and is derived from spent coffee grounds.

The beverage may further comprise at least one ester selected from, but not limited to, ethyl (*E*)-4-hexenoate, isoamyl acetate, 3-methylbutyl octanoate, ethyl nonanoate, ethyl 9-decenoate, phenethyl acetate.

According to a particular aspect, the beverage may be a fermented beverage. The beverage may have a suitable ethanol content. For example, the beverage may have an ethanol content of 0.5-8% by volume.

The beverage may have any suitable pH. For example, the pH of the beverage may be 3.5-6.5.

The beverage may have a suitable Brix. For example, the Brix of the beverage may be 5-26°Bx.

According to a second aspect of the present invention, there is provided a method of forming an undistilled alcoholic beverage derived from spent coffee grounds, the method comprising:

- hydrolysing a liquid mixture of spent coffee grounds to obtain a hydrolysed liquid mixture;
- adding yeast to the hydrolysed liquid mixture; and
- fermenting the hydrolysed liquid mixture comprising yeast at a pre-determined temperature for a pre-determined period of time to form the beverage.

In particular, the alcoholic beverage formed from the method may comprise esters, the esters comprising at least one ester selected from: ethyl 2,4-hexadienoate and ethyl decanoate.

The hydrolysing may comprise acid hydrolysing the liquid mixture. In particular, the acid hydrolysing may be with a food-grade acid. The food-grade acid may be any suitable acid, such as, but not limited to, citric acid, malic acid, lactic acid, or a combination thereof.

The hydrolysing may further comprise enzyme hydrolysing following the acid hydrolysing. The enzyme hydrolysing may comprise hydrolysing the liquid mixture with any suitable enzyme. For example, the enzyme may comprise, but is not limited to, carbohydrase.

The yeast may be any suitable yeast. According to a particular aspect, the yeast may be, but not limited to, *Saccharomyces* yeast, non-*Saccharomyces* yeast, or a combination thereof. For example, the yeast may be, but not limited to, *Kluyveromyces* (*K.*) *thermotolerans*, *Saccharomyces* (*S.*) *cerevisiae*, *Torulaspora* (*T.*) *delbrueckii*, *Pichia* (*P.*) *kluyveri*, *Lachancea* (*L.*) *thermotolerans*, or a combination thereof.

The adding may comprise adding any suitable amount of yeast. According to a particular aspect, the adding may comprise adding yeast to obtain an initial yeast count of 5-7 log CFU yeast/mL.

5 The pre-determined period of time may be any suitable period of time for the purposes of the present invention. According to a particular aspect, the pre-determined period of time may be 2-29 days.

The pre-determined temperature may be any suitable temperature for the purposes of the present invention. According to a particular aspect, the pre-determined temperature may be 15-37°C.

10 According to a particular aspect, the method may further comprise adding sugar to the hydrolysed liquid mixture prior to the adding yeast to the hydrolysed liquid mixture. The sugar added may be any suitable sugar for the purposes of the present invention. In particular, the adding sugar to the hydrolysed liquid mixture may comprise adding sugar such that the hydrolysed liquid mixture has a Brix of 5-26 °Bx.

15 According to a particular aspect, the method may further comprise heating the hydrolysed liquid mixture prior to the adding yeast to the hydrolysed liquid mixture.

The method may further comprise adding yeast extract prior to the adding yeast to the hydrolysed liquid mixture. The method may further comprise adjusting pH of the hydrolysed liquid mixture prior to the adding yeast to the hydrolysed liquid mixture.

20 According to a particular aspect, the adding yeast may further comprise adding lactic acid bacteria. Any suitable amount of lactic acid bacteria may be added. In particular, the adding may comprise adding lactic acid bacteria to obtain an initial lactic acid bacteria count of 4-7 log CFU/mL.

25 The lactic acid bacteria may be any suitable lactic acid bacteria. According to a particular aspect, the lactic acid bacteria may be, but not limited to, *Oenococcus* (*O.*) *oeni*, *Lactobacillus* (*L.*) *plantarum*, or a combination thereof.

Brief Description of the Drawings

In order that the invention may be fully understood and readily put into practical effect there shall now be described by way of non-limitative example only exemplary

embodiments, the description being with reference to the accompanying illustrative drawings. In the drawings:

Figure 1 shows growth of *K. thermotolerans* Concerto during fermentation of two types of SCG hydrolysate media - KC: *K. thermotolerans* Concerto with 0% yeast extract and
5 KY: *K. thermotolerans* Concerto with 0.25% yeast extract. Yeast cell counts shown are the mean values of triplicate fermentations with error bars indicating the standard deviations of the mean values;

Figure 2 shows the ethanol content of the fermented SCG hydrolysate at day 22. KC: *K. thermotolerans* Concerto with 0% yeast extract, KY: *K. thermotolerans* Concerto
10 with 0.25% yeast extract. The values shown are the mean of triplicate fermentation (n=3), with error bar representing the standard deviation of the mean. a: statistical analysis using ANOVA at 95% confidence interval;

Figure 3 shows the total sugar content changes of SCG hydrolysate media fermented with *K. thermotolerans* Concerto. The concentrations are the mean values of triplicate
15 fermentations (n=3) with error bars indicating the standard deviations of the mean values;

Figure 4 shows growth of *S. cerevisiae* MERIT during fermentation of two types of SCG hydrolysate media - SC: *S. cerevisiae* MERIT with 0% yeast extract and SY: *S. cerevisiae* MERIT with 0.25% yeast extract. Yeast cell count shown are the mean
20 values of triplicate fermentations with error bars indicating the standard deviation of the mean values;

Figure 5 shows the ethanol content of the fermented SCG hydrolysate at day 22. SC: *S. cerevisiae* MERIT with 0% yeast extract and SY: *S. cerevisiae* MERIT with 0.25% yeast extract. The values are mean of triplicate fermentation (n=3), with error bar
25 representing the standard deviation of the mean. a: statistical analysis using ANOVA at 95% confidence interval;

Figure 6 shows the total sugar content changes of SCG hydrolysate media fermented with *S. cerevisiae* MERIT. SC represents *S. cerevisiae* MERIT with 0% yeast extract and SY represents *S. cerevisiae* MERIT with 0.25% yeast extract. The concentrations

shown are the mean values of triplicate fermentations (n=3) with error bars indicating the standard deviation of the mean values;

Figure 7 shows growth of *T. delbrueckii* Biodiva during fermentation of SCG hydrolysates. TC represents *T. delbrueckii* Biodiva with 0% added yeast extracts and
5 TY represents *T. delbrueckii* Biodiva with 0.25% added yeast extracts. Yeast cell counts are the mean values of triplicate fermentations with error bars indicating the standard deviations of the mean values;

Figure 8 shows ethanol content of the fermented SCG hydrolysate from day 0 to day 14. TC represents *T. delbrueckii* Biodiva with 0% yeast extracts and TY represents *T.*
10 *delbrueckii* Biodiva with 0.25% yeast extracts. The values are the mean values of triplicate fermentations (n=3) with error bars indicating the standard deviations of the mean;

Figure 9 shows growth of *P. kluyveri* FrootZen during fermentation of SCG hydrolysates. PC represents *P. kluyveri* FrootZen with 0% yeast extracts and PY
15 represents *P. kluyveri* FrootZen with 0.25% yeast extracts. Yeast cell counts are the mean values of triplicate fermentations with error bars indicating the standard deviations of the mean values;

Figure 10 shows ethanol content of the fermented SCG from day 0 to day 14. PC represents *P. kluyveri* FrootZen with 0% yeast extracts and PY represents *P. kluyveri*
20 FrootZen with 0.25% yeast extracts. The values are the mean values of triplicate fermentations (n=3) with error bars indicating the standard deviations of the mean;

Figure 11A shows production of ethyl acetate, Figure 11B shows production of isoamyl acetate and Figure 11C shows production of 2-phenethyl acetate, during fermentation
25 with *P. kluyveri* FrootZen. The concentration is the mean values of triplicate fermentations (n = 3), with error bar representing the standard deviation of the mean. PC represents *P. kluyveri* FrootZen with 0% yeast extract and PY represents *P. kluyveri* FrootZen with 0.25% yeast extract

Figure 12A shows growth of yeast strains and Figure 12B shows growth of *O. oeni* strains (b) during SCG hydrolysates fermentation. C represents (●) *L. thermotolerans*
30 Concerto monoculture, CP represents (⊗) Co-culture of *L. thermotolerans* Concerto

and *O. oeni* PN4, CB represents (▲) Co-culture of *L. thermotolerans* Concerto and *O. oeni* Enoferm Beta, and CL represents (▼) Co-culture of *L. thermotolerans* Concerto and *O. oeni* Lalvin 31. The values are the mean values of triplicate fermentations (n=3) with error bars indicating the standard deviations of the mean; and

- 5 Figure 13 shows ethanol contents of fermented SCG hydrolysates. C represents (●) *L. thermotolerans* Concerto monoculture, CP represents (※) Co-culture of *L. thermotolerans* Concerto with *O. oeni* PN4, CB represents (▲) Co-culture of *L. thermotolerans* Concerto with *O. oeni* Enoferm Beta and CL represents (▼) Co-culture of *L. thermotolerans* Concerto with *O. oeni* Lalvin 31. The values are the mean values
10 of triplicate fermentations (n=3) with error bars indicating the standard deviations of the mean.

Detailed Description

- As explained above, there is a need for processing spent coffee grounds (SCG) to better utilise it. Accordingly, the present invention relates to the valorization of SCG by
15 forming a spent coffee grounds-derived beverage.

- In general terms, the present invention relates to an alcoholic beverage derived from spent coffee grounds and a method of forming the same. The SCG-derived beverage may be formed from a method which is environmentally friendly and is able to valorize the SCG, thereby converting the SCG into a novelty beverage. In particular, the
20 beverage may have a range of flavour compounds, organic acids and a certain amount of ethanol. The beverage may have a pleasant aroma due to greater amounts of fruity esters and other unique aroma compounds.

- According to a first aspect, there is provided an alcoholic beverage comprising esters and having an ethanol content of $\leq 10\%$ by volume, wherein the esters comprise at
25 least one of: ethyl 2,4-hexadienoate and ethyl decanoate; and wherein the alcoholic beverage is an undistilled alcoholic beverage and is derived from spent coffee grounds.

- The beverage may be any suitable spent coffee ground-derived beverage. For the purposes of the present invention, an alcoholic beverage is defined as a beverage which comprises alcohol. In particular, the alcohol may be ethanol. The beverage may
30 have an ethanol content of $\geq 0.5\%$ by volume. For example, the beverage may have an ethanol content of 0.5-10%, 0.7-9.8%, 0.9-9.5%, 1.0-9.0%, 1.2-8.5%, 1.5-8.0%, 2.0-

7.0%, 2.5-6.5%, 3.0-6.0%, 3.5-5.5%, 4.0-5.0%, 4.2-4.8%, 4.3-4.5% by volume. Even more in particular, the beverage may have an ethanol content of 0.5-8.0% by volume.

According to a particular aspect, the beverage does not comprise methanol. This is advantageous since methanol may be harmful when consumed.

- 5 The beverage may be an undistilled alcoholic beverage. In particular, the beverage may be a fermented beverage. A fermented beverage may be defined as a beverage which has undergone fermentation.

According to a particular aspect, the beverage may be any suitable alcoholic beverage. For example, the alcoholic beverage may include, but is not limited to, wine, ciders,
10 beers and spirits.

The esters comprised in the beverage may confer a suitable flavour and taste to the beverage. The presence of esters in the beverage show that the beverage has undergone fermentation. In particular, the ester comprised in the beverage may impart a fruity and/or floral characteristic to the beverage.

- 15 The average total ester content in the beverage may be 15-6000 µg/L. In particular, the average total ester content may be 20-6000 µg/L, 40-5800 µg/L, 60-5700 µg/L, 80-5600 µg/L, 90-5500 µg/L, 100-5700 µg/L, 200-5400 µg/L, 220-5450 µg/L, 240-5350 µg/L, 280-5250 µg/L, 300-5250 µg/L, 400-5000 µg/L, 800-4500 µg/L, 1000-4000 µg/L, 1400-3500 µg/L, 1600-3000 µg/L, 2000-2500 µg/L.

- 20 The beverage may comprise at least one of: ethyl 2,4-hexadienoate and ethyl decanoate. According to a particular aspect, the beverage may further comprise at least one ester selected from, but not limited to: ethyl (*E*)-4-hexenoate, isoamyl acetate, 3-methylbutyl octanoate, ethyl nonanoate, ethyl 9-decenoate, phenethyl acetate. Other esters which may be present include, but is not limited to, ethyl acetate,
25 ethyl octanoate, ethyl hexanoate, 2-phenethyl acetate, 2-furanmethyl acetate, methyl acetate, methyl 2-furoate, methyl lactate, diacetyl, acetoin, 2-nonanone or mixtures thereof.

The beverage may have a suitable pH. For example, the pH of the beverage may be 3.5-6.5. In particular, the pH may be 3.0-5.0.

The beverage may have a suitable Brix or its specific gravity equivalent. Brix is a measure of the amount of sugars in the beverage. For example, 1 °Bx refers to 1 g of sucrose in 100 g of the beverage. Accordingly, the higher the Brix, the higher the amount of sugars in the beverage which in turn may relate to a higher alcohol content
5 in the beverage.

The Brix of the beverage may be 5-26 °Bx. For the purposes of the present invention, reference to Brix of the alcoholic beverage refers to the measure of the Brix of the spent coffee grounds comprised in the beverage. The Brix of the beverage may be 7-25 °Bx, 10-20 °Bx, 12-18 °Bx, 13-15 °Bx. In particular, the Brix may be 5-20 °Bx.

10 The beverage may further comprise additional additives. The additive may be any suitable additive for giving a more finished consumer product. According to a particular aspect, the additive may be, but not limited to, sweetener, flavouring, stabilizer, thickeners, colourant, preservatives, acidity regulator, or a combination thereof.

