



(51) International Patent Classification:

A23L 11/30 (2016.01) C12G 3/02 (2006.01)  
A23C 11/02 (2006.01)

(21) International Application Number:

PCT/SG2018/050193

(22) International Filing Date:

18 April 2018 (18.04.2018)

(25) Filing Language:

English

(26) Publication Language:

English

(30) Priority Data:

10201703194V 19 April 2017 (19.04.2017) SG

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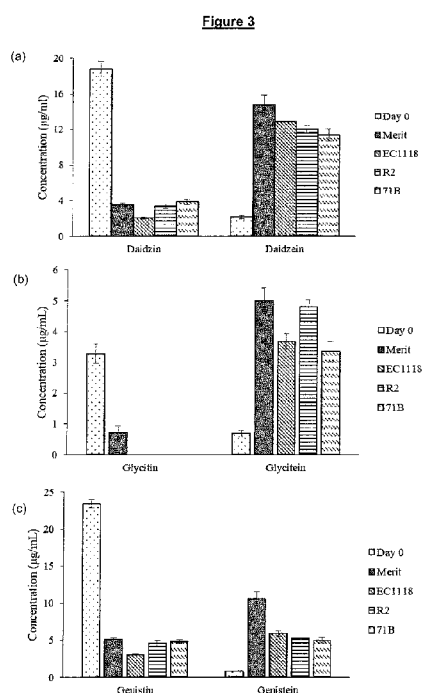
(81) Designated States (unless otherwise indicated, for every kind of national protection available): AE, AG, AL, AM, AO, AT, AU, AZ, BA, BB, BG, BH, BN, BR, BW, BY, BZ, CA, CH, CL, CN, CO, CR, CU, CZ, DE, DJ, DK, DM, DO, DZ, EC, EE, EG, ES, FI, GB, GD, GE, GH, GM, GT, HN, HR, HU, ID, IL, IN, IR, IS, JO, JP, KE, KG, KH, KN, KP, KR, KW, KZ, LA, LC, LK, LR, LS, LU, LY, MA, MD, ME, MG, MK, MN, MW, MX, MY, MZ, NA, NG, NI, NO, NZ, OM, PA, PE, PG, PH, PL, PT, QA, RO, RS, RU, RW, SA, SC, SD, SE, SG, SK, SL, SM, ST, SV, SY, TH, TJ, TM, TN, TR, TT, TZ, UA, UG, US, UZ, VC, VN, ZA, ZM, ZW.

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

Declarations under Rule 4.17:

— of inventorship (Rule 4.17(iv))

(54) Title: A SOY WHEY-DERIVED BEVERAGE



(57) Abstract: There is provided a soy whey-derived beverage comprising 12-30 mg/L free soy isoflavones. There is also provided a method for forming the soy whey-derived beverage comprising: providing soy whey; adding a microorganism to the soy whey; and fermenting the soy whey at a predetermined temperature and period of time. In various embodiments, the beverage has an alcohol content of <0.5% by volume or >0.5% by volume or a total isoflavone content of > 20 mg/L. In a preferred embodiment, the microorganism is a yeast and/or the beverage is preferably wine.



**Published:**

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

## A soy whey-derived beverage

### Technical Field

The present invention relates to a soy whey-derived beverage and a method of forming  
5 the same. In particular, the soy whey-derived beverage may be a fermented beverage.

### Background

Soy whey is a liquid waste stream generated from soy beancurd manufacturing. Soy  
whey itself is a nutritive medium. Due to the high biological oxygen demand (BOD) and  
chemical oxygen demand (COD) in view of the nutrient content comprising mainly  
10 protein and soluble sugars, direct disposal of the soy whey into a drainage system will  
result in environmental pollution. Hence, it is necessary for soy whey to be pre-treated  
before it is discharged into the drainage system. Current soy whey treating methods  
still result in generation of a considerable amount of waste at the end of the methods.

### Summary of the invention

15 The present invention seeks to address these problems, and/or provides a soy whey-  
derived beverage, as well as an improved method for biotransforming soy whey into a  
suitable beverage without generating any waste stream. The beverage according to  
the present invention may have health benefits in view of the presence of free  
isoflavones within the beverage. In particular, free isoflavones are known to confer  
20 health benefits to consumers in view of their antioxidant properties and in reducing the  
risk of cancer and cardiovascular diseases.

According to a first aspect, the present invention provides a soy whey-derived  
beverage comprising  $\geq 10$  mg/L. In particular, the soy whey-derived beverage may  
comprise 12-30 mg/L free soy isoflavones. Further, the beverage may have a total soy  
25 isoflavone content of  $\geq 20$  mg/L. In particular, the soy whey-derived beverage may  
have a total soy isoflavone content of 25-55 mg/mL.

The beverage may be any suitable beverage. According to a particular aspect, the  
beverage may be a fermented beverage.

The beverage may be an alcoholic or non-alcoholic beverage. In particular, if the  
30 beverage is a non-alcoholic beverage, the beverage may have an alcohol content of  $<$   
0.5% by volume. If the beverage is an alcoholic beverage, the beverage may have an

alcohol content of  $\geq 0.5\%$  by volume. In particular, the beverage may have an alcohol content of 5-40% by volume. For example, the alcohol content may be 7-38%, 10-35%, 12-30%, 15-28%, 17-25%, 20-22%. Even more in particular, the alcohol content may be 5-15%.

5 According to a particular aspect, the beverage may be an alcoholic beverage. In particular, the beverage may be an alcoholic beverage such as, but not limited to, a soy whey-derived wine.

The beverage may have any suitable pH. For example, the pH of the beverage may be 2-6. In particular, the pH may be 2.5-5.5, 3-5, 3.5-4.5, 3.75-4.0. Even more in  
10 particular, the pH may be about 3.5-5.

The beverage may have a suitable Brix. For example, the Brix of the beverage may be 3-20 °Bx. In particular, the Brix may be 5-18 °Bx, 6-15 °Bx, 7-12 °Bx, 8-10 °Bx, 9-9.5 °Bx. Even more in particular, the Brix may be about 5-15 °Bx.

The beverage may have a suitable ester content. For example, the ester content of the  
15 beverage may be about 5-10 mg/L.

According to a second aspect of the present invention, there is provided a method of forming a soy whey-derived beverage described above, the method comprising:

- providing soy whey;
- adding a microorganism to the soy whey; and
- 20 - fermenting the soy whey at a pre-determined temperature for a pre-determined period of time to form the beverage.