According to a second aspect of the present invention, there is provided a method of
15 forming an undistilled alcoholic beverage derived from spent coffee grounds (SCG), the method comprising:

- hydrolysing a liquid mixture of spent coffee grounds to obtain a hydrolysed liquid mixture;
- adding yeast to the hydrolysed liquid mixture; and
- 20 - fermenting the hydrolysed liquid mixture comprising yeast at a pre-determined temperature for a pre-determined period of time to form the beverage.

The SCG may be any suitable spent coffee grounds and may be obtained from any
25 suitable source. The SCG may be treated to convert the SCG into a suitable form for use in the method of the present invention. Therefore, the method may further comprise treating the SCG prior to the hydrolysing.

The treating may comprise defatting the SCG. The defatting of the SCG may be by any suitable method. According to a particular aspect, the defatting may comprise defatting
30 the SCG with a suitable solvent such as, but not limited to, hexane, methanol, or a combination thereof. The treating may further comprise grinding the SCG into powder

form. The powdered SCG may then be dissolved in a suitable solvent to form a SCG liquid mixture. For example, the solvent may be, but not limited to, water.

The concentration of the SCG in the liquid mixture may be any suitable concentration. For example, the concentration of SCG in the liquid mixture may be 2-40 w/v %. In particular, the concentration may be 2-37 w/v %, 4-35 w/v %, 6-33 w/v %, 8-31 w/v %, 10-29 w/v %, 12-27 w/v %, 14-25 w/v %, 16-23 w/v %, 18-21 w/v %, 20-37 w/v %, 18-37 w/v %, 4-30 w/v %. Even more in particular, the concentration may be 10-20 w/v %.

The treating may further comprise drying the SCG following the defatting. The drying may be under suitable conditions. In particular, the drying may comprise drying the SCG to enable the SCG to be ground into a suitable form such as a powder form.

The hydrolysing may be by any suitable method to hydrolyse and convert the insoluble macromolecules, such as but not limited to polysaccharides, into smaller soluble molecules, such as sugars, to facilitate fermentation. In particular, the hydrolysing may enable recovery of up to 30% of sugars to be recovered from the SCG.

According to a particular aspect, the hydrolysing may comprise acid hydrolysing the liquid mixture. The acid hydrolysing may be by any suitable acid. In particular, the acid hydrolysing may be using a food-grade acid. For the purposes of the present invention, a food-grade acid is defined as an acid which is suitable for being consumed. The food-grade acid may be any suitable acid, such as, but not limited to, citric acid, malic acid, lactic acid, or a combination thereof.

The hydrolysing may be under suitable conditions. For example, the conditions may comprise the hydrolysing time and hydrolysing at a suitable temperature. According to a particular aspect, the acid hydrolysing may be for 10-120 min. For example, the acid hydrolysing may be for 15-110 min, 20-100 min, 30-90 min, 35-80 min, 40-70 min, 45-60 min, 50-55 min. In particular, the acid hydrolysing may be for 40-60 min.

According to a particular aspect, the acid hydrolysing may be at a temperature of 75-140°C. For example, the temperature may be 77-135°C, 79-130°C, 80-120°C, 90-110°C, 100-105°C. In particular, the temperature may be 90-130°C.

The hydrolysing may further comprise enzyme hydrolysing the liquid mixture following the acid hydrolysing. The enzyme hydrolysing may comprise hydrolysing the liquid

mixture with any suitable enzyme. For example, the enzyme may comprise, but is not limited to, carbohydrase. The carbohydrase may be any suitable carbohydrase, such as but not limited to, cellulase, hemicellulase, pectinase, or a combination thereof. A suitable amount of carbohydrase may be added to the acid hydrolysed liquid mixture.

- 5 The enzyme hydrolysing may be under suitable conditions. For example, the enzyme hydrolysing may be for a suitable period of time. In particular, the enzyme hydrolysing may be for 2-50 hours, 4-45 hours, 6-40 hours, 8-35 hours, 10-30 hours, 12-24 hours, 15-20 hours. Even more in particular, the enzyme hydrolysing may be for 4-24 hours.

- The enzyme hydrolysis may be at a suitable temperature. In particular, the enzyme
10 hydrolysing may be at 18-60°C. For example, the temperature may be 20-57°C, 22-55°C, 25-50°C, 30-45°C, 35-40°C. Even more in particular, the temperature may be 40-50°C.

The enzyme hydrolysis may be at a suitable pH. In particular, the enzyme hydrolysing may be at a pH of 4-8. Even more in particular, the pH may be 4-6.

- 15 Following the hydrolysing, a hydrolysed liquid mixture of SCG may be formed. The hydrolysed liquid mixture may be cooled prior to the adding of yeast. Accordingly, the method may further comprise cooling the hydrolysed liquid mixture prior to the adding yeast.

- According to a particular aspect, the method may further comprise adding sugar to the
20 hydrolysed liquid mixture prior to the adding yeast to the hydrolysed liquid mixture. The addition of the sugar may be for increasing carbohydrate content in the SCG liquid mixture. The sugar added may be any suitable sugar for the purposes of the present invention. For example, the sugar added may comprise, but is not limited to, glucose, sucrose, fructose, or a combination thereof. In particular, the sugar added may be
25 sucrose.

The adding the sugar may be to adjust the Brix of the hydrolysed liquid mixture. In particular, the Brix of the hydrolysed liquid mixture may be adjusted to 5-26 °Bx. Even more in particular, the Brix of the hydrolysed liquid mixture may be adjusted to about 15 °Bx.

The method may further comprise adjusting pH of the hydrolysed liquid mixture prior to the adding yeast to the hydrolysed liquid mixture. The adjusting the pH enables the yeast to grow in the hydrolysed liquid mixture. For example, the pH may be adjusted to a pH of 3.5-6.5. In particular, the pH may be adjusted to a pH of about 5.

- 5 The adjusting the pH may be by any suitable method. For example, the adjusting may comprise adding a suitable acid. The acid used for the adjusting must be an acid which is suitable for being consumed. In particular, the acid may be, but not limited to, malic acid, citric acid, lactic acid, or a combination thereof. Even more in particular, the acid may be citric acid, malic acid, or a combination thereof.
- 10 According to a particular aspect, the method may further comprise heating the hydrolysed liquid mixture prior to the adding yeast to the hydrolysed liquid mixture. The heating may comprise mild pasteurization of the hydrolysed liquid mixture. The heating may extend the shelf life of the liquid mixture prior to the fermenting and may also reduce the risk of contamination during the fermenting. In particular, the heating may
- 15 eliminate or reduce indigenous microbial activity in the liquid mixture.

The heating may be carried out under suitable conditions. In particular, the heating may be under suitable conditions. For example, the heating may be carried out at a temperature of about 40-80°C. In particular, the temperature may be about 60-80°C. Even more in particular, the temperature may be about 60°C.

- 20 The heating may be carried out for a suitable period of time. For example, the heating may be for 10-75 minutes. In particular, the heating may be for about 10-60 minutes, 15-45 minutes, 20-40 minutes, 25-30 minutes. Even more in particular, the heating may be for about 30 minutes.

- The heating may be by suitable means. For example, the heating may be in a water bath.
- 25

The method may further comprise cooling the hydrolysed liquid mixture following the heating. The cooling may comprise cooling the hydrolysed liquid mixture to room temperature.

- The adding may comprise adding any suitable yeast. According to a particular aspect,
- 30 the yeast may be, but not limited to, *Saccharomyces* yeast, non-*Saccharomyces* yeast,

or a combination thereof. For example, the yeast may be, but not limited to, *Kluyveromyces (K.) thermotolerans*, *Saccharomyces (S.) cerevisiae*, *Torulaspora (T.) delbrueckii*, *Pichia (P.) kluyveri*, *Lachancea (L.) thermotolerans*, or a combination thereof. In particular, the yeast may be *K. thermotolerans* Concerto, *S. cerevisiae* MERIT, *T. delbrueckii* Biodiva, *P. kluyveri* FrootZen, *O. oeni* Lalvin 31, *O. oeni* PN4, *O. oeni* Enoferm Beta, or a combination thereof.

The adding may comprise adding any suitable amount of yeast. For example, the amount of yeast added may be 1-9 log CFU/mL. In particular, the amount may be about 4-7 log CFU/mL. Even more in particular, the amount may be about 5-7 log CFU/mL. According to a particular aspect, the adding may comprise adding yeast to obtain an initial yeast count of 5 log CFU yeast/mL.

According to a particular aspect, the method may further comprise adding nitrogen compounds to the hydrolysed liquid mixture prior to the adding yeast to the hydrolysed liquid mixture. The addition of nitrogen compounds may be for increasing nitrogen content in the hydrolysed liquid mixture. The nitrogen compound added may be any suitable substance containing available nitrogen. For example, the nitrogen compound added may comprise, but it is not limited to, ammonia, free amino acids, or a combination thereof. In particular, the nitrogen compound may be yeast extracts.

The subsequently added yeasts may convert the yeast extract components into volatile compounds, such as esters. Accordingly, the addition of yeast extracts may enhance the pleasant aroma of the final beverage formed from the method.

According to a particular aspect, the adding yeast may further comprise adding lactic acid bacteria. Any suitable amount of lactic acid bacteria may be added. In particular, the adding may comprise adding lactic acid bacteria to obtain an initial lactic acid bacteria count of 4-7 log CFU/mL. Even more in particular, the adding may comprise adding lactic acid bacteria to obtain an initial lactic acid bacteria count of 4-7 log CFU/mL, 5-7 log CFU/mL, or 6-7 log CFU/mL.

The lactic acid bacteria may be any suitable lactic acid bacteria. According to a particular aspect, the lactic acid bacteria may be, but not limited to, *Oenococcus (O.) oeni*, *Lactobacillus (L.) plantarum*, or a combination thereof.

The fermenting may be carried out under suitable conditions. For example, the fermenting may be at a pre-determined temperature for a pre-determined period of time. The pre-determined period of time may be any suitable period of time for the purposes of the present invention. According to a particular aspect, the pre-determined
5 period of time may be 2-29 days. In particular, the pre-determined period of time may be about 3-25 days, 5-22 days, 7-20 days, 9-18 days, 10-20 days, 12-18 days, 14-15 days. Even more in particular, the pre-determined period of time may be about 14-22 days. According to a particular aspect, the pre-determined period of time may be about 14 days. According to another particular aspect, the pre-determined period of time may
10 be about 22 days.

The pre-determined temperature may be any suitable temperature for the purposes of the present invention. According to a particular aspect, the pre-determined temperature may be 15-37°C. In particular, the pre-determined temperature may be 15-37°C, 17-35°C, 20-30°C, 22-25°C. Even more in particular, the pre-determined temperature may
15 be about 20-30°C. According to a particular aspect, the pre-determined temperature may be about 30°C. According to another particular aspect, the pre-determined temperature may be about 20°C.

According to a particular aspect, the fermenting may be conducted statically, such that the liquid mixture is not subjected to any shaking or movement.

20 During the fermenting, the yeasts metabolise the fermentable sugars and convert the existing substances such as aldehydes and acids into esters, or fructose and glucose into ethanol, thereby producing an alcoholic beverage and enriching the aroma profile of the beverage.

The method of the present invention provides a method of preparing a spent coffee
25 grounds-derived beverage which may have lower alcohol content, a pleasant taste and aroma. The beverage formed from the method of the present invention utilises yeast and optionally lactic acid bacteria. Use of yeast in the fermenting may generate a large amount of aroma compounds that make the spent coffee ground transform into a beverage with improved taste and highly fruity aroma. In particular, the alcoholic
30 beverage formed from the method may comprise esters, wherein the esters comprise at least one ester selected from: ethyl 2,4-hexadienoate and ethyl decanoate. The beverage formed from the method may further comprise at least one ester selected

from, but not limited to: ethyl (*E*)-4-hexenoate, isoamyl acetate, 3-methylbutyl octanoate, ethyl nonanoate, ethyl 9-decenoate, phenethyl acetate. Other esters which may be present include, but is not limited to, ethyl acetate, ethyl octanoate, ethyl hexanoate, 2-phenethyl acetate, 2-furanmethyl acetate, methyl acetate, methyl 2-furoate, methyl lactate, diacetyl, acetoin, 2-nonanone, or mixtures thereof.

The flavour/aroma may be considered to be the most important attribute to define the quality of an alcoholic beverage such as wine. The wine aroma comes from the volatile aroma compounds that may be generated during yeast fermentation. During fermentation, yeasts generate a large number of aroma compounds such as volatile fatty acids, higher alcohols, esters that greatly influence the aroma of wine. Hence, the choice of yeast and fermentation conditions that is suitable for the type of medium and wine style are important.

Having now generally described the invention, the same will be more readily understood through reference to the following examples which are provided by way of illustration, and are not intended to be limiting.

EXAMPLE

Preparation of SCG hydrolysates

To obtain SCG hydrolysates, SCG were defatted with hexane (1:10, w/v) and dried in a fume hood overnight. The defatted SCG were ground into fine powder and dissolved in water (15%, w/v). These liquid mixtures were sequentially hydrolyzed by acid (citric acid, 200 mM, 121°C, 1 h) and enzyme (Viscozyme®L, 6%, v/w, 50°C, 24 h, pH 5).

The SCG hydrolysates were cooled to room temperature before adding 93.4 g/L sucrose into the SCG with the final °Brix value of 15. Yeast extract (either 0% or 0.25%, w/v) was added into the SCG medium before pH values were adjusted to 5.

The hydrolysates were subsequently pasteurized at 60°C for 30 min. The effectiveness of pasteurization was checked by plate counting, as is known in the art. Then pure yeast was added to the pasteurized SCG medium with an initial cell count of about 5 log CFU/mL. Fermentation was conducted statically at 20°C for 22 days. The final

product obtained was then further analyzed for the sugars, ethanol, organic acids, phenolics, amino acids and aroma contents.

Example 1 - Formation of beverage from a non-*Saccharomyces* yeast

Two types of SCG hydrolysate media (one with added 0% yeast extract and one with
5 0.25% yeast extract) were prepared as described above. As mentioned above, the pH of the media was adjusted to pH 5 and pasteurized at 60°C for 30 min, followed by cooling to room temperature.

Fermentation of SCG was performed by inoculating *K. thermotolerans* Concerto to obtain an initial cell population of approximately 5 log CFU/mL. Mixture of yeast and
10 SCG medium were cultured statically at 20°C for 22 days. Both fermented SCG media were analysed for sugars, ethanol, organic acids, phenolics, amino acids and aroma compound contents.

Results

Yeast growth

15 As can be seen in Figure 1, *K. thermotolerans* Concerto grew well in the SCG media with an overall cell population increase of 1.95-1.98 log CFU/mL, especially with the addition of yeast extract.

Ethanol content

Ethanol was the main compound produced by the fermentation of SCG medium with *K.*
20 *thermotolerans* Concerto. As shown in Figure 2, the ethanol content of final SCG beverages after 22 days of fermentation with 0% yeast extract and 0.25% yeast extract was 4.41% and 4.58%, respectively.

Non-volatile components

Besides ethanol, other compounds such as the amount of organic acids, phenolics,
25 sugars, and amino acids were also measured. Table 1 provides the values of the different components measured.

Compound (g/L)	Day 0	Day 22	
	Pre-treated SCG	Concerto (KC)	Concerto + YE (KY)
Organic acids			
Citric acid	34.87 ± 0.78a	36.98 ± 0.087b	35.42 ± 0.79ab
Malic acid	0.80 ± 0.01c	0.27 ± 0.02b	0.22 ± 0.00a
Quinic acid	0.71 ± 0.01a	1.75 ± 0.14c	1.56 ± 0.06b
Succinic acid	1.84 ± 0.01a	1.74 ± 0.12a	2.23 ± 0.17b
Acetic acid	0.24 ± 0.02a	0.32 ± 0.01b	0.55 ± 0.02c
Alkaloids (mg/L)			
Trigonelline	451.87 ± 13.14b	451.87 ± 13.14b	376.11 ± 34.97a
Theobromine	93.30 ± 2.25b	7.36 ± 1.00a	18.89 ± 0.16a
Theophylline	28.82 ± 3.13c	9.12 ± 0.25a	8.64 ± 0.92a
Caffeine	1.25 ± 0.01b	1.18 ± 0.02ab	1.14 ± 0.05a
Phenolic acids (mg/L)			
Chlorogenic acids	133.73 ± 2.54b	43.31 ± 1.27a	43.58 ± 1.09a
Caffeic acid	606.06 ± 9.68a	655.87 ± 3.78b	643.25 ± 7.20b
Ferulic acid	9.99 ± 1.55a	20.34 ± 1.09b	19.53 ± 1.85b
<i>p</i> -Coumaric acid	4.26 ± 0.63a	9.19 ± 0.81c	9.46 ± 0.18c
Sugars			
Fructose	25.18 ± 0.04b	4.17 ± 0.02a	4.26 ± 0.03a
Glucose	71.35 ± 0.06b	4.90 ± 0.02a	4.90 ± 0.03a
Sucrose	12.38 ± 0.01b	2.38 ± 0.05a	2.34 ± 0.01a
Mannose	21.67 ± 0.00b	4.25 ± 0.00a	4.10 ± 0.01a
Galactose	23.02 ± 0.00b	4.54 ± 0.01a	4.53 ± 0.01a
Arabinose	2.44 ± 0.05b	2.33 ± 0.03a	2.41 ± 0.05ab
Total sugar	155.59 ± 1.66b	22.56 ± 0.14a	22.56 ± 0.14a

SCG: spent coffee grounds; YE: adding 0.25% yeast extract; KC: *K. thermotolerans* Concerto with 0% yeast extract, KY: *K. thermotolerans* Concerto with 0.25% (w/v) yeast extract; a, b, c: statistical analysis using ANOVA (n=3) at 95% confidence interval. Same letters indicate no significant difference between samples

Table 1: Measured components contained in fermented SCG alcoholic beverage formed from fermentation of SCG hydrolysate using *K. thermotolerans* Concerto

- Several organic acids such as citric acid, malic acid, quinic acid, succinic acid, and acetic acid were quantified (see Table 1). The amounts of citric acid (36.98 g/L), quinic acid (1.75 g/L), and acetic acid (0.32 g/L) increased significantly after fermentation, while the amount of malic acid decreased significantly after fermentation. Moreover, the

production of succinic acid (2.23 g/L) and acetic acid (0.55 g/L) were further increased when the SCG media was supplemented with yeast extract.

Phenolic compounds and alkaloids were also quantified, as provided in Table 1. For alkaloids, theophylline was produced significantly after fermentation while others remained stable. For phenolic acids, caffeic acid, ferulic acid and p-coumaric acid were generated significantly after fermentation. However, the supplementation of yeast extract to the SCG hydrolysate showed no effect on the generation of phenolic acids.

The SCG hydrolysates contained various sugars including fructose, glucose, sucrose (added), mannose, galactose and arabinose. The total sugar content declined significantly from 150 g/L to around 22 g/L, as can be seen in Figure 3. Almost all the sugars were significantly consumed by yeast during fermentation, and this consumption was accelerated in when the SCG hydrolysate media was supplemented with yeast extract.

The concentrations of amino acids before and after SCG fermentation with *K. thermotolerans* Concerto was also measured. It was found that after fermentation, most amino acids, except for proline and histidine, significantly declined to low levels. The consumed amino acids played important roles in production of aroma compounds in the formed alcoholic beverage.

Volatile compounds

The changes in the volatile composition of the SCG hydrolysate pre and post fermentation are shown in Table 2. Among the volatile compounds measured and quantified, esters were the main contributors to the fruity aroma of the alcoholic beverage formed.

Phenylethyl alcohol, ethyl acetate, ethyl (*E*)-4-hexenoate, ethyl octanoate, ethyl decanoate, isoamyl acetate played significant roles in providing a fruity aroma in the fermented SCG hydrolysate medium. Production of several esters was boosted by the addition of yeast extract.

Compound	Identification methods	LRI	Pre-Treated SCG (Day 0)		SCG beverage (Day 22)	
			0% YE	0.25% YE	Concerto (KC)	Concerto + YE (KY)
Acids						
Acetic acid	MS, LRI	1452	26.01 ± 0.85c	24.11 ± 0.93c	8.94 ± 0.30a	13.47 ± 0.19b
Isobutyric acid	MS, LRI	1568	0.32 ± 0.03b	0.47 ± 0.06c	0.22 ± 0.02a	0.26 ± 0.00ab
Pentanoic acid	MS, LRI	1669	14.51 ± 0.30c	13.54 ± 0.24b	2.47 ± 0.11a	2.30 ± 0.09a
Hexanoic acid	MS, LRI	1847	0.46 ± 0.02b	0.46 ± 0.02b	0.33 ± 0.06a	0.78 ± 0.01c
4-Hexenoic acid	MS, LRI	1923	0.00 ± 0.00a	0.00 ± 0.00a	0.92 ± 0.02b	1.20 ± 0.21b
Alcohols						
1-Pentanol	MS, LRI	1226	1.60 ± 0.67a	2.99 ± 0.08a	22.31 ± 1.02b	20.34 ± 0.32b
Furfuryl alcohol	MS, LRI	1680	0.00 ± 0.00a	0.00 ± 0.00a	0.56 ± 0.05b	0.74 ± 0.00c
Phenylethyl alcohol	MS, LRI	1958	0.94 ± 0.20a	1.33 ± 0.10a	23.17 ± 0.09b	29.86 ± 0.76c
Esters						
Ethyl acetate	MS, LRI		0.00 ± 0.00a	0.00 ± 0.00a	18.32 ± 0.00c	13.86 ± 0.11b
Ethyl (E)-4-hexenoate	MS, LRI	1289	0.00 ± 0.00a	0.00 ± 0.00a	1.98 ± 0.07b	2.10 ± 0.38b
Ethyl octanoate	MS, LRI	1433	0.00 ± 0.00a	0.00 ± 0.00a	0.73 ± 0.00b	1.63 ± 0.16c
Ethyl 2,4-hexadienoate	MS, LRI	1515	0.73 ± 0.17b	0.50 ± 0.03b	0.18 ± 0.04a	0.42 ± 0.05ab
Ethyl decanoate	MS, LRI	1637	0.00 ± 0.00a	0.00 ± 0.00a	0.14 ± 0.03a	1.25 ± 0.12b
Isoamyl acetate	MS, LRI	1119	0.00 ± 0.00a	0.00 ± 0.00a	3.17 ± 0.56b	3.76 ± 0.31b
Aldehydes						
Benzaldehyde	MS, LRI	1544	2.13 ± 0.17b	1.86 ± 0.04b	0.69 ± 0.14a	0.68 ± 0.02a
Benzeneacetaldehyde	MS, LRI	1663	14.51 ± 0.30c	13.54 ± 0.24b	1.18 ± 0.00a	1.15 ± 0.05a
Furfural	MS, LRI	1480	42.42 ± 0.93c	33.79 ± 0.70c	0.99 ± 0.19a	0.80 ± 0.05a
Ketones						
2,3-Pentanedione	MS, LRI	1064	1.07 ± 0.05b	1.47 ± 0.01c	0.00 ± 0.00a	0.00 ± 0.00a
2-Acetyl furan	MS, LRI	1522	1.12 ± 0.16b	1.02 ± 0.05b	0.51 ± 0.07a	0.50 ± 0.02a
2-Formyl-5-methylfuran	MS, LRI	1591	15.32 ± 0.52c	13.45 ± 0.54b	0.49 ± 0.03a	0.31 ± 0.03a
Acetoin	MS, LRI	1308	0.00 ± 0.00a	0.00 ± 0.00a	0.27 ± 0.01b	0.35 ± 0.07b
Butyrolactone	MS, LRI	1655	0.63 ± 0.06b	0.52 ± 0.03b	0.00 ± 0.00a	0.00 ± 0.00a
Furans						
2,5-Dimethylfuran	MS, LRI		0.00 ± 0.00a	0.00 ± 0.00a	3.94 ± 0.03b	3.76 ± 0.31d
2-Acetyl-5-methylfuran	MS, LRI	1634	0.32 ± 0.06bc	0.40 ± 0.02c	0.18 ± 0.03a	0.24 ± 0.01ab
2-Vinylfuran	MS, LRI	2023	0.60± 0.04b	0.62 ± 0.02b	0.42 ± 0.05a	0.45 ± 0.00a
Benzene, 1,3-bis(1,1-dimethylethyl)-	MS, LRI	1427	2.67 ± 0.23a	3.79 ± 0.12b	2.26 ± 0.31a	2.51 ± 0.12a
Others						
3-Acetylpyrrole	MS, LRI	1995	0.41 ± 0.06a	0.53 ± 0.01a	0.31 ± 0.04a	0.36 ± 0.02b
α-pyrone-6-carboxylic acid	MS, LRI	1366	0.00 ± 0.00a	0.00 ± 0.00a	2.29 ± 0.36b	2.86 ± 0.16c
cis-Linalool oxide	MS, LRI	1476	0.54 ± 0.03b	0.50 ± 0.03b	0.38 ± 0.04a	0.39 ± 0.04a
Guaiacol	MS, LRI	1879	0.25 ± 0.03ab	0.33 ± 0.02b	0.21 ± 0.03a	0.25 ± 0.01ab

5 LRI: linear retention index; determined on a DB-FFAF column relative to C7-C40 hydrocarbons; YE: adding 0.25% yeast extract; KC: *K. thermotolerans* Concerto without yeast extract, KY: *K. thermotolerans* Concerto with 0.25% (w/v) yeast extract; Values are the mean of triplicate fermentations (± standard deviation); a,b,c Statistical analysis using ANOVA (n=3) at 95% confidence interval. Same letter indicates no significant difference between samples

Table 2: Selected volatile compounds and their concentrations (µg/L) before and after fermentation with *K. thermotolerans* Concerto

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In conclusion, SCG hydrolysates fermented with non-*Saccharomyces* yeast *K. thermotolerans* Concerto may be used for producing SCG-derived alcoholic beverage with health benefit compounds and a pleasant aroma profile.

Example 2 - Formation of beverage from a *Saccharomyces* yeast

Two types of SCG hydrolysate media (one with added 0% yeast extract and one with 0.25% yeast extract) were prepared as described above. As mentioned above, the pH of the media was adjusted to pH 5 and pasteurized at 60°C for 30 min, followed by cooling to room temperature.

- 5 Fermentation of SCG was performed by inoculating SCG hydrolysates with *S. cerevisiae* MERIT to obtain an initial cell population of approximately 5 log CFU/mL. Mixture of yeast and SCG medium were cultured statically at 20°C for 22 days. Both fermented SCG media were analysed for sugars, ethanol, organic acids, phenolics, amino acids and aroma compound contents.

10 *Results*

Yeast growth

As can be seen in Figure 4, *S. cerevisiae* MERIT grew well in the SCG media with an overall cell population increase of 2.40-2.81 log CFU/mL from day 0 to day 22, and this was further improved by the addition of yeast extract.

15 Ethanol content

Ethanol was the main compound produced by the fermentation of SCG medium with *S. cerevisiae* MERIT. As shown in Figure 5, the ethanol content of final SCG beverages after 22 days of fermentation with 0% yeast extract and 0.25% yeast extract was 4.75% and 4.64%, respectively.

20 Non-volatile components

Besides ethanol, other compounds such as the organic acids, phenolics, sugars, and amino acids produced were also measured. Table 3 provides the values of the different components measured.