The soy whey may be any suitable soy whey for the purposes of the present invention.

The microorganism may be any suitable microorganism. For example, the  
25 microorganism may be yeast, *Zymomonas mobilis*, lactic acid bacteria or acetic acid bacteria.

According to a particular aspect, the microorganism may be yeast. The yeast may be any suitable yeast for the purposes of the present invention. For example, the yeast may be *Saccharomyces* yeast, non-*Saccharomyces* yeast, or a combination thereof. In

particular, the yeast may be *Saccharomyces (S.) cerevisiae*, *Torulaspota (T.) delbrueckii*, *Kluyveromyces (K.) thermotolerans*, *Metschnikowia (M.) pulcherrima*, *Pichia (P.) kluyveri*, *Williopsis (W.) saturnus*, or a combination thereof.

The pre-determined period of time may be any suitable period of time for the purposes  
5 of the present invention. According to a particular aspect, the pre-determined period of time may be 2-21 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 13-35°C.

10 According to a particular aspect, the method may further comprise adjusting the pH of the soy whey prior to the adding a microorganism to the soy whey. For example, the pH may be adjusted to a pH of 3-5.

According to a particular aspect, the method may further comprise adding sugars to the soy whey prior to the adding a microorganism to the soy whey. The sugars added may  
15 be any suitable sugars for the purposes of the present invention.

According to a particular aspect, the method may further comprise heating the soy whey prior to the adding a microorganism to the soy whey.

#### Brief Description of the Drawings

In order that the invention may be fully understood and readily put into practical effect  
20 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 four *Saccharomyces cerevisiae* strains during soy whey fermentation;

25 Figure 2(a) shows pH change and Figure 2(b) shows soluble solid content change during soy whey fermentation;

Figure 3(a) shows changes in isoflavone glycoside (daidzin) and free isoflavone (daidzein) concentration, Figure 3(b) shows changes in isoflavone glycoside (glycitin)

and free isoflavone (glycitein) concentration, Figure 3(c) shows changes in isoflavone glycoside (genistin) and free isoflavone (genistein) concentration and Figure 3(d) shows changes in antioxidant capacity of the soy whey and soy whey wine after fermentation;

5 Figure 4(a) shows changes in the ethanol content and Figure 4(b) shows changes in the isoamyl alcohol, 1-hexanol and 2-phenylethanol content, in the soy whey before and after fermentation;

Figure 5(a) shows changes in the content of Ethyl acetate, isoamyl acetate, hexyl acetate and 2-phenylethyl acetate and Figure 5(b) shows changes in the content of  
10 ethyl hexanoate and ethyl octanoate, in the soy whey before and after fermentation;

Figure 6 shows the changes in the hexanal (aldehyde) content before and after fermentation;

Figure 7 shows the changes in 2-pentylfuran content before and after fermentation;

Figure 8 shows the growth of the five non-Saccharomyces strains during soy whey  
15 fermentation;

Figure 9(a) shows pH change and Figure 9(b) shows soluble solid content change during soy whey fermentation;

Figure 10(a) shows changes in the isobutanol and phenylethanol content, Figure 10(b) shows changes in the isoamyl alcohol content, Figure 10(c) shows the changes in the  
20 1-pentanol, 1-hexanol and 1-octen-3-ol content, in the soy whey before and after fermentation;

Figure 11(a) shows changes in the acetic acid and hexanoic acid content, Figure 11(b) shows changes in the content of isobutyric acid and 3-methylbutanoic acid and Figure  
25 11(c) shows changes in the content of octanoic acid, of the soy whey before and after fermentation;

Figure 12(a) shows changes in the content of Ethyl hexanoate, ethyl decanoate, Figure 12(b) shows changes in the content of ethyl octanoate, ethyl 9-decenoate and Figure

12(c) shows changes in the content of 2-phenylethyl acetate, ethyl heptanoate and ethyl nonanoate, of the soy whey before and after fermentation;

Figure 13(a) shows changes in the content of hexanal, Figure 13(b) shows changes in the content of octanal and 2-decenal, Figure 13(c) shows changes in the content of 2-pentenal, 2-hexenal and 2-heptenal, of the soy whey before and after fermentation;

Figure 14 shows changes to the phytate concentration (expressed as phytic acid-phosphate) in the soy whey before and after fermentation;

Figure 15 shows growth of *Torulaspora delbrueckii* Biodiva during soy whey fermentation;

Figure 16(a) shows pH change and Figure 16(b) shows soluble solid content change during soy whey fermentation;

Figure 17 shows changes to the sucrose content during fermentation;

Figure 18(a) shows changes in the malic acid content, Figure 18(b) shows changes in the content of alpha-ketoglutaric acid, Figure 18(c) shows changes in the content of succinic acid and Figure 18(d) shows changes in the content of pyruvic acid, of the soy whey during fermentation;

Figure 19 shows the glycerol content in the soy whey-derived alcoholic beverage;

Figure 20 shows the ethanol content in the soy whey-derived alcoholic beverage;

Figure 21 shows changes to the soluble protein content in the soy whey during fermentation;

Figure 22(a) shows changes daidzin concentration, Figure 22(b) shows changes in the daidzein concentration, Figure 22(c) shows changes in genistin concentration, Figure 22(d) shows changes in genistein concentration, of the soy whey during fermentation and Figure 22(e) shows changes in antioxidant capacity of the soy whey wine after fermentation;

Figure 23 shows the growth of *Torulaspora delbreuckii* Biodiva in the soy whey at different fermentation temperatures;

Figure 24(a) shows pH change and Figure 24(b) shows soluble solid content change during soy whey fermentation;

Figure 25 shows the growth of *Torulaspora delbreuckii* Biodiva in the soy whey at different sugar concentrations; and

5 Figure 26(a) shows pH change and Figure 26(b) shows soluble solid content change during soy whey fermentation.

#### Detailed Description

As explained above, there is a need for processing soy whey which can otherwise be environmentally damaging. The present invention relates to a soy whey-derived  
10 beverage which fully utilises soy whey, which is a waste stream of soybean manufacturing. The soy whey-derived beverage may be formed in an environmentally friendly manner and is also a simple method, making scale up of the method possible. In particular, the method of forming the soy whey-derived beverage of the present invention fully utilises soy whey and achieves practically zero waste.