- 25 Several organic acids such as citric acid, malic acid, quinic acid, succinic acid, and acetic acid were quantified (see Table 3) on day 0 and day 22. The amount of quinic acid (1.75 g/L) and acetic acid (0.61 g/L) increased significantly after fermentation, while the amount of malic acid declined significantly after fermentation. Moreover, the generation of succinic acid (2.56 g/L) and acetic acid (0.77 g/L) were further increased

with the supplementation of yeast extract in the SCG hydrolysate, while the concentration of malic acid further declined with the addition of yeast extract.

Compound (g/L)	Day 0	Day 22	
	Pre-treated SCG	Merit (SC)	Merit + YE (SY)
Organic acids			
Citric acid	34.87 ± 0.78a	36.05 ± 1.28a	36.01 ± 0.34a
Malic acid	0.80 ± 0.01c	0.37 ± 0.02b	0.21 ± 0.00a
Quinic acid	0.71 ± 0.01a	1.75 ± 0.05b	1.84 ± 0.05b
Succinic acid	1.84 ± 0.01a	1.92 ± 0.10a	2.56 ± 0.06b
Acetic acid	0.24 ± 0.02a	0.61 ± 0.02b	0.77 ± 0.01c
Alkaloids			
Trigonelline	0.27 ± 0.00a	0.28 ± 0.00a	0.25 ± 0.01a
Theobromine	0.02 ± 0.01b	0.01 ± 0.00a	0.01 ± 0.00
Theophylline	0.02 ± 0.01a	0.08 ± 0.00c	0.05 ± 0.02b
Caffeine	3.45 ± 0.02a	3.58 ± 0.05a	3.46 ± 0.03a
Phenolic acids			
Chlorogenic acids	0.03 ± 0.01a	0.02 ± 0.00a	0.02 ± 0.00a
Caffeic acid	0.26 ± 0.02a	0.60 ± 0.00b	0.51 ± 0.02b
Ferulic acid	0.01 ± 0.00a	0.02 ± 0.00b	0.02 ± 0.01b
<i>p</i> -coumaric acid	0.00 ± 0.00a	0.01 ± 0.00b	0.01 ± 0.00b
Sugars			
Fructose	25.18 ± 0.04b	4.38 ± 0.00a	4.37 ± 0.05a
Glucose	71.35 ± 0.06c	4.29 ± 0.03b	3.09 ± 0.06a
Sucrose	12.38 ± 0.01b	2.21 ± 0.04a	2.46 ± 0.00a
Mannose	21.67 ± 0.00b	4.25 ± 0.00a	4.21 ± 0.00a
Galactose	23.02 ± 0.00c	4.34 ± 0.02b	3.76 ± 0.00a
Arabinose	2.44 ± 0.05b	2.50 ± 0.04b	2.22 ± 0.00a
Total sugar	155.59 ± 1.66b	21.96 ± 0.23a	20.11 ± 0.11a

SCG: spent coffee grounds; YE: adding 0.25% yeast extract; SC represents *S. cerevisiae* MERIT without yeast extract and SY represents *S. cerevisiae* MERIT with 0.25% (w/v) yeast extract. a, b, c: statistical analysis using ANOVA (n=3) at 95% confidence interval. Same letters indicate no significant difference between samples

Table 3: Measured components contained in fermented SCG alcoholic beverage formed from fermentation of SCG hydrolysate using *S. cerevisiae* MERIT

Phenolic compounds and alkaloids were also quantified, as provided in Table 3. For alkaloids, theophylline was produced significantly after fermentation while the amount of theobromine decreased significantly after fermentation with others remaining stable. The addition of yeast extract to the SCG hydrolysate only affected the decline of theophylline. For phenolic acids, caffeic acid, ferulic acid and *p*-coumaric acid were

generated significantly after fermentation. However, the addition of yeast extract showed no effect on the generation of phenolic acids.

Various sugar profiles in the SCG hydrolysates were measured. In particular, the amounts of fructose, glucose, sucrose (added), mannose, galactose and arabinose were quantified. The total sugar content declined significantly from 155.59 g/L to around 21 g/L, as can be seen in Figure 6. All the sugars were significantly consumed by yeast during fermentation, regardless of whether the SCG hydrolysate was supplemented with or without yeast extract. However, the consumption of glucose, galactose and arabinose decreased significantly when yeast extract was added.

The concentration of amino acids before and after SCG fermentation with *S. cerevisiae* MERIT was also measured. It was found that the addition of yeast extract enriched amino acids contents of SCG hydrolysate. In total, eighteen kinds of amino acids were quantified. After fermentation, most of the amino acids, except for histidine, significantly decreased to low levels. The consumed amino acids played important roles in the production of aroma compounds in the formed alcoholic beverage. In particular, the content of amino acids in the final SCG beverages remained in the range of 0.08 – 73.01 mg/L.

Volatile compounds

The changes in the volatile composition of SCG hydrolysate before and after fermentation are shown in Table 4. Among the volatile compounds measured and quantified, esters were the main contributors to the fruity aroma of the alcoholic beverage formed. Production of 3-methylbutyl octanoate, ethyl acetate, ethyl hexanoate, ethyl (*E*)-4-hexenoate, ethyl octanoate, ethyl 2,4-hexadienoate, ethyl nonanoate, ethyl decanoate, ethyl 9-decenoate, isoamyl acetate and phenethyl acetate played significant roles in contributing to the fruity aroma of the fermented SCG hydrolysate medium.

The addition of yeast extract had a positive effect on the production of some volatile compounds including the production of phenylethyl alcohol, ethyl octanoate, ethyl 9-decenoate, isoamyl acetate and phenethyl acetate, as seen from Table 4. The high amounts of these volatile compounds enriched the aroma of the formed SCG-derived beverage.

Compound	Identification methods	LRI	Day 0		Day 22	
			Pre-Treated SCG		SCG beverage	
			0% YE	0.25% YE	Merit (SC)	Merit + YE (SY)
Acids						
Acetic acid	MS, LRI	1452	26.01 ± 0.85b	24.11 ± 0.93b	11.36 ± 0.85a	24.36 ± 0.20b
Isobutyric acid	MS, LRI	1568	0.32± 0.03b	0.47 ± 0.06c	0.19 ± 0.02a	0.17 ± 0.01a
Pentanoic acid	MS, LRI	1669	14.51 ± 0.30d	13.54 ± 0.24c	0.19 ± 0.04a	1.79 ± 0.20b
Hexanoic acid	MS, LRI	1847	0.46 ± 0.02a	0.46 ± 0.02a	1.85 ± 0.15b	1.73 ± 0.14b
4-Hexenoic acid	MS, LRI	1923	0.00 ± 0.00a	0.00 ± 0.00a	1.85 ± 0.35c	1.29 ± 0.21b
Alcohols						
1-Pentanol	MS, LRI	1226	1.60 ± 0.67a	2.99 ± 0.08a	22.05 ± 0.56c	16.45 ± 0.83b
Furfuryl alcohol	MS, LRI	1680	0.00 ± 0.00a	0.00 ± 0.00a	0.47 ± 0.01b	0.56 ± 0.02c
Phenylethyl Alcohol	MS, LRI	1958	0.94 ± 0.20a	1.33 ± 0.10a	48.22 ± 1.57b	67.72 ± 0.67c
Esters						
3-Methylbutyl octanoate	MS, LRI	1658	0.00 ± 0.00a	0.00 ± 0.00a	0.25 ± 0.0b	0.47 ± 0.08c
Ethyl Acetate	MS, LRI		0.00 ± 0.00a	0.00 ± 0.00a	13.94 ± 0.88b	15.10 ± 0.27b
Ethyl hexanoate	MS, LRI	1224	0.00 ± 0.00a	0.00 ± 0.00a	2.95 ± 0.00b	2.94 ± 0.29b
Ethyl (E)-4-hexenoate	MS, LRI	1289	0.00 ± 0.00a	0.00 ± 0.00a	5.50 ± 0.31b	4.96 ± 0.33b
Ethyl octanoate	MS, LRI	1433	0.00 ± 0.00a	0.00 ± 0.00a	15.76 ± 0.31b	30.11 ± 0.27c
Ethyl 2,4-hexadienoate	MS, LRI	1515	0.73 ± 0.17a	0.50 ± 0.03a	0.55 ± 0.04a	0.70 ± 0.01a
Ethyl nonanoate	MS, LRI	1538	0.00 ± 0.00a	0.00 ± 0.00a	0.40 ± 0.02b	0.48 ± 0.06b
Ethyl decanoate	MS, LRI	1637	0.00 ± 0.00a	0.00 ± 0.00a	3.40 ± 0.00b	9.08 ± 0.74c
Ethyl 9-decenoate	MS, LRI	1693	0.00 ± 0.00a	0.00 ± 0.00a	0.45 ± 0.04b	0.89 ± 0.20c
Isoamyl acetate	MS, LRI	1119	0.00 ± 0.00a	0.00 ± 0.00a	2.79 ± 0.24b	3.97 ± 0.03b
Phenethyl acetate	MS, LRI	1829	0.00 ± 0.00a	0.00 ± 0.00a	1.85 ± 0.15b	4.84 ± 0.69c
Aldehydes						
Benzaldehyde	MS, LRI	1544	2.13 ± 0.17c	1.86 ± 0.04b	0.56 ± 0.00a	0.48 ± 0.05a
Benzeneacetaldehyde	MS, LRI	1663	14.51 ± 0.30d	13.54 ± 0.24c	1.68 ± 0.16b	0.31 ± 0.04a
Furfural	MS, LRI	1480	42.42 ± 0.93c	33.79 ± 0.70b	0.33 ± 0.00a	0.64 ± 0.00a
Ketones						
2,3-Pentanedione	MS, LRI	1064	1.07 ± 0.05b	1.47 ± 0.01c	0.00 ± 0.00a	0.00 ± 0.00a
2-Acetyl-furan	MS, LRI	1522	1.12 ± 0.16b	1.02 ± 0.05b	0.40 ± 0.02a	0.50 ± 0.04a
2-Formyl-5-methylfuran	MS, LRI	1591	15.32 ± 0.52c	13.45 ± 0.54b	0.26 ± 0.00a	0.23 ± 0.03a
Acetoin	MS, LRI	1308	0.00 ± 0.00a	0.00 ± 0.00a	0.55 ± 0.01b	0.91 ± 0.07c
Butyrolactone	MS, LRI	1655	0.63 ± 0.06d	0.52 ± 0.03c	0.00 ± 0.00b	0.00 ± 0.00a
Furans						
2,5-Dimethylfuran	MS, LRI		0.00 ± 0.00a	0.00 ± 0.00a	2.99 ± 0.18b	3.81 ± 0.17c
2-Acetyl-5-methylfuran	MS, LRI	1634	0.32 ± 0.06b	0.40 ± 0.02b	0.21 ± 0.01a	0.16 ± 0.00a
2-Vinylfuran	MS, LRI	2023	0.60± 0.04b	0.62 ± 0.02b	0.35 ± 0.03a	0.41 ± 0.08a
Benzene, 1,3-bis(1,1-dimethylethyl)-	MS, LRI	1427	2.67 ± 0.23b	3.79 ± 0.12c	1.55 ± 0.37a	2.10 ± 0.12ab
Others						
3-Acetylpyrrole	MS, LRI	1995	0.41 ± 0.06a	0.53 ± 0.01a	0.48 ± 0.02a	0.41 ± 0.09a
α-pyrone-6-carboxylic acid	MS, LRI	1366	0.00 ± 0.00a	0.00 ± 0.00a	3.69 ± 0.71c	2.172 ± 0.72b
cis-Linalool oxide	MS, LRI	1476	0.54 ± 0.03c	0.50 ± 0.03c	0.42 ± 0.02b	0.28 ± 0.01a
Guaiacol	MS, LRI	1879	0.25 ± 0.03a	0.33 ± 0.02a	1.32 ± 0.14b	0.27 ± 0.04a

5 LRI: linear retention index; determined on a DB-FFAF column relative to C7-C40 hydrocarbons; YE: adding 0.25% yeast extract; SC represents *S. cerevisiae* MERIT without yeast extract and SY represents *S. cerevisiae* MERIT with 0.25% yeast extract. Values are the mean of triplicate fermentations (± standard deviation); a,b,c,d Statistical analysis using ANOVA (n=3) at 95% confidence interval. Same letter indicates no significant difference between samples

Table 4: Selected volatile compounds and their concentrations (µg/L) before and after fermentation with *S. cerevisiae* MERIT

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In conclusion, SCG hydrolysates fermented with *Saccharomyces* yeast *S. cerevisiae* MERIT may be used for producing SCG-derived alcoholic beverage with a pleasant aroma profile.

Example 3 - Formation of beverage from another non-*Saccharomyces* yeast

Two types of SCG hydrolysates were prepared as described above, with one having 0% yeast extract and the other having 0.25% (w/v) yeast extract. As mentioned above, pH was adjusted to 5 using 10 M NaOH and subsequently pasteurized at 60°C for 30 min, followed by cooling to room temperature.

Fermentation of the hydrolysates was performed by inoculating the hydrolysates with *Torulaspora delbrueckii* Biodiva (Lallemand Inc, Brooklyn Park, Australia) with an initial cell population of approximately 5 log CFU/mL. The fermentation was terminated after static incubation for 14 days at 20°C. Sugars, ethanol, organic acids, phenolics, amino acids, and volatiles in the fermented media were analysed.

Results**Yeast growth**

As can be seen in Figure 7, *T. delbrueckii* Biodiva grew well in the SCG hydrolysates with increased cell populations of 1.74-2.43 log CFU/mL and the yeast population was markedly increased when yeast extracts were added.

Ethanol content

The production of ethanol increased along with the progression of fermentation and reached a plateau by day 10 in the presence of added yeast extracts but ethanol production continued until day 14 of termination for the hydrolysates without added yeast extracts. As seen in Figure 8, the final ethanol content of fermented SCG hydrolysates were 3.65% (v/v, with 0% yeast extracts) and 3.75% (v/v, with 2.5% yeast extracts). Yeast extracts addition markedly enhanced ethanol production during fermentation from day 2 to day 10.

Non-volatile components

Besides ethanol, other compounds such as the organic acids, phenolics, sugars, and alkaloids produced were also measured. Table 5 provides the values of the different components measured.

Compound	Day 0	Day 14	
	SCG hydrolysate	Biodiva (TC)	Biodiva + YE (TY)
Sugars (g/L)			
Fructose	14.41 ± 0.27b	0.00 ± 0.00a	0.00 ± 0.00a
Glucose	26.37 ± 0.47b	0.00 ± 0.00a	0.00 ± 0.00a
Sucrose	8.06 ± 0.06b	0.33 ± 0.01a	0.30 ± 0.00a
Mannose	7.69 ± 0.19b	2.32 ± 0.06a	2.46 ± 0.07a
Galactose	14.94 ± 0.20b	7.63 ± 0.51a	7.26 ± 0.30a
Arabinose	2.49 ± 0.30a	2.27 ± 0.03a	2.29 ± 0.05a
Total	74.98 ± 1.23b	12.74 ± 1.11a	12.51 ± 0.74a
Organic acids (g/L)			
Citric acid	38.35 ± 0.13b	36.29 ± 0.02a	36.96 ± 0.04ab
α-Ketoglutaric acid (mg/L)	30.89 ± 2.40c	23.05 ± 0.53b	16.59 ± 0.61a
Malic acid	0.97 ± 0.05b	0.29 ± 0.01a	0.24 ± 0.01a
Succinic acid	1.83 ± 0.12a	2.40 ± 0.06b	3.05 ± 0.00c
Lactic acid	0.09 ± 0.00a	0.55 ± 0.04b	0.71 ± 0.05c
Acetic acid	0.04 ± 0.00a	0.58 ± 0.02b	0.57 ± 0.02b
Phenolic acids (mg/L)			
Chlorogenic acids	120.86 ± 2.27b	92.51 ± 5.09a	100.69 ± 4.66ab
Caffeic acid	469.88 ± 8.27a	874.83 ± 13.01b	896.66 ± 30.67b
Ferulic acid	18.64 ± 0.85a	18.71 ± 0.70a	19.41 ± 0.67a
p-Coumaric acid	7.40 ± 0.21a	7.13 ± 0.29a	7.74 ± 0.13a
Alkaloids (mg/L)			
Trigonelline	523.19 ± 15.71a	699.28 ± 6.07b	725.95 ± 31.53b
Theobromine	126.01 ± 3.33b	103.18 ± 2.90a	108.20 ± 4.63ab
Theophylline	73.23 ± 2.17b	54.87 ± 1.89a	57.93 ± 2.48a
Caffeine	1386.29 ± 39.10a	1328.01 ± 47.39a	1435.86 ± 25.26a

Note: SCG: spent coffee grounds; TC: *T. delbrueckii* Biodiva without yeast extracts; TY: *T. delbrueckii* Biodiva with 0.25% (w/v) yeast extracts. a, b, c: Statistical analysis using ANOVA (n=3) at 95% confidence interval. Same letters indicate no significant difference between samples.

5 **Table 5: Selected non-volatile compounds and their concentrations before and after fermentation with *T. delbrueckii* Biodiva**

Sugars such as fructose, glucose, sucrose (added), mannose, galactose and arabinose were detected in the SCG hydrolysates. The total sugar contents declined significantly from 74.98 g/L to around 12.74 g/L after fermentation. All sugars were significantly consumed by yeast during fermentation, accelerated by the addition of yeast extracts. Among the detected organic acids (citric acid, α-ketoglutaric acid, malic acid, succinic acid, lactic acid and acetic acid), succinic acid (2.40 g/L), lactic acid (0.55 g/L) and acetic acid (0.71 g/L) increased significantly, while malic acid decreased significantly

after fermentation. Moreover, the generation of succinic acid (3.05 g/L) was further increased with addition of yeast extracts.

Phenolic acids and alkaloids were also quantified, as shown in Table 5. For phenolic acids, caffeic acid was increased significantly while chlorogenic acid decreased after
5 fermentation. Ferulic acid and p-coumaric acid kept stable before and after fermentation. Supplementation of yeast extracts had a positive effect on the generation of caffeic acid while no significant effects were observed on other phenolic acids. Among the four alkaloids, trigonelline was produced significantly after fermentation while others decreased slightly.

10 Amino acids were taken up by the *T. delbrueckii* Biodiva when compared to the contents of amino acids before and after fermentation. Eighteen kinds of amino acids were quantified. The addition of yeast extracts increased the amount of amino acids in SCG hydrolysates. In general, a decreasing trend in the contents of most amino acids was observed. After fermentation, most amino acids except aspartic acid, glutamic acid
15 and alanine significantly decreased to low levels. Major utilization of the amino acids corresponded to the high levels of volatile compounds generated in the alcoholic beverage (Table 6). The contents of amino acids in the final SCG beverages ranged from 1.22-126.14 mg/L.

Volatile components

20 The identified volatile compounds of SCG hydrolysates before and after fermentation are shown in Table 6. Volatile acids (6), alcohols (3), esters (13), aldehydes (3), furan (3) and other types of volatile compounds (3) were detected. Among these volatile compounds, esters were the largest group and played a significant role in the pleasant and fruity aroma in the formed beverage, including ethyl acetate, ethyl hexanoate, ethyl
25 (*E*)-4-hexenoate, ethyl octanoate, ethyl decanoate, ethyl (*Z*)-4-decenoate, ethyl dodecanoate, ethyl 2,4-hexadienoate, Isoamyl acetate, propyl acetate, octyl acetate, 2-phenethyl acetate, and 2-phenethyl propionate. Moreover, the production of several esters was increased with addition of yeast extracts. In particular, ethyl dodecanoate was only generated in the SCG hydrolysate with added yeast extracts. Also, three new
30 esters (ethyl dodecanoate, ethyl (*Z*)-4-decenoate, octyl acetate, phenethyl propionate) were detected, which were not detected in the beverages of Examples 2 and 3.

Compound	Identification methods	LRI	Day 0		Day final	
			Pre-Treated SCG		SCG beverage	
			0% YE	0.25% YE	Biodiva (TC)	Biodiva + YE (TY)
Acids						
Acetic acid	MS, LRI	1463	250.85 ± 25.71b 102.74 ± 17.81b	242.01 ± 19.59b 100.99 ± 15.71b	158.238 ± 12.12a	195.70 ± 13.67a
3-Methylbutanoic acid	MS, LRI	1611			0.00 ± 0.00a	0.00 ± 0.00a
Pentanoic acid	MS, LRI	1623	0.00 ± 0.00a	0.00 ± 0.00a	48.69 ± 3.91b	46.51 ± 7.99b
4-Hexenoic acid	MS, LRI	1918	0.00 ± 0.00a	0.00 ± 0.00a	26.12 ± 2.74b	27.75 ± 0.78b
Hexanoic acid	MS, LRI	1834	145.32 ± 7.45a 128.89 ± 25.52c	148.73 ± 17.63a	0.00 ± 0.00b	0.00 ± 0.00b
Benzoic acid	MS, LRI	2452		36.98 ± 3.34b	0.00 ± 0.00a	0.00 ± 0.00a
Alcohols						
Ethanol	MS, LRI	N.A.	0.00 ± 0.00a	0.00 ± 0.00a	8058.07 ± 1010.54b	8077.29 ± 622.19b 909.25 ± 61.06b 183.43 ± 10.44b
2-Phenylethyl alcohol	MS, LRI	1934	34.27 ± 2.70a	40.94 ± 5.17a	861.22 ± 20.02b	
1-Pentanol	MS, LRI	1228	0.00 ± 0.00a	0.00 ± 0.00a	172.18 ± 14.38b	
Esters						
Ethyl acetate	MS, LRI	N.A.	0.00 ± 0.00a	0.00 ± 0.00a	215.30 ± 9.80b	277.12 ± 13.11c
Ethyl hexanoate	MS, LRI	1252	0.00 ± 0.00a	0.00 ± 0.00a	59.30 ± 8.92b	88.97 ± 3.88c
Ethyl (E)-4-hexenoate	MS, LRI	1298	0.00 ± 0.00a	0.00 ± 0.00a	56.80 ± 8.15b	82.16 ± 10.35c 141.92 ± 18.68b 292.67 ± 31.48c
Ethyl octanoate	MS, LRI	1447	0.00 ± 0.00a	0.00 ± 0.00a	66.95 ± 6.80b	
Ethyl decanoate	MS, LRI	1653	0.00 ± 0.00a	0.00 ± 0.00a	84.82 ± 9.57b	
Ethyl (Z)-4-decenoate	MS, LRI	1601	0.00 ± 0.00a	0.00 ± 0.00a	10.05 ± 0.96b	13.80 ± 0.30c
Ethyl dodecanoate	MS, LRI	1829	0.00 ± 0.00a	0.00 ± 0.00a	0.00 ± 0.00a	12.43 ± 1.18b 106.50 ± 10.08c 144.90 ± 17.49c
Ethyl 2,4-hexadienoate	MS, LRI	1509	0.00 ± 0.00a	0.00 ± 0.00a	85.51 ± 5.52b	
Octyl acetate	MS, LRI	1477	0.00 ± 0.00a	0.00 ± 0.00a	66.95 ± 6.80b	
2-Phenethyl acetate	MS, LRI	1817	0.00 ± 0.00a	0.00 ± 0.00a	33.37 ± 2.57b	42.61 ± 6.57c
2-Phenethyl propionate	MS, LRI	1882	0.00 ± 0.00a	0.00 ± 0.00a	24.53 ± 0.96b	38.35 ± 5.33c
Aldehydes						
Benzaldehyde	MS, LRI	1540	127.92 ± 21.65b	125.21 ± 4.36b	73.53 ± 4.86a	80.83 ± 13.49a
Benzeneacetaldehyde	MS, LRI	1629	0.00 ± 0.00a	0.00 ± 0.00a	30.41 ± 3.59b	50.95 ± 0.04c
3-Furaldehyde	MS, LRI	1488	1425.06 ± 209.81a	1362.87 ± 264.83a	23.168 ± 2.09b	21.48 ± 4.27b
Furan						
2-Pentylfuran	MS, LRI	1247	195.44 ± 15.12b 602.72 ± 59.27b	216.44 ± 15.55b 575.27 ± 76.01b	0.00 ± 0.00a	0.00 ± 0.00a
2-Formyl-5-methylfuran	MS, LRI	1574			11.29 ± 1.15a	8.12 ± 0.51a
2-Vinylfuran	MS, LRI	2001	15.15 ± 1.80b	11.72 ± 2.33a	11.15 ± 0.63a	10.07 ± 0.51a
Phenolics						
Guaiacol	MS, LRI	1892	12.70 ± 2.23c	10.23 ± 1.20ab	7.06 ± 1.09a	7.39 ± 1.00a
Others						
2,4-Decadienal	MS, LRI	1866	99.54 ± 10.02b	92.14 ± 10.29b	0.00 ± 0.00a	0.00 ± 0.00a
3-Acetylpyrrole	MS, LRI	1992	20.56 ± 2.79b	19.48 ± 1.55b	12.94 ± 1.87a	10.92 ± 1.67a

LRI: linear retention index; determined on a DB-FFAF column relative to C7-C40 hydrocarbons; YE: adding 0.25% yeast extract; TC: *T. delbrueckii* Biodiva without yeast extracts; TY: *T. delbrueckii* Biodiva

with 0.25% (w/v) yeast extracts. Values are the mean of triplicate fermentations ($\pm$ standard deviation); a,b,c Statistical analysis using ANOVA (n=3) at 95% confidence interval. Same letter indicates no significant difference between samples.

5 **Table 6: Selected volatile compounds and their concentrations ($\mu\text{g/L}$) before and after fermentation with *T. delbrueckii* Biodiva**

Example 4 - Formation of beverage from another non-*Saccharomyces* yeast

The preparation of SCG hydrolysates was as described in Example 3. Pasteurized SCG hydrolysates were inoculated with *P. kluyveri* FrootZen (Chr. Hansen, Horsholm, Denmark) with an initial cell population of approximately 5 log CFU/mL and was statically fermented at 20°C for 14 days. Analyses were conducted on the sugars, ethanol, organic acids, phenolics, amino acids, and volatiles contents as described in Example 3.

Yeast cell growth

15 As seen in Figure 9, *P. kluyveri* FrootZen grew well in the SCG hydrolysates with increased cell populations by 2.28-2.46 log CFU/mL and it markedly increased when yeast extracts were added.

Ethanol content

20 The content of ethanol (1.98%) in the SCG hydrolysates with addition of 0.25% yeast extract was higher than that (1.47%) in the SCG hydrolysates without yeast extracts. Different alcohol contents were detected from day 2 to day 14 ranging from 0.2% to 1.98%, as seen in Figure 10.

Non-volatile components

25 Non-volatile compounds containing sugars, organic acids, phenolic acids, and alkaloids, were measured and their amounts are as shown in Table 7.

Sugars including fructose, glucose, sucrose (added), mannose, galactose and arabinose were detected in the SCG hydrolysates. The total sugar contents declined significantly from 74.98 g/L to around 20.49-35.06 g/L after fermentation. All sugars were significantly consumed by yeast during the fermentation, accelerated by the addition of yeast extracts.