15 In general terms, the invention relates to a soy whey-derived beverage and a method of forming the same. The soy whey-derived beverage may have health benefits as the beverage may comprise isoflavones in free form which are bioavailable. The bioavailability of free isoflavones in a human is known to impart good health benefits such as antioxidance and reduction of risks of diseases such as cancer and  
20 cardiovascular diseases.

According to a first aspect, the present invention provides a soy whey-derived beverage comprising free soy isoflavones. The free isoflavones may also be referred to as isoflavone aglycone. The soy whey-derived beverage may comprise  $\geq 10$  mg/L free  
25 soy isoflavones. In particular, the soy whey-derived beverage may comprise 10-35 mg/L, 12-30 mg/L, 15-28 mg/L, 17-25 mg/L, 20-23 mg/L, 21-22 mg/L free soy isoflavones. Even more in particular, the beverage comprises 12-30 mg/L free soy isoflavones.

For the purposes of the present invention, free soy isoflavones is defined as a plant-based bioactive phytoestrogenic compound without a sugar molecule attached to it.  
30 The free soy isoflavones may include, but is not limited to, daidzein, glycitein and

genistein. The free soy isoflavones comprised in the beverage are beneficial as they enhance the absorption of the isoflavones in the gastrointestinal tract of the consumer of the beverage.

According to a particular aspect, the beverage may have a total soy isoflavone content of  $\geq 20$  mg/L. The total soy isoflavones measures the total amount of isoflavones consisting of free isoflavones and bound isoflavones (isoflavone glycoside) in the beverage. In particular, the total soy isoflavone content may be 25-55 mg/mL, 27-52 mg/L, 30-50 mg/L, 32-48 mg/L, 25-45 mg/L, 30-40 mg/L, 32-38 mg/L, 33-35 mg/L. Even more in particular, the beverage comprises 27-50 mg/L total soy isoflavones.

The beverage may be any suitable soy whey-derived beverage. According to a particular aspect, the beverage may be a fermented beverage. A fermented beverage may be defined as a beverage which has undergone fermentation.

The beverage may be an alcoholic or non-alcoholic beverage. According to a particular aspect, the beverage may be a non-alcoholic beverage. In particular, the beverage may have an alcohol content of  $< 0.5\%$  by volume. The beverage may be any suitable non-alcoholic beverage. Examples of a non-alcoholic beverage may include, but is not limited to, soy whey probiotic beverage, soy whey vinegar beverage and other non-alcoholic fermented beverage.

According to another particular aspect, the beverage may be an alcoholic beverage. For the purposes of the present invention, an alcoholic beverage is defined as a beverage which comprises an alcohol. The alcohol may include ethanol. In particular, the beverage may have an alcohol content of  $\geq 0.5\%$  by volume.

In particular, the beverage may have an alcohol content of 5-40% by volume. For example, the alcohol content may be 7-38%, 10-35%, 12-30%, 15-28%, 17-25%, 20-22%. Even more in particular, the alcohol content may be 5-15%.

According to a particular aspect, the beverage may be any suitable alcoholic beverage. Examples of an alcoholic beverage may include, but is not limited to, wine, ciders, beers and spirits. In particular, the beverage may be a soy whey-derived wine.

The beverage may have a suitable pH. For example, the pH of the beverage may be 2-6. In particular, the pH may be 2.5-5.5, 3-5, 3.5-4.5, 3.75-4.0. Even more in particular, the pH may be about 3.5-5.

The beverage may have a suitable Brix or its specific gravity equivalent. Brix is a  
5 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 in the beverage.

The Brix of the beverage may be 3-20 °Bx. For the purposes of the present invention,  
10 reference to Brix of the alcoholic beverage refers to the measure of the Brix of the soy-whey comprised in the beverage. In particular, the Brix of the beverage may be 5-18°Bx, 6-15 °Bx, 7-12 °Bx, 8-10 °Bx, 9-9.5 °Bx. Even more in particular, the Brix may be about 5-15 °Bx.

According to a particular aspect, the beverage may have a suitable ester content. For  
15 example, the ester content of the beverage may be 5-10 mg/L. 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 floral characteristic to the beverage. This may be advantageous as the esters may help to mask the beany and/or grassy odour which may have been originally present in the soy whey.

20 The esters comprised in the beverage may be any suitable ester. For example, the ester may be, but not limited to, ethyl esters, acetate esters, or a combination thereof. In particular, the esters may be, but not limited to, ethyl acetate, isoamyl acetate, hexyl acetate, 2-phenylethyl acetate, ethyl hexanoate, ethyl octanoate, or a combination thereof.

25 According to a second aspect of the present invention, there is provided a method of forming a soy whey-derived beverage described above, the method comprising:

- providing soy whey;
  - adding a microorganism to the soy whey; and
  - fermenting the soy whey at a pre-determined temperature for a pre-
- 30 determined period of time to form the beverage.

The soy whey may be any suitable soy whey for the purposes of the present invention.

The soy whey may undergo treatment before the adding a microorganism to the soy whey. According to a particular aspect, the method may further comprise adjusting the pH of the soy whey prior to the adding. The adjusting the pH enables the microorganism to grow in the soy whey. For example, the pH may be adjusted to a pH of 3-5. 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, tartaric acid, gluconic acid, succinic acid, or a combination thereof.

According to a particular aspect, the method may further comprise adding sugars to the soy whey prior to the adding a microorganism to the soy whey. The addition of the sugars may be for increasing carbohydrate content in the soy whey. The sugars added may be any suitable sugars for the purposes of the present invention. For example, the sugars added may comprise, but is not limited to, glucose, sucrose, fructose, or a combination thereof. The adding the sugars may be to adjust the Brix of the soy whey. In particular, the Brix of the soy whey may be adjusted to 3-30 °Bx. Even more in particular, the Brix of the soy whey may be adjusted to 10-15 °Bx.

According to a particular aspect, the method may further comprise heating the soy whey prior to adding a microorganism to the soy whey. The heating may comprise mild pasteurization of the soy whey. The heating may extend the shelf life of the soy whey prior to the fermenting and may also reduce the risk of contamination during the fermenting. The heating may be carried out under suitable conditions. For example, the heating may be carried out at a temperature of about 40-140°C. In particular, the temperature may be about 60-80°C.

The heating may be carried out for a suitable period of time. For example, the heating may be for 2 seconds-60 minutes. In particular, the heating may be for about 10-30 minutes. Even more in particular, the heating may be for about 20 minutes.