Compound	Day 0	Day 14	
	Pre-treated SCG	FrootZen (PC)	FrootZen + YE (PY)
Sugars (g/L)			
Fructose	14.41 ± 0.27c	11.98 ± 0.22b	4.33 ± 0.16a
Glucose	26.37 ± 0.47c	5.49 ± 0.17b	0.00 ± 0.00a
Sucrose	8.06 ± 0.06b	6.54 ± 0.33a	6.00 ± 0.23a
Mannose	7.69 ± 0.19b	2.16 ± 0.09a	1.35 ± 0.05a
Galactose	14.94 ± 0.20b	6.629 ± 0.19a	6.60 ± 0.19a
Arabinose	2.49 ± 0.30a	2.22 ± 0.13a	2.21 ± 0.20a
In total	74.98 ± 1.23c	35.06 ± 0.47b	20.49 ± 0.52a
Organic acids (g/L)			
Citric acid	38.35 ± 0.13b	36.48 ± 0.06a	36.85 ± 0.04a
α-Ketoglutaric acid (mg/L)	30.89 ± 2.40a	34.96 ± 0.96ab	36.45 ± 5.93b
Malic acid	0.97 ± 0.05c	0.33 ± 0.01b	0.25 ± 0.00a
Succinic acid	1.83 ± 0.12a	2.18 ± 0.07b	3.17 ± 0.02c
Lactic acid	0.09 ± 0.00a	0.71 ± 0.06b	1.13 ± 0.12c
Acetic acid	0.04 ± 0.00a	0.52 ± 0.02b	0.48 ± 0.03b
Phenolic acids (mg/L)			
Chlorogenic acids	120.86 ± 2.27b	99.57 ± 9.53a	66.11 ± 3.77ab
Caffeic acid	469.88 ± 8.27a	340.83 ± 17.73b	219.57 ± 6.62b
Ferulic acid	18.64 ± 0.85a	16.83 ± 1.63a	11.96 ± 0.32a
p-Coumaric acid	7.40 ± 0.21a	7.44 ± 0.017a	18.13 ± 0.43b
Alkaloids (mg/L)			
Trigonelline	523.19 ± 15.71a	716.28 ± 18.57b	594.11 ± 12.62b
Theobromine	126.01 ± 3.33b	111.95 ± 3.61a	99.89 ± 1.67ab
Theophylline	73.23 ± 2.17a	60.48 ± 1.94b	53.29 ± 1.01b
Caffeine	1386.29 ± 39.10a	1412.35 ± 21.69a	1395.78 ± 53.78a

Note: SCG: spent coffee grounds; PC: *P. kluyveri* FrootZen without yeast extract; PY: *P. kluyveri* FrootZen with 0.25% (w/v) yeast extracts. a, b, c: Statistical analysis using ANOVA (n=3) at 95% confidence interval. Same letters indicate no significant difference between samples.

Table 7: Selected non-volatile compounds and their concentrations before and after fermentation with *P. kluyveri* FrootZen

5

Among the detected organic acids (citric acid, α-ketoglutaric acid, malic acid, succinic acid, lactic acid and acetic acid), succinic acid (2.18 g/L), lactic acid (0.71 g/L) and acetic acid (0.52 g/L) increased significantly, while citric acid and malic acid decreased significantly after fermentation. Moreover, the generation of succinic acid (3.17 g/L) and lactic acid (1.13 g/L) was further increased with addition of yeast extracts.

10

Phenolic acids and alkaloids were also quantified in Table 7. All detected phenolic acids declined (some significantly) after fermentation except for p-coumaric acid when yeast extracts was added. The supplementation of yeast extracts accelerated the generation of trigonelline and p-coumaric acid and accelerated the consumption of

15

other detected phenolic compounds. Among the four alkaloids, trigonelline was produced significantly after fermentation while others decreased slightly, especially for significantly decreased caffeine when yeast extracts was added.

Changes of amino acids in the SCG hydrolysates fermented with *P. kluyveri* FrootZen were also measured (not shown). Eighteen kinds of amino acids were quantified. The addition of yeast extracts significantly enhanced the amount of amino acids in SCG hydrolysates. In general, a decreasing trend in the contents of most amino acids was observed. After fermentation, most amino acids except threonine, serine and cystine significantly decreased to low levels. Major utilization of the amino acids corresponded to the high levels of volatile compounds generated in the alcoholic beverage (see Table 8 below). The contents of threonine, serine and cystine increased after fermentation. The content of amino acids in the final SCG beverages ranged from 0-34.89 mg/L.

Volatile compounds

Volatile compounds quantified and measured are summarized in Table 8. In summary, there were acids (4), alcohols (2), esters (13), aldehydes (3), furan & ketones (4) and other odour-active substances (3). Among these compounds, 13 esters contribute to the pleasant and fruity aroma of SCG beverages which consist of ethyl acetate, ethyl hexanoate, ethyl octanoate, ethyl decanoate, ethyl dodecanoate, ethyl 2,4-hexadienoate, isoamyl acetate, propyl acetate, octyl acetate, benzyl acetate, 2-phenethyl acetate, 2-phenethyl propionate and furfuryl acetate. Moreover, the addition of yeast extracts significantly enhanced the generation of ethyl acetate, ethyl hexanoate, isoamyl acetate, and 2-phenethyl acetate.

In contrast with Example 3, two new esters (benzyl acetate, furfuryl acetate) were also detected in the beverage formed and the contents of some esters (e.g. ethyl acetate, isoamyl acetate and phenethyl acetate) were much higher than that in Example 3. Hence, the aroma characters of volatile compounds in Example 4 would be distinguished from that of other SCG related beverages formed in the other examples.

Compound	Identification methods	LRI	Day 0		Day 14	
			SCG hydrolysate		SCG beverage	
			0% YE	0.25% YE	FrootZen (PC)	FrootZen+ 0.25% YE (PY)
Acids						
Pentanoic acid	MS, LRI	1623	0.00 ± 0.00a	0.00 ± 0.00a	48.86± 4.41b	73.40 ± 5.03c
Hexanoic acid	MS, LRI	1834	0.45 ± 0.05a 128.89 ±	14.88 ± 1.76b	0.00 ± 0.00a	0.00 ± 0.00a
Benzoic acid	MS, LRI	2452	25.52c	36.98 ± 3.34b	0.00 ± 0.00a	0.00 ± 0.00a
Octanoic acid	MS, LRI	2038	0.00 ± 0.00a	0.00 ± 0.00a	30.84 ± 1.70b	93.64 ± 5.45c
Alcohols						
1-Pentanol	MS, LRI	1228	0.00 ± 0.00a	0.00 ± 0.00a	0.00 ± 0.00a	51.06 ± 2.95b
2-Phenylethyl alcohol	MS, LRI	1934	34.27 ± 2.70a	40.94 ± 5.17a	267.93 ± 12.26c	195.91 ± 11.17b
Esters						
Ethyl acetate	MS, LRI	N.A.	0.00 ± 0.00a	0.00 ± 0.00a	5850.99 ± 337.23b	10482.89 ± 966.95c
Ethyl hexanoate	MS, LRI	1252	0.00 ± 0.00a	0.00 ± 0.00a	54.40 ± 6.82b	84.83 ± 6.53c
Ethyl octanoate	MS, LRI	1447	0.00 ± 0.00a	0.00 ± 0.00a	40.99 ± 5.54a 125.66 ±	321.14 ± 41.91b
Ethyl decanoate	MS, LRI	1653	0.00 ± 0.00a	0.00 ± 0.00a	11.01b	292.22 ± 25.92c
Ethyl dodecanoate	MS, LRI	1829	0.00 ± 0.00a	0.00 ± 0.00a	0.00 ± 0.00a	50.11 ± 1.32b
Ethyl 2,4-hexadienoate	MS, LRI	1509	0.00 ± 0.00a	0.00 ± 0.00a	67.62 ± 5.12b 1713.15 ±	80.19 ± 1.90c
Isoamyl acetate	MS, LRI	1147	0.00 ± 0.00a	0.00 ± 0.00a	121.23b	3387.50 ± 534.03c
Propyl acetate	MS, LRI	N.A.	0.00 ± 0.00a	0.00 ± 0.00a	0.00 ± 0.00a	51.07 ± 3.09b
Octyl acetate	MS, LRI	1477	0.00 ± 0.00a	0.00 ± 0.00a	18.88 ± 2.49b	21.19 ± 3.06b
Benzyl acetate	MS, LRI	1744	0.00 ± 0.00a	0.00 ± 0.00a	48.48 ± 7.02b	50.48 ± 5.42b
2-Phenethyl acetate	MS, LRI	1817	0.00 ± 0.00a	0.00 ± 0.00a	20470.10 ± 925.96b	30988.75 ± 1127.51b
2-Phenethyl propionate	MS, LRI	1882	0.00 ± 0.00a	0.00 ± 0.00a	79.53 ± 6.20b 315.74 ±	86.53 ± 5.12b
Furfuryl acetate	MS, LRI	1520	0.00 ± 0.00a	0.00 ± 0.00a	46.15b	286.55 ± 10.12b
Aldehydes						
Benzaldehyde	MS, LRI	1540	127.92 ± 21.65b	125.21 ± 4.36b	40.19 ± 7.43a	120.71 ± 11.69b
Benzeneacetaldehyde	MS, LRI	1629	0.00 ± 0.00a 14.25 ±	0.00 ± 0.00a	141.31 ± 21.10b	401.67 ± 20.10c
Furfural	MS, LRI	1488	2.09a	13.63 ± 2.65a	14.19 ± 1.67a	15.86 ± 0.54a
Furan & Ketones						
2,5-Dimethylfuran	MS, LRI	1033	0.00 ± 0.00a 195.44 ±	0.00 ± 0.00a	43.17 ± 3.81b	74.79 ± 4.27b
2-Pentylfuran	MS, LRI	1247	15.12a 602.72 ±	15.55a 575.27 ±	0.00 ± 0.00b	0.00 ± 0.00b
2-Formyl-5-methylfuran	MS, LRI	1574	59.27b 15.15 ±	76.01b	0.00 ± 0.00a	0.00 ± 0.00a
2-Vinylfuran	MS, LRI	2001	1.80a	11.72 ± 2.33a	13.21 ± 0.95a	17.26 ± 1.16a
Phenolics						
Guaiacol	MS, LRI	1892	12.70 ± 2.23c	10.23 ± 1.20bc	5.67 ± 0.54ab	8.49 ± 0.56a
Others						
2,4-Decadienal	MS, LRI	1866	99.54 ± 10.02b 20.56 ±	92.14 ± 10.29b	0.00 ± 0.00a	0.00 ± 0.00a
3-Acetylpyrrole	MS, LRI	1992	2.79ab	19.48 ± 1.55a	17.84 ± 1.16b	24.19 ± 0.51a

LRI: linear retention index; determined on a DB-FFAF column relative to C7-C40 hydrocarbons; YE: adding 0.25% yeast extract; Values are the mean of triplicate fermentations ($\pm$ standard deviation); a,b,c Statistical analysis using ANOVA (n=3) at 95% confidence interval. PC: *P. kluyveri* FrootZen without yeast extract; PY: *P. kluyveri* FrootZenFrootZen with 0.25% (w/v) yeast extracts. Same letter indicates no significant difference between samples

Table 8: Selected volatile compounds and their concentrations ($\mu\text{g/L}$) before and after fermentation with *P. kluyveri* FrootZen

In this Example, the production of ethyl acetate, isoamyl acetate and 2-phenethyl acetate was significantly higher than other esters as seen in Figures 11A to C. Ethyl acetate presents an ethereal fruity smell when less than 10% in solution; isoamyl acetate gives a sweet fruity banana smell even at 1% solution; 2-phenethyl acetate contributes to a floral, rose like aroma, and the smell can last for 16 hours at 100%. Therefore, the high generation of ethyl acetate, isoamyl acetate and 2-phenethyl acetate could also be a safe and natural way to obtain condensed flavour and fragrance.

Examples 5, 6 and 7 - Formation of beverage from a non-Saccharomyces yeast and lactic acid bacteria

In these examples, beverages were formed by utilizing a non-*Saccharomyces* yeast (e.g. *Lachancea thermotolerans* Concerto) together with three different strains of a lactic acid bacterium (LAB), *Oenococcus oeni* to ferment the SCG hydrolysates with added yeast extracts to modulate flavour.

SCG hydrolysates prepared as described above. pH was adjusted to 5 by adding 10 M NaOH and subsequently, the SCG hydrolysates were pasteurized at 60°C for 30 min and cooled to room temperature before being fermented with *L. thermotolerans* Concerto (Chr. Hansen, Horsholm, Denmark) and *O. oeni* (Chr. Hansen, Horsholm Denmark) with an initial cell count of approximately 5 log CFU/mL for *L. thermotolerans* Concerto and approximately 6 log CFU/mL for *O. oeni*.

Three co-culture fermentations were conducted, including *L. thermotolerans* Concerto and *O. oeni* Lalvin 31, *L. thermotolerans* Concerto and *O. oeni* Enoferm Beta and *L. thermotolerans* Concerto and *O. oeni* PN4. The hydrolysates have been defined as follows: Example 5 - Concerto + Lalvin; Example 6 - Concerto + Beta; and Example 7 - Concerto + PN4. The monoculture of *L. thermotolerans* Concerto (Concerto) was used

as a control. The SCG hydrolysate fermentations were terminated after static incubation for 14 days at 20°C. Ethanol, sugars, organic acids, phenolics, alkaloids, amino acids and volatiles were analyzed.

The growth of yeast and *O. oeni*

- 5 The growth of *L. thermotolerans* Concerto under the four different combinations of LAB described above is shown in Figure 12A.

Yeast cell populations increased by 0.5 to 1.34 Log CFU/mL from day 0 to day 1 and decreased after day 1 in both control and co-culture fermentations. *L. thermotolerans* Concerto slightly decreased when co-inoculated with *O. oeni* Lalvin 31, while the
10 population of *L. thermotolerans* Concerto decreased sharply when co-inoculated with *O. oeni* PN4 and *O. oeni* Enoferm Beta from day 1 and no viable yeast cell could be detected by day 7.

The changes of the three *O. oeni* strains population showed similar trends as can be seen in Figure 12B. Cell growth increased gradually from day 0 to day 2 and decreased
15 from day 2. The cell counts for *O. oeni* PN4 (initial 7.69 Log CFU/mL) and Enoferm Beta (initial 7.72 Log CFU/mL) increased to 9.41 Log CFU/mL and 9.30 Log CFU/mL at day 2, respectively. The population of *O. oeni* Lalvin 31 (initial 6.26 Log CFU/mL) increased to 8.09 Log CFU/mL at day 2. The lower initial biomass of *O. oeni* Lalvin 31 was ascribed to its slow growth in pre-culture. The increments in cell counts were
20 approximately 1.7 Log CFU/mL for the three *oenococcal* strains.

Ethanol content

As seen in Figure 13, the ethanol content in the SCG hydrolysates fermented with the co-culture of *L. thermotolerans* Concerto and *O. oeni* Lalvin 31 (Example 5), co-culture of *L. thermotolerans* Concerto and *O. oeni* Enoferm Beta (Example 6), and co-culture
25 of *L. thermotolerans* Concerto and *O. oeni* PN4 (Example 7) were 1.55%, 0.58%, and 0.58%, respectively, which were significantly lower than that (4.94%, v/v) of *L. thermotolerans* Concerto monoculture fermentation.

Non-volatile components

Non-volatile compounds such as sugars, organic acids, phenolic acids, alkaloids, and amino acids were measured. Sugars including fructose, glucose, sucrose, mannose, galactose and arabinose were detected in the SCG hydrolysates. The results are as shown in Table 9.

5

Compound	Day 0	Fermented SCG (Day 14)			
	SCG hydrolysates	Concerto	Concerto + Lalvin	Concerto + Beta	Concerto + PN4
Sugars (g/L)					
Fructose	18.70 ± 0.62c	3.92 ± 0.14b	2.64 ± 0.63a	2.18 ± 0.19a	2.22 ± 0.12a
Glucose	52.68 ± 2.12b	3.85 ± 0.29a	4.15 ± 0.04a	6.90 ± 0.92a	6.95 ± 0.13a
Sucrose	10.83 ± 0.31c	2.48 ± 0.08a	4.76 ± 0.07b	4.55 ± 0.38b	4.94 ± 0.28b
Mannose	16.27 ± 0.74c	4.05 ± 0.08a	4.54 ± 0.25a	7.11 ± 0.82b	7.16 ± 0.12b
Galactose	16.88 ± 0.49c	4.40 ± 0.50a	6.65 ± 0.28b	7.13 ± 0.71b	6.88 ± 0.39b
Arabinose	2.59 ± 0.16a	2.49 ± 0.10a	2.60 ± 0.19a	2.54 ± 0.06a	2.51 ± 0.07a
Total	117.96 ± 2.99c	21.19 ± 0.77a	25.34 ± 1.33a	30.42 ± 2.96b	30.65 ± 0.77b
Organic acids (g/L)					
Citric acid	35.05 ± 0.47b	33.73 ± 0.12b	27.32 ± 1.08a	32.54 ± 0.15b	33.45 ± 0.07b
α-Ketoglutaric acid (mg/L)	34.55 ± 4.14c	9.74 ± 0.09a	20.32 ± 0.71b	19.58 ± 1.04b	14.19 ± 0.32a
Malic acid	0.38 ± 0.02c	0.04 ± 0.00a	0.17 ± 0.01b	0.36 ± 0.02c	0.34 ± 0.01c
Pyruvic acid	0.01 ± 0.00a	0.25 ± 0.01e	0.16 ± 0.01d	0.04 ± 0.00b	0.06 ± 0.00c
Succinic acid	2.67 ± 0.04a	3.75 ± 0.17a	14.86 ± 0.63b	16.36 ± 2.01b	14.34 ± 0.70b
Lactic acid	0.12 ± 0.00a	0.53 ± 0.02a	18.46 ± 0.16b	20.24 ± 2.04bc	22.37 ± 1.10c
Acetic acid	0.14 ± 0.00a	0.45 ± 0.01b	5.04 ± 0.19d	4.67 ± 0.09c	5.16 ± 0.05d
Phenolic acids (mg/L)					
Chlorogenic acid	137.35 ± 4.80c	45.011 ± 5.64a	56.78 ± 4.46a	78.80 ± 5.59b	81.86 ± 5.74b
	596.96 ±	649.66 ±	640.01 ±		
Caffeic acid	28.69b	27.75c	11.86bc	55.07 ± 5.89a	73.25 ± 2.03a
Ferulic acid	12.44 ± 0.14a	25.21 ± 0.89b	24.94 ± 2.63b	24.88 ± 2.39b	29.40 ± 1.58b
p-Coumaric acid	5.14 ± 0.05a	10.95 ± 0.34d	8.27 ± 0.23c	6.68 ± 0.77b	6.72 ± 0.18b
Alkaloids (mg/L)					
	507.23 ±		471.31 ±	506.18 ±	511.27 ±
Trigonelline	10.19b	441.84 ± 7.20a	1.40ab	27.70b	23.55b
Theobromine	131.18 ± 7.64b	26.26 ± 2.08a	34.30 ± 1.84a	33.67 ± 1.46a	26.72 ± 7.84a
Theophylline	68.83 ± 2.13b	26.40 ± 1.53a	47.86 ± 5.72a	36.71 ± 5.61a	38.44 ± 1.85a
	1199.99 ±	1158.66 ±	1273.93 ±	1032.26 ±	1004.66 ±
Caffeine	37.21ab	36.11ab	185.42b	71.70ab	48.79a

Note: SCG: spent coffee grounds; Concerto: monoculture of *L. thermotolerans* Concerto; Concerto+Lalvin: Co-culture of *L. thermotolerans* Concerto and *O. oeni* Lalvin 31; Concerto+Beta: Co-culture of *L. thermotolerans* Concerto and *O. oeni* Enoferm Beta; Concerto+PN4: Co-culture of *L. thermotolerans* Concerto and *O. oeni* PN4. a, b, c: Statistical analysis using ANOVA (n=3) at 95% confidence interval. Same letters indicate no significant difference between samples

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Table 9: Selected non-volatile compounds and their concentrations before and after fermentation with *L. thermotolerans* Concerto and *O. oeni*

Non-volatile components in Example 5 (Concerto+Lalvin)

In Example 5 (Concerto + Lalvin), the total sugar content declined significantly from 117.96 g/L to around 25.34 g/L after fermentation. All sugars were significantly consumed by yeast and/or bacteria during fermentation except for arabinose. The total
5 sugar content (25.34 g/L) remaining in the fermented SCG hydrolysates in Example 5 was similar to that of monoculture fermentation of *L. thermotolerans* Concerto.

Organic acids such as citric acid, α -ketoglutaric acid, malic acid, succinic acid, lactic acid and acetic acid were detected. Succinic acid (14.86 g/L), lactic acid (18.46 g/L), acetic acid (5.04 g/L) and pyruvic acid (0.16 g/L) increased significantly, while citric
10 acid, α -Ketoglutaric acid and malic acid decreased significantly after fermentation. Moreover, succinic acid and lactic acid produced in the co-culture of *L. thermotolerans* Concerto with *O. oeni* Lalvin 31 were significantly higher than those in the monoculture of *L. thermotolerans* Concerto.

Phenolic acids and alkaloids were also quantified in Table 9. After fermentation, ferulic
15 acid and *p*-coumaric acid increased significantly; chlorogenic acids decreased significantly, while caffeic acid kept stable. Among the four alkaloids, theobromine and theophylline decreased significantly while others kept stable after fermentation.

Amino acids especially threonine, serine and tyrosine were markedly consumed by *L. thermotolerans* Concerto and *O. oeni* Lalvin 31 as compared with the consumption of
20 amino acids in the monoculture fermentation of *L. thermotolerans* Concerto. In general, a decreasing trend for most amino acids was observed except for glycine. The remaining content of glycine was higher before fermentation. The contents of amino acids in the final SCG beverages ranged from 1.20-78.75 mg/L after fermentation.

Non-volatile compounds in Example 6 (Concerto+Beta)

25 In Example 6 (Concerto + Beta), the total sugar contents declined significantly from 117.96 g/L to around 30.42 g/L after fermentation, which was higher than that (21.19 g/L) remained monoculture fermentation of *L. thermotolerans* Concerto. All sugars were significantly consumed by yeast and/or bacteria during fermentation except for arabinose.

Organic acids including citric acid, α -ketoglutaric acid, malic acid, succinic acid, lactic acid and acetic acid were detected. Succinic acid (16.36 g/L), lactic acid (20.24 g/L), acetic acid (4.67 g/L) and pyruvic acid (0.04 g/L) increased significantly; α -Ketoglutaric acid decreased significantly while malic acid kept stable before and after fermentation.

- 5 Moreover, the contents of succinic acid and lactic acid produced in the co-culture of *L. thermotolerans* Concerto with *O. oeni* Enoferm Beta were significantly higher than those generated in the monoculture of *L. thermotolerans* Concerto.

Phenolic acids and alkaloids in Example 6 were also quantified as shown in Table 9. For phenolic acids, *p*-coumaric acid and ferulic acid increased significantly, chlorogenic acids and caffeic acid decreased significantly.

Among the four alkaloids, theobromine and theophylline decreased significantly, while trigonelline and caffeine kept stable when taking SCG hydrolysates before fermentation as a reference. The amount of trigonelline was significantly produced as compared with the monoculture of *L. thermotolerans* Concerto.

- 15 Most amino acids were consumed by *L. thermotolerans* Concerto and *O. oeni* Enoferm Beta during fermentation. Among the detected amino acids, serine and alanine were markedly consumed by *L. thermotolerans* Concerto and *O. oeni* Enoferm beta. In general, a decreasing trend for most amino acids was observed. Interestingly, the remaining contents of phenylalanine, lysine and arginine markedly were significantly higher than those remained in the monoculture of *L. thermotolerans* Concerto after fermentation. The contents of amino acids in the final SCG beverages ranged from 3.43-90.39 mg/L.

Non-volatile compounds in Example 7 (Concerto+PN4)

- 25 In Example 7 (Concerto + PN4), the total sugar contents declined significantly from 117.96 g/L to around 30.65 g/L after fermentation, which was higher than that (21.19 g/L) of monoculture fermentation of *L. thermotolerans* Concerto. All sugars were significantly consumed by yeast and/or bacteria during fermentation except for arabinose.

- 30 Among all detected organic acids, succinic acid (14.34 g/L), lactic acid (22.37 g/L), acetic acid (5.16 g/L) and pyruvic acid (0.06 g/L) increased significantly; α -Ketoglutaric

acid decreased significantly, while malic acid and citric acid kept stable before and after fermentation.

Phenolic acids and alkaloids in Example 7 were quantified in Table 9. For phenolic acids, *p*-coumaric acid increased significantly, chlorogenic acids, caffeic acid and
5 ferulic acid decreased significantly.

Among the four alkaloids, theophylline and theophylline decreased significantly after fermentation. Meanwhile, trigonelline decreased significantly as compared with that of the monoculture fermentation of *L. thermotolerans* Concerto.

Most amino acids were consumed by *L. thermotolerans* Concerto and *O. oeni* PN4
10 during fermentation. Among the detected amino acids, serine and tyrosine were markedly consumed by *L. thermotolerans* Concerto and *O. oeni* PN4. In general, a declining trend for most amino acids was observed compared with SCG hydrolysates before fermentation. Interestingly, the contents of amino acids such as valine, Leucine, phenylalanine and arginine after fermentation were markedly higher than those
15 remained in the monoculture fermentation of *L. thermotolerans* Concerto. The contents of amino acids in the final SCG beverages ranged from 2.67-88.54 mg/L.

Volatile compounds

Volatile compounds play a significant role on the aroma and flavour of fermented SCG beverages such as methyl acetate (fragrant, fruity), methyl lactate (strawberry flavour),
20 furfural (almond fragrant), acetoin (buttery odor), diacetyl (buttery odor). The differences between the kinds and concentrations of volatile compounds could contribute to the different characteristics for the fermented SCG beverages.

The identified volatile compounds of SCG hydrolysates before and after fermentation in Examples 5, 6 and 7 are shown in Table 10. In particular, volatile acids (13), alcohols
25 (6), esters (13), aldehydes (4), ketones (6), furans (3), phenols (6), terpenoids (3), pyrazines (3) and pyrroles (3) were detected.

In Example 5 (Concerto + Lalvin), there was a higher content of methyl acetate, methyl lactate, furfural, acetoin, and diacetyl in the coculture fermented SCG hydrolysates when compared with that of the monoculture fermentation of *L. thermotolerans*
30 Concerto.

In Example 6 (Concerto + Beta), the contents of 2-furanmethanol, methyl acetate, methyl lactate, furfural, acetoin, diacetyl, and volatile phenols (e.g. *p*-cresol) were significantly higher than those in the single culture fermentation of *L. thermotolerans* Concerto.

- 5 In Example 7 (Concerto + PN4), the fermented SCG hydrolysates showed significantly higher contents of 2-furanmethanol, methyl acetate, methyl lactate, furfural, diacetyl, 4-ethylguaiacol, 2,5-dimethylfuran and pyrrole-2-carboxaldehyde compared with those in the single culture fermentation of *L. thermotolerans* Concerto.

In summary, the prototypes of SCG beverages of Examples 5, 6 and 7 were different
10 from each other in the contents of sugars, organic acids, phenolic acids, and volatile compounds as discussed above. Moreover, large amounts of organic acids (e.g. succinic acid and lactic acid) were generated from SCG fermentation.

Whilst the foregoing description has described exemplary embodiments, it will be understood by those skilled in the technology concerned that many variations may be
15 made without departing from the present invention.