The microorganism may be any suitable microorganism. For example, the microorganism may be yeast, *Zymomonas mobilis*, lactic acid bacteria or acetic acid bacteria.

In particular, the microorganism may be yeast. The yeast may be any suitable yeast for the purposes of the present invention. The yeast may be *Saccharomyces* yeast, non-*Saccharomyces* yeast, or a combination thereof.

According to a particular aspect, the microorganism may be *Saccharomyces* yeast. Examples of *Saccharomyces* yeast include, but are not limited to, *Saccharomyces* (*S.*) *cerevisiae*, *S. pastorianus*, *S. boulardii*.

According to another particular aspect, the microorganism may be non-*Saccharomyces* yeast. Examples of non-*Saccharomyces* yeast include, but are not limited to, *Torulaspota* (*T.*) *delbrueckii*, *Kluyveromyces* (*K.*) *thermotolerans*, *Metschnikowia* (*M.*) *pulcherrima*, *Pichia* (*P.*) *kluyveri*, *Williopsis* (*W.*) *saturnus*, or a combination thereof.

The adding a microorganism may comprise adding a suitable amount of microorganism. For example, when the microorganism is yeast, the amount of yeast added may be 1-9 log CFU/mL. In particular, the amount may be 5-7 log CFU/mL.

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 period of time may be 2-21 days. In particular, the pre-determined period of time may be about 7-14 days. Even more in particular, the pre-determined period of time may be about 10 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 13-35°C. In particular, the pre-determined temperature may be 15-25°C. Even more in particular, the pre-determined temperature may be 20°C. The temperature may be changed at any point during the fermenting.

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

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

#### *Soybean curd production and soy whey collection*

For all the examples, the soy whey was generated through small scale production of soybean curd. In particular, soymilk was generated by blending 100 g of soybeans (from Canada) obtained from a supermarket with 800 mL of water for 16 hours. After that, the soaked beans were drained and blended with deionized (DI) water using a blender (Model MX-J210GN, Panasonic, Osaka, Japan) in a 1:8 dry weight to volume ratio to obtain a slurry. The slurry was then filtered through a cheese cloth to obtain soymilk. The residue (okara) was rinsed with deionized water in a 1:2 dry weight to volume ratio. The soymilk was boiled for 5 minutes and cooled to 87°C prior to the addition of 2% (w/w with respect to the dry weight of the soybean) commercial calcium sulfate (Home Brew Ohio, Sandusky, USA) suspended in deionized water. The mixture was stirred to ensure proper mixing of the coagulant with the soymilk before it was allowed to stand for 15 minutes. The calcium ions served as bridging ions to facilitate the cross-linking between soy proteins to form the gel (soybean curd). The coagulated soymilk (tofu) was transferred onto a meshed mold overlaid with a cheese cloth. The tofu was pressed thrice for 5 minutes each at maximum pressure using a manual press and the tofu whey was collected below the meshed mold. The pH of the tofu whey (initial pH of 5.78) was first adjusted to pH 4.0 using 1 M D-/L-malic acid. The soluble solids content (°Brix; an initial value of 2.55) was then adjusted to 15 °Brix using commercial sucrose. The pre-treated tofu whey was pasteurized at 60°C for 20 minutes and the effectiveness of the pasteurization was verified via plate counting.

#### *Fermentation*

Fermentations were conducted in 500-mL Erlenmeyer flasks containing 300 mL of pasteurized soy whey. Each flask was inoculated with 1% (v/v) of each pre-culture of

yeast. The fermentation was carried out statically at 20°C for 10 days and samples were withdrawn periodically for analysis. Yeast cell count was determined by spread plating on potato dextrose agar (PDA; Oxoid, Hampshire, United Kingdom).

#### *Analysis methodology*

5 The pH and °Brix values were measured using a pH meter (827 pH Lab, Metrohm, Herisau, Switzerland) and a refractometer (RX-5000α, ATAGO, Tokyo, Japan), respectively. The yeast cell count was enumerated via spread plating on potato dextrose agar (PDA; Oxoid, Hampshire, United Kingdom).

10 Sugars, glycerol, organic acids, amino acids and isoflavones were analyzed and quantified using high performance liquid chromatography (HPLC; Shimadzu, Kyoto, Japan). Both sugars and glycerol were separated using a Zorbax carbohydrate column (150 × 4.6 mm; Agilent, Santa Clara, CA, USA) connected to evaporative light scattering detector (ELSD). Sugars were eluted at 40°C with acetonitrile/water (80:20 v/v) as mobile phase flowing at 1.4 mL/min. Glycerol was eluted using the same mobile  
15 phase at 30°C and flow rate of 0.5 mL/min. Organic acids were separated using Supelcogel C-160 H column (300 × 7.8 mm; Supelco, Bellefonte, PA, USA) with 0.1 % (v/v) sulfuric acid (H<sub>2</sub>SO<sub>4</sub>) as the mobile phase at 0.4 mL/min and column temperature of 40°C. Organic acid were identified using photodiode array (PDA) set at 210 nm as the detector.

20 Amino acids and ammonia content were analyzed by using reversed-phase Waters AccQ-Tag Nova-Pak C18 column (150 × 3.9 mm; Waters, Dublin, Ireland) and reagents from Waters. Isoflavones were analysed using Zorbax Eclipse Plus C18 Column (150 × 4.6 mm; Agilent, Santa Clara, CA, USA) with 0.1% acetic acid in DI water (solvent A) and 0.1% acetic acid in methanol (solvent B). The analysis started  
25 with 15% solvent B with gradual increments of solvent B till 50% within 46 minutes and held at 50% solvent B for additional 10 minutes before equilibrating back to 15% solvent B. Isoflavones were detected and identified using PDA set at 262 nm. All analytes were identified and quantified using analytical grade sugars, glycerol, organic acids, amino acids and isoflavones as standards.

30 Soluble protein determination was carried using the Bradford method (Bradford, 1976) and the Bradford protein assay kit was purchased from Bio-Rad (California, United

States of America). Five mL of the diluted protein reagent was added to 0.1 mL of the sample/BSA standard and incubated for five minutes before absorbance measurement at 595 nm using a UV-Vis spectrophotometer (UVmini-1240, Shimadzu, Kyoto, Japan).

5 Antioxidant capacity of the alcoholic beverage was determined using 2,2-diphenyl-1-picrylhydrazyl (DPPH) analysis. Pre-treated samples and Trolox standards (0.1 mL) were mixed with 3.9 mL of 25 mg/L methanolic DPPH solution and left to stand in the dark for two hours. The analysis was carried out using a UV-Vis spectrophotometer (UVmini-1240, Shimadzu, Kyoto, Japan) set at 515 nm.

10 Volatile components were determined using headspace solid-phase micro-extraction (HS-SPME) gas chromatography (GC) coupled with mass spectrometry (MS) and flame ionization detector (FID) (Agilent; Santa Clara, CA, USA). The samples were adjusted to pH 2.5 using 1M hydrochloric acid and then subjected to HS-SPME extraction at 60°C for 50 minutes using a carboxen/poly(dimethylsiloxane) fiber (Supelco, Bellefonte, PA, USA) at 250 rpm/min. The SPME fiber was desorbed at 15 250°C for 3 minutes in the injection port. Helium (carrier gas) flow rate was set at 1.2 mL/min and the temperature program was set to increase from 50°C (5 minutes) to 230°C (30 minutes) at a rate of 5°C/min. Identification of the volatile compounds was done by comparing their individual mass spectra with the NIST08 Library and Wiley275 Library as well as linear retention indices (LRI). Ethanol quantification was performed 20 using external standards dissolved in 10% (v/v) unfermented soy whey.

Phytate analysis were carried out by first adjusting the samples to pH 2.0 – 2.5 using 0.8 M HCl, followed by the addition of 10% (w/v) NaCl to the acidified samples. The samples were shake at 250 rpm for 30 minutes before putting into a 4°C refrigerator for 60 minutes to allow the sediments to settle. The samples were then centrifuged at 25 14000 g for 5 minutes and the supernatant was diluted by a factor of 25 and 250 µL of the diluted samples were mixed with 83.3 µL of the modified Wade reagent (0.3 g FeCl<sub>3</sub>.6H<sub>2</sub>O with 3 g 5'-sulphosalicylic acid in 1 litre of DI water). Absorbance of the coloured samples were measured at 500nm using a microplate reader and the concentrations of the phytate in the samples were determined using a standard 30 calibration curve (expressed in mg PA-P/L).

**Example 1 – Effect of different *S. cerevisiae* yeast on beverage formed**

Four different species of commercial *Saccharomyces* (*S.*) *cerevisiae* yeasts were used in the example. The species were *S. cerevisiae* Merit, *S. cerevisiae* EC1118, *S. cerevisiae* R2, and *S. cerevisiae* 71B.

- 5 The different strains of yeasts were inoculated at 1% (v/v) ratio into the soy whey and fermented for 10 days at 20°C. Samples were withdrawn periodically for analysis.

*Yeast viability*

Figure 1 shows the yeast viability of all four *Saccharomyces* yeasts used in this example. All the yeasts used showed growth and remained viable till the end of the  
10 fermentation in the soy whey medium. This shows that the soy whey contained sufficient nutrient for the growth of the yeast.

*pH and soluble solid content*

Figure 2(a) shows the pH of the beverage, i.e. soy wine over the course of the fermentation and Figure 2(b) shows the soluble solid content changes during the  
15 fermentation.

As can be seen in Figure 2(a), all four *Saccharomyces* yeasts were able to further acidify the soy wine beverage, with Merit strain producing the most acidic wine at the end of the fermentation. The higher the acidity, the more sour the alcoholic beverage will be. The four *Saccharomyces* yeasts shared similar Brix profiles throughout the  
20 fermentation as can be seen in Figure 2(b). The decrease in the Brix value means that the yeasts were able to utilize the nutrients in the soy whey which is consistent with the results as shown in Table 1.

*Changes in sugar, glycerol and organic acids*

Table 1 shows the properties of the beverages formed using the different strains of  
25 yeast.

	Day 0		Soy whey wine		
	Pre-treated soy whey				
		Merit	EC1118	R2	71B
pH	3.99 ± 0.01a	3.75 ± 0.01b	3.85 ± 0.00c	3.81 ± 0.02d	3.91 ± 0.01e
°Brix	14.77 ± 0.03a	5.04 ± 0.06b	5.10 ± 0.03bc	5.20 ± 0.09c	5.09 ± 0.07bc
Ethanol (% v/v)	0.00 ± 0.00a	7.15 ± 1.06b	8.00 ± 0.45b	7.82 ± 0.38b	7.33 ± 0.48b
Total Sugars (g/100 mL)	12.754 ± 0.106a	0.371 ± 0.072bc	0.523 ± 0.065cd	0.605 ± 0.062d	0.340 ± 0.045b
Total Organic Acids (g/100 mL)	0.455 ± 0.040a	0.772 ± 0.047b	0.705 ± 0.027b	0.739 ± 0.053b	0.704 ± 0.012b

**Table 1 – Enological parameters of the spy whey and the respective soy whey wine formed from the different monoculture *S. cerevisiae***

It can be seen from Table 1 that the sugars present in the soy whey were metabolized to low levels at the end of the fermentation which demonstrated that the yeasts were able to use the supplemented sucrose for growth. The decrease in the amount of sugars corresponded with the increase in the ethanol content of the soy wine formed following the fermentation. The four *Saccharomyces* yeasts produced glycerol during the fermentation. Glycerol production is essential to wine production as it impart mild sweetness, smoothness and viscosity to the wine.

*Changes in soluble protein and amino acids*

As shown in Table 2, a substantial amount of soluble protein content was observed in the soy whey prior to fermentation. A decrease in the final concentration of the soluble protein was observed for all the samples at the end of the fermentation. It is known that *S. cerevisiae* yeasts possess proteolytic activity. The conversion of the protein to amino acids is beneficial for the yeast growth. The different types of amino acids shown in Table 2 generally showed a decrease in the concentration at the end of the fermentation with few exceptions. The utilization of the amino acids is essential for the growth of the yeasts.

	Day 0	Day 10			
	Pre-treated soy whey	Merit	EC118	R2	71B
Soluble protein (mg/mL)	1.041±0.039a	0.803±0.061b	0.490±0.083c	0.597±0.043cd	0.681±0.020d
Total amino acid (mg/100 mL)	18.737±1.823a	2.730±0.266b	2.314±0.136b	2.593±0.191b	2.286±0.264b

**Table 2 – Concentration of the total soluble protein content and amino acid content on Day 0 and Day 10 of the fermentation**

*Changes in isoflavone content and antioxidant capacity*

Figure 3 shows the changes in isoflavone glucoside and free isoflavone concentrations in the pre-treated soy whey and at the end of the fermentation of the soy whey. Soy isoflavones are naturally present in abundant amount in soybeans. These isoflavones, in both the glycosides and the aglycones (free) form, may remain soluble after coagulation of soybean and migrate to the soy whey. All isoflavone glycosides (i.e. isoflavone bound to sugars, examples being daidzin, glycitin, genistin) decreased in concentration while all the free isoflavones (aglycones) such as daidzein, glycitein and genistein, increased in concentration at the end of the fermentation as reflected in Figure 3. The increase in isoflavone aglycone is beneficial as it enhances the absorption of the isoflavone in the gastrointestinal tract when consumed.

*Changes in volatile profile*

With the sugar supplementation prior to the fermentation, the ethanol content of the soy wine ranged between 7-8%. Indigenous undesirable off-flavour imparting hexanol present in the soy whey was undetectable while new alcohols were produced. The disappearance of the indigenous alcohols improves soy whey flavour through reducing the grassy and beany off-flavour characteristic of the soy product. The results are as shown in Figure 4.

Figure 5 shows the changes in the ester content after fermentation. No esters were detected in the original soy whey. Esters were only detected in the soy wine after fermentation. The formation of esters by the yeasts is beneficial as esters impart fruity and floral characteristic to the final product and this can help to mask the beany and grassy odour originally present in the soy whey.

Figure 6 shows the changes in the aldehyde content before and after fermentation. It can be seen that endogenous aldehydes, particularly hexanal, were metabolized to undetectable levels after the fermentation. This may be beneficial to the aroma profile of the soy wine as the disappearance of the aldehydes will reduce the beany and grassy odour of the soy whey.

Figure 7 provides the changes in 2-pentylfuran content before and after fermentation. It can be seen that following fermentation, 2-pentylfuran is not present in any of the

samples. 2-pentylfuran, which has a licorice-like flavour on its own, is formed from the breakdown of linoleic acid by the soy lipoxygenase. Together with hexanol, 2-hexenal, ethyl vinyl ketone, 2-pentylfuran can contribute to the grassy and beany odour of soy products. The metabolism of 2-pentylfuran is beneficial to the aroma profile of the soy  
5 whey as it reduces the grassy and beany odour of the soy wine.

### **Example 2 – Effect of non-Saccharomyces yeasts on beverage formed**

Five different species of commercial non-Saccharomyces yeasts (*Torulaspota* (*T.*) *delbrueckii* Biodiva, *Kluyveromyces* (*K.*) *thermotolerans* Concerto, *Metschnikowia* (*M.*) *pulcherrima* Flavia, *Pichia* (*P.*) *Kluyveri*, Frootzen and *Williopsis* (*W.*) *saturnus*  
10 NCYC2251) were used in the experiment. Different strains of yeasts were inoculated 1% (v/v) ratio into the soy whey and fermented for 10 days at 20°C. Samples were withdrawn periodically for analysis.

Similar analysis as carried out in Example 1 was carried out in this example.

#### *Yeast viability*

15 It can be seen from Figure 8 that all five yeasts were able to grow in the soy whey and experience a 2-2.5 log CFU/mL growth at the end of the fermentation. This shows that the soy whey comprised sufficient nutrients for the growth of non-Saccharomyces yeasts.

#### *pH and soluble solid content*

20 Figure 9(a) shows the pH of the beverage, i.e. soy wine over the course of the fermentation and Figure 9(b) shows the soluble solid content changes during the fermentation.

As can be seen in Figure 9, two distinct trends can be observed from Figure 9(a). Biodiva and Concerto experienced a slight drop in pH while the other three yeasts  
25 experienced an increase in the pH. The increase in pH shows that the alcoholic beverage for the latter three yeasts will be less sour as compared to the alcoholic beverage fermented from Biodiva and Concerto. As for the soluble solid content, Biodiva and Concerto experienced a continuous decrease in the °Brix value while the

other three yeasts had a constant °Brix value. This shows that Flavia, Frootzen and NCYC2251 were unable to significantly utilize the supplemented sucrose for growth.

*Changes in sugar, glycerol, ethanol and organic acids*

5 Table 3 shows the properties of the beverages formed using the different strains of yeast.

	Day 0	Day 10				
	Pre-treated soy whey	Biodiva	Concerto	Flavia	Frootzen	NCYC2251
pH	4.02±0.01a	3.79±0.01b	3.88±0.02c	4.19±0.05d	4.75±0.05e	4.44±0.03f
°Brix	14.94±0.08a	6.84±0.08b	6.78±0.11b	14.71±0.04c	14.68±0.07c	14.68±0.03c
Ethanol content (v/v %)	0.00±0.000a	7.12±0.60b	6.20±1.03b	0.00±0.00a	0.00±0.00a	0.00±0.00a
Total sugars (g/L)	112.60±11.91a	22.57±1.11b	23.50±1.12b	99.97±5.46a	98.08±4.62a	104.16±8.57a
Glycerol (g/L)	0.00±0.000a	4.18±0.18b	3.42±0.09c	0.00±0.00a	0.00±0.00a	0.00±0.00a
Total organic acids (g/L)	4.77±0.25ab	7.27±0.16c	7.29±0.12c	5.22±0.08b	3.99±0.23d	4.58±0.37a

**Table 3 – Enological parameters of soy whey and the respective soy wine conducted by various monocultures of non-Saccharomyces yeasts**

10 Sugars added to the soy whey prior to the fermentation were metabolized to a large extent in Biodiva and Concerto samples, while insignificant changes to the sugar content were observed in the other three yeasts samples. This observation shows that Flavia, Frootzen and NCYC2251 were not able to metabolize the added sugars in the soy whey. The extent of sugar utilization corresponded to the amount of ethanol produced, with Biodiva and Concerto producing between 6-7% of ethanol while no ethanol was detected in the other three yeasts samples (not suitable for alcoholic beverage production). Glycerol was only detected in Biodiva and Concerto samples and glycerol is known to contribute to smoothness and viscosity of the product

20 For organic acid production, Biodiva and Concerto showed significant increases in the organic acid content which corresponded to the decrease in pH. Frootzen showed a significant decrease in the organic acid content which corresponded to the greatest increase in the pH at the end of the fermentation. The increase in organic acid content

in Biodiva and Concerto samples is beneficial in creating a hurdle for the growth of spoilage microorganism that will affect the shelf life of the product.

*Changes in soluble protein, amino acids and gamma amino-butyric acid content*

The five non-*Saccharomyces* yeasts used in this example had differing levels of protein utilization, with Flavia having the highest protein utilization at the end of the fermentation. The results are as shown in Table 4.

High protein utilization shows that Flavia has a high proteolytic activity to convert protein to amino acids for growth. The five yeasts also showed differing levels of amino acid utilization, with Biodiva and Concerto having the lowest amino acid content at the end of the fermentation. High amino acid utilization supports both the yeast growth and flavour production. Gamma amino-butyric acid (GABA) is commonly found in legumes and was detected in the soy whey. Biodiva, Concerto and NCYC2251 utilized the GABA for their metabolism while Flavia and Frootzen did not utilize the GABA. GABA is a bioactive compound that can act as an anti-hypertensive agent and can serve as a functional ingredient in the product.

	Day 0	Day 10				
	Pre-treated soy whey	Biodiva	Concerto	Flavia	Frootzen	NCYC2251
Soluble protein (g/L)	1.33±0.08a	0.65±0.04b	0.38±0.07c	0.09±0.01d	0.45±0.02ce	0.56±0.05be
Total amino acid (mg/L)	197.79±21.27a	23.12±2.39b	21.98±1.33b	94.65±2.54c	59.04±7.81d	58.87±7.01d
GABA (mg/L)	26.82±3.43a	5.58±0.94b	5.45±1.04b	27.74±0.97a	26.56±2.12a	16.53±2.25c

**Table 4 – Concentration of the soluble protein and amino acid content of the soy whey before and after fermentation**

*Changes in isoflavone content and antioxidant capacity*

Isoflavones, both the aglycones and glucosides, were detected in the soy whey prior to fermentation. The five yeasts showed differing levels of hydrolysis, with NCYC2251 having the highest  $\beta$ -glucosidase activity. The increase in isoflavone aglycone concentration after the fermentation resulted in an increase in the antioxidant capacity of the respective wine. Hence, the conversion of isoflavone glucoside to isoflavone aglycone is beneficial in increasing the health benefit of the resulting alcoholic beverage. The results of the analysis are as shown in Table 5.

### *Changes in volatile profile*

The changes in the alcohol content before and after fermentation is shown in Figures 10 (a) to (c). In particular, endogenous alcohols such as 1-pentanol, 1-hexanol and 1-octen-3-ol were metabolized to low or trace levels at the end of the fermentation by the five yeasts. The reduction of indigenous alcohol is beneficial in reducing the beany and grassy odour of the alcoholic beverage. New alcohols, such as ethanol (refer to Table 3), isobutanol, phenylethanol and isoamyl alcohol were produced by the five yeasts that can contribute to the aroma profile of the alcoholic beverage.

Figures 11 (a) to (c) show the changes in the volatile acid content of the soy whey before and after fermentation. In particular, hexanoic acid was the only acid detected in the soy whey. New volatile acids were produced by the yeasts and the type of acids produced differed among the yeast strains. The different types of volatile acids produced will affect the aroma profile differently.

Figures 12 (a) to (c) on the other hand shows the ester content of the soy whey before and after fermentation. Esters were not detected in the soy whey prior to fermentation. The esters detected in the wine samples were the result of the fermentation process and the esters will impart a fruity character to the alcoholic beverage.

The changes in the aldehyde content of the soy whey before and after fermentation is shown in Figures 13 (a) to (c). Indigenous aldehydes such as hexanal, 2-pentenal, 2-hexenal and 2-heptenal were detected in the soy whey prior to the fermentation and these aldehydes were metabolized to low or trace levels at the end of the fermentation. The metabolism of these indigenous aldehydes is beneficial as the grassy and beany odour of the soy whey will be reduced. New aldehydes such as octanal and 2-decenal were detected in Frootzen samples that can impart aroma profile of the beverage.

### *Changes in phytate content*

Soybeans are known to contain anti-nutrients such as phytate which binds minerals, reducing mineral bioavailability. Phytate had been detected in the soy whey pre-fermentation. However, the phytate concentration decreased in all the samples after the fermentation. This is therefore advantageous.

	Day 0					Day 10				
	Pre-Treated Soy Whey	Biodiva	Concerto	Flavia	Frootzen	NCYC2251				
<b>Isoflavones (mg/L)</b>										
Daidzin	17.66 ± 0.72	5.30 ± 0.26	7.35 ± 0.64	16.14 ± 1.73	11.62 ± 1.76	0.61 ± 0.06				
Glycitin	3.77 ± 0.19	0.34 ± 0.03	0.35 ± 0.03	0.16 ± 0.04	0.19 ± 0.02b	0.13 ± 0.02				
Genistin	25.99 ± 1.28	6.37 ± 0.58	9.16 ± 0.38	20.93 ± 2.24	14.62 ± 0.99	0.71 ± 0.06				
<b>Total glucosides</b>	<b>47.42 ± 2.07</b>	<b>12.01 ± 0.82</b>	<b>16.86 ± 0.90</b>	<b>37.24 ± 3.99</b>	<b>26.42 ± 2.47</b>	<b>1.45 ± 0.13</b>				
Daidzein	1.88 ± 0.11	11.65 ± 0.94	11.67 ± 0.46	5.32 ± 0.80	8.78 ± 0.48	13.12 ± 0.32				
Glycitein	0.38 ± 0.08	4.66 ± 0.41	5.02 ± 0.22	3.66 ± 0.13	4.23 ± 0.24	4.40 ± 0.05				
Genistein	1.16 ± 0.08	9.27 ± 1.59	9.95 ± 0.76	3.92 ± 0.58	7.98 ± 0.64	9.61 ± 1.05				
<b>Total alycones</b>	<b>3.42 ± 0.19</b>	<b>25.58 ± 2.93</b>	<b>26.64 ± 1.38</b>	<b>12.90 ± 1.33</b>	<b>20.99 ± 1.27</b>	<b>27.13 ± 1.19</b>				
<b>Total Isoflavones (mg/mL)</b>	<b>50.84 ± 2.23</b>	<b>37.59 ± 3.71</b>	<b>43.50 ± 2.16</b>	<b>50.14 ± 2.87</b>	<b>47.41 ± 2.52</b>	<b>28.58 ± 1.26</b>				
Trolox equivalent*	0.014 ± 0.001	0.023 ± 0.001	0.022 ± 0.001	0.024 ± 0.000	0.020 ± 0.001	0.023 ± 0.002				

**Table 5 – Changes to the isoflavone content and antioxidant capacity of the soy whey before and after fermentation**

### Example 3 – Effect of coagulant in soy whey on beverage formed

In this example, the soy whey produced by the method described above was used. In addition to this, other soy wheys were also used in which a different coagulant was used. In particular, the method of making the soy whey was the same, except that the  
5 coagulant used in making the initial soybean was different.

Accordingly, soybean was made using three commercially available coagulants (gypsum, glucono-delta-lactone and nigari) using the method explained above (Soybean curd whey production) and the respective soy whey generated was used for fermentation using *T. delbrueckii* Biodiva for 10 days at 20°C. Samples were withdrawn  
10 periodically for analysis.

#### *Yeast viability*

As can be seen from Figure 15, the yeast grew well in the tofu whey regardless of the coagulant used and the yeast grew by approximately 2 log CFU/mL by the end of the fermentation period. This shows that the presence of different coagulants (or ions) do  
15 not affect the growth of the yeast.

#### *pH and soluble solid content*

Figure 16(a) shows the pH of the beverage, i.e. soy wine over the course of the fermentation and Figure 16(b) shows the soluble solid content changes during the fermentation.

20 As can be seen in Figure 16, all the samples experienced a decrease in the pH by the end of the fermentation period. The soluble solid content for the four samples experienced a continuous decrease in the °Brix value throughout the fermentation. This shows that the different coagulants did not affect the acid production and sugar utilization.

#### *Changes in sugar, glycerol, ethanol and organic acids*

25 The changes in the sucrose content during fermentation is shown in Figure 17 while Figures 18(a) to (d) show the changes to the organic acid content. It can be seen that the yeasts were able to utilize the fermentable sugars in the respective soy whey. The

presence of higher calcium in the gypsum soy whey may have contributed to the slightly faster sucrose utilization as shown in Figure 17(a).

The presence of the different coagulants also did not affect the production and metabolism of malic acid, succinic acid and pyruvic acid as can be seen from Figures 18(a), (c) and (d). However, the presence of GDL significantly increased the production of alpha-ketoglutaric acid during the fermentation, as shown in Figure 18(b). Accordingly, the presence of GDL has impact on the physiological function of the yeast to induce higher alpha-ketoglutaric acid production.

Glycerol content present in the respective soy whey beverage is shown in Figure 19. The presence of magnesium ions in the Nigari samples (both nigari standard and nigari equivalent) produced slightly more glycerol than the gypsum and GDL samples. The higher glycerol content may provide more mouthfeel and viscosity to the alcoholic beverage fermented with nigari soy whey.

Ethanol content present in the respective soy whey beverage is shown in Figure 20. It can be seen that the presence of different coagulants did not affect the ethanol production across the different samples. However, it is interesting to note that nigari samples have slightly, but insignificantly, lower alcohol content but higher glycerol content.

#### *Changes in soluble protein content*

As can be seen from Figure 21, the soluble protein content in all the samples dropped after the fermentation. The presence of GDL induced higher protein utilization while the presence of nigari induced lower protein utilization. Higher protein utilization indicates higher amino acids production which can aid in yeast growth and flavour production.

#### *Changes in isoflavone content and antioxidant activity*

Figure 22 shows the changes in the isoflavone content. Isoflavone glucosides (daidzin and genistin) concentration decreased and isoflavone aglycones increased in concentration after fermentation, indicating that the yeasts were able to hydrolyze the glucosides to their corresponding aglycone. The increase in aglycone concentration resulted in an increase in the antioxidant capacity as shown in Figure 23(e).

#### Example 4 – Effect of temperature on soy whey wine fermentation

In this example, the effect of temperature on the fermentation kinetics of wine yeast *Torulaspora delbrueckii* Biodiva in tofu whey wine fermentation was evaluated.

The fermentation method described above was used except that for each sample, the fermentation temperature was adjusted to 15°C, 20°C and 30°C. The fermentation was carried out for 10 days with the soy whey having a starting sugar content of 15 °Brix.

Figure 23 shows the cell count of yeast over the course of fermentation. As can be seen from the figure, the yeasts were able to grow and survive in the soy whey under different fermentation temperature albeit minor differences. The yeast in the 15°C samples grew at a slower rate during the first two days while the yeast in the 30°C samples experience a drop in the cell count after day 4. The high temperature (30°C), combined with other factors such as alcohol, could have accelerated the cell death in the tofu whey wine samples.

Figures 24(a) and (b) shows the pH changes and the changes in the soluble solid content over the course of the fermentation. A distinct trend was observed from the pH and soluble solid content. With increasing fermentation temperature, the drop in pH was greater and the decrease in the soluble solid content, which corresponds to sugar consumption, was faster. Higher fermentation temperature induces the yeast to have higher metabolic activity which in turn promoted faster sugar consumption and higher acid production.

Other properties of the resulting soy whey wine after fermentation is provided in Table 6.

Sample		Specific Gravity	Brix	Alcohol content (1)	Alcohol content (2)
Unfermented		1.0629	14.915	N.A	N.A
Fermented	15 °C	1.0146	8.53	6.34	6.57
	20 °C	0.9984	5.555	8.47	8.77
	30 °C	0.9998	5.48	8.29	8.58
Formula				(OS-FS)*131.25	(OS-FS)/7.36

N.A: Not applicable

**Table 6 – Specific gravity and alcohol content of the resulting soy whey wine after fermentation**

Ethanol content in the soy whey wine samples were calculated based on the specific gravity of the samples using two different formulas. The incomplete sugar utilization in 15°C samples (indicated by the high ending soluble solid content at Day 10) resulted in a lower ethanol content as compared to 20°C and 30°C samples.

5 **Example 5 – Effect of different sugar concentration on soy whey fermentation**

In this example, the effect of different sugar concentration on the fermentation kinetics in soy whey wine fermentation was evaluated.

The soy whey production method described above was used except that for each sample, the starting sugar content of the soy whey was adjusted to 5, 15, 25 and 30  
10 °Brix. The fermentation was carried out for 18 days at 20°C and the yeast used for the fermentation was *Torulasporea