Compounds	Identification methods	LRI	Day 0	Fermented SCG hydrolysates (Day 14)			
			Unfermented SCG hydrolysates	CC	CL	CB	CP
Acids							
α -Pyrone-6-carboxylic acid	MS, LRI	1364	0.00 $\pm$ 0.00a	72.39 $\pm$ 0.54c	21.53 $\pm$ 0.32b	1.23 $\pm$ 0.01a	0.98 $\pm$ 0.00a
Propanoic acid	MS, LRI	1542	20.48 $\pm$ 0.11d	4.11 $\pm$ 0.04a	4.67 $\pm$ 0.05a	14.64 $\pm$ 0.21c	10.90 $\pm$ 0.09b
2-Propenoic acid	MS, LRI	1642	5.26 $\pm$ 0.04a	3.60 $\pm$ 0.32a	2.43 $\pm$ 0.20a	11.30 $\pm$ 0.12b	2.54 $\pm$ 0.00a
Butanoic acid	MS, LRI	1630	30.19 $\pm$ 0.12d	5.36 $\pm$ 0.03b	7.54 $\pm$ 0.11b	2.52 $\pm$ 0.03a	19.03 $\pm$ 0.09c
Pentanoic acid	MS, LRI	1631	4.72 $\pm$ 0.03e	2.89 $\pm$ 0.01d	0.50 $\pm$ 0.00a	1.56 $\pm$ 0.02b	2.23 $\pm$ 0.03c
3-Methylcrotonic acid	MS, LRI	1803	8.72 $\pm$ 0.03c	3.84 $\pm$ 0.02a	3.92 $\pm$ 0.04a	7.53 $\pm$ 0.04b	7.01 $\pm$ 0.03b
Hexanoic acid	MS, LRI	1848	15.81 $\pm$ 0.09b	7.37 $\pm$ 0.09a	9.45 $\pm$ 0.08a	17.14 $\pm$ 0.11b	21.26 $\pm$ 0.12c
(E)-3-Hexenoic acid	MS, LRI	1922	0.00 $\pm$ 0.00a	8.06 $\pm$ 0.09c	3.58 $\pm$ 0.01b	3.04 $\pm$ 0.03b	3.70 $\pm$ 0.01b
Octanoic acid	MS, LRI	2063	1.50 $\pm$ 0.01a	2.76 $\pm$ 0.03a	6.53 $\pm$ 0.07b	19.23 $\pm$ 0.07d	16.81 $\pm$ 0.04c
Nonanoic acid	MS, LRI	2169	0.87 $\pm$ 0.01b	1.75 $\pm$ 0.00c	0.26 $\pm$ 0.00a	0.99 $\pm$ 0.01b	0.93 $\pm$ 0.00b
Decanoic acid	MS, LRI	2276	0.00 $\pm$ 0.00a	0.81 $\pm$ 0.01b	1.79 $\pm$ 0.03c	2.11 $\pm$ 0.00cd	2.42 $\pm$ 0.02d
Benzoic acid	MS, LRI	2454	3.71 $\pm$ 0.05ab	6.03 $\pm$ 0.03c	2.96 $\pm$ 0.03a	6.21 $\pm$ 0.03c	4.27 $\pm$ 0.04b
Benzeneacetic acid	MS, LRI	2578	1.24 $\pm$ 0.02b	0.82 $\pm$ 0.01a	0.89 $\pm$ 0.02ab	1.16 $\pm$ 0.01ab	0.94 $\pm$ 0.01ab
Alcohols							
Isoamyl alcohol	MS, LRI	1219	1.19 $\pm$ 0.01a	240.72 $\pm$ 1.84d	91.54 $\pm$ 1.00c	61.36 $\pm$ 0.40b	45.55 $\pm$ 1.82b
2-Heptanol	MS, LRI	1323	57.31 $\pm$ 0.32d	5.04 $\pm$ 0.05a	6.21 $\pm$ 0.03a	25.93 $\pm$ 0.02c	17.48 $\pm$ 0.11b
2,3,3-Trimethyl-2-butanol	MS, LRI	1394	1.98 $\pm$ 0.02a	5.20 $\pm$ 0.06b	6.21 $\pm$ 0.04b	13.17 $\pm$ 0.02c	36.98 $\pm$ 0.09d
Furfuryl	MS, LRI	1677	4.11 $\pm$ 0.02a	32.02 $\pm$ 0.39b	30.84 $\pm$ 0.46b	77.02 $\pm$ 0.13d	68.88 $\pm$ 0.27c
Benzyl alcohol	MS, LRI	1910	2.44 $\pm$ 0.01a	4.66 $\pm$ 0.02c	3.58 $\pm$ 0.03b	4.07 $\pm$ 0.02b	5.34 $\pm$ 0.03d
2-Phenylethyl alcohol	MS, LRI	1952	0.00 $\pm$ 0.00a	647.87 $\pm$ 3.12d	154.76 $\pm$ 1.60c	108.50 $\pm$ 0.23b	97.64 $\pm$ 0.71b

Aldehydes									
2-Propenal	MS, LRI	1120	3.37 ± 0.03a	93.99 ± 1.14b	1.21 ± 0.01a	0.00 ± 0.00a	5.85 ± 0.04a		
Furfural	MS, LRI	1477	1.24 ± 0.12a	11.59 ± 0.09ab	20.97 ± 0.13b	88.17 ± 0.85c	93.92 ± 0.19c		
Benzaldehyde	MS, LRI	1538	126.56 ± 0.99b	29.44 ± 0.38a	22.53 ± 0.16a	22.25 ± 0.08a	26.67 ± 0.22a		
2,4-dimethyl benzaldehyde	MS, LRI	1835	10.18 ± 0.03d	5.54 ± 0.03a	5.43 ± 0.03a	7.44 ± 0.03b	9.06 ± 0.05c		
Esters									
Ethyl acetate	MS, LRI	/	0.00 ± 0.00a	94.27 ± 0.81b	318.452 ± 3.49c	69.82 ± 0.80b	80.49 ± 0.89b		
Ethyl hexanoate	MS, LRI	1226	0.00 ± 0.00a	4.11 ± 0.05b	2.21 ± 0.03b	19.87 ± 0.16d	16.81 ± 0.10c		
Ethyl 2,4-hexadienoate	MS, LRI	1512	0.00 ± 0.00a	5.98 ± 0.01d	0.68 ± 0.01c	0.67 ± 0.00c	0.27 ± 0.00b		
Ethyl octanoate	MS, LRI	1431	0.00 ± 0.00a	19.95 ± 0.25c	7.42 ± 0.03b	4.64 ± 0.01b	5.59 ± 0.07b		
Ethyl nonanoate	MS, LRI	1534	0.00 ± 0.00a	2.72 ± 0.00c	0.54 ± 0.00b	0.00 ± 0.00a	0.00 ± 0.00a		
Ethyl decanoate	MS, LRI	1637	0.00 ± 0.00a	17.12 ± 1.87d	10.45 ± 0.67c	0.55 ± 0.05a	6.87 ± 0.14b		
Ethyl benzoate	MS, LRI	1627	0.00 ± 0.00a	0.00 ± 0.00a	0.92 ± 0.00d	0.26 ± 0.00b	0.49 ± 0.01c		
Isoamyl acetate	MS, LRI	1117	0.00 ± 0.00a	9.94 ± 0.06c	8.02 ± 0.15c	3.51 ± 0.02b	2.17 ± 0.01b		
Methyl acetate	MS, LRI	1313	0.00 ± 0.00a	0.00 ± 0.00a	29.42 ± 0.07d	13.41 ± 0.09c	11.12 ± 0.10b		
Methyl 2-furoate	MS, LRI	1589	0.00 ± 0.00a	5.12 ± 0.03b	5.00 ± 0.06b	9.42 ± 0.07c	8.87 ± 0.08c		
Methyl lactate	MS, LRI	1324	0.00 ± 0.00a	7.26 ± 0.08b	44.67 ± 0.00c	60.05 ± 0.63d	82.87 ± 1.02e		
2-furanmethyl acetate	MS, LRI	1242	0.00 ± 0.00a	1.97 ± 0.03b	3.86 ± 0.04c	7.38 ± 0.08d	8.33 ± 0.09d		
2-Phenethyl acetate	MS, LRI	1828	0.00 ± 0.00a	3.01 ± 0.04cd	3.50 ± 0.03d	2.21 ± 0.02b	2.63 ± 0.03bc		
Furans									
2,5-Dimethylfuran	MS, LRI	/	22.53 ± 0.01ab	25.36 ± 0.13ab	20.14 ± 0.15ab	62.65 ± 13.72b	77.98 ± 4.83b		
2-Acetylfuran	MS, LRI	1518	36.51 ± 0.47b	15.56 ± 0.26a	15.64 ± 0.11a	32.83 ± 0.24b	30.92 ± 0.10b		
2-Acetyl-5-methylfuran	MS, LRI	1629	14.02 ± 0.09c	6.58 ± 0.02a	6.5 ± 0.03a	11.30 ± 0.12b	11.10 ± 0.07b		
Ketones									
2-Heptanone	MS, LRI	1205	2.63 ± 0.02a	0.00 ± 0.00a	16.64 ± 0.23b	44.31 ± 0.17d	35.31 ± 0.19c		
Diacetyl	MS, LRI	1750	0.00 ± 0.00a	0.00 ± 0.00a	1.49 ± 0.02a	3.31 ± 0.01c	2.50 ± 0.03b		

Acetoin	MS, LRI	1301	0.00 ± 0.00a	7.34 ± 0.09a	197.95 ± 1.45c	76.69 ± 1.75b	101.49 ± 1.06b
2-Nonanone	MS, LRI	1387	3.96 ± 0.01a	0.00 ± 0.00a	45.52 ± 0.40b	52.35 ± 0.35b	88.93 ± 0.99c
2,5-Hexanedione	MS, LRI	1516	0.00 ± 0.00a	0.00 ± 0.00a	2.35 ± 0.01a	26.37 ± 0.24c	22.02 ± 0.12b
Methyl cyclopentenolone	MS, LRI	1842	4.13 ± 0.19b	0.82 ± 0.14a	2.43 ± 0.11ab	3.32 ± 0.01ab	4.02 ± 0.04b
Pyrazines							
2,6-Dimethylpyrazine	MS, LRI	1337	5.40 ± 0.03d	2.00 ± 0.02a	2.91 ± 0.03b	3.98 ± 0.02c	3.82 ± 0.05c
2-Ethyl-6-methylpyrazine	MS, LRI	1396	7.76 ± 0.07b	2.63 ± 0.04a	2.41 ± 0.02a	2.78 ± 0.02a	3.07 ± 0.02a
2-Ethyl-3,5-dimethylpyrazine	MS, LRI	1449	6.36 ± 0.01c	2.92 ± 0.01b	2.99 ± 0.05b	1.37 ± 0.02a	1.44 ± 0.01a
Pyrroles							
2-Acetylpyrrole	MS, LRI	1989	14.47 ± 0.14c	9.79 ± 0.03ab	8.22 ± 0.05a	12.33 ± 0.08bc	12.84 ± 0.16c
Pyrrole-2-carboxaldehyde	MS, LRI	2048	21.98 ± 0.16c	8.65 ± 0.07a	10.15 ± 0.03a	19.50 ± 0.01b	19.08 ± 0.11b
1-Methyl-2-formylpyrrole	MS, LRI	2127	3.98 ± 0.01d	2.21 ± 0.00b	1.54 ± 0.01a	3.45 ± 0.01cd	3.23 ± 0.05c
Terpenoids							
trans-Linalool oxide	MS, LRI	1444	15.65 ± 0.08c	3.40 ± 0.03a	1.98 ± 0.01a	8.75 ± 0.11b	9.83 ± 0.04b
Linalool	MS, LRI	1546	5.03 ± 0.05a	3.40 ± 0.03a	4.06 ± 0.04a	20.31 ± 0.09b	19.72 ± 0.18b
Terpineol	MS, LRI	1655	1.17 ± 0.01b	0.69 ± 0.01a	1.06 ± 0.01ab	2.04 ± 0.02c	2.54 ± 0.02d
Volatile phenols							
m-Cresol	MS, LRI	1145	3.17 ± 0.01a	21.50 ± 0.02bc	17.44 ± 0.12b	34.79 ± 0.28d	26.05 ± 0.23c
Guaiacol	MS, LRI	1876	6.52 ± 0.07b	3.51 ± 0.02a	3.67 ± 0.02a	4.44 ± 0.03a	5.90 ± 0.03b
Phenol	MS, LRI	2020	11.31 ± 0.07c	8.10 ± 0.06b	6.10 ± 0.02a	9.80 ± 0.08b	9.32 ± 0.09b
4-Ethylguaiacol	MS, LRI	2047	1.78 ± 0.01a	3.80 ± 0.04b	5.67 ± 0.02c	15.58 ± 0.10d	15.88 ± 0.05d
p-Cresol	MS, LRI	2097	2.21 ± 0.02c	1.17 ± 0.01a	0.81 ± 0.01a	1.92 ± 0.02bc	1.68 ± 0.02b

LRI: linear retention index; determined on a DB-FFAP column relative to C7-C40 hydrocarbons; Concerto: monoculture of *L. thermotolerans* Concerto; Concerto+Lalvin: Co-culture of *L. thermotolerans* Concerto and *O. oeni* Lalvin 31; Concerto+Beta: Co-culture of *L. thermotolerans* Concerto and *O. oeni* Enoform Beta; Concerto+PN4: Co-culture of *L. thermotolerans* Concerto and *O. oeni* PN4; Values are the mean of triplicate fermentations (± standard deviation); a,b,c,d,e Statistical analysis using ANOVA (n=3) at 95% confidence interval. Same letter indicates no significant difference between samples

Table 10: Selected volatile compounds and their concentrations (µg/L) before and after fermentation with *L. thermotolerans* and *O. oeni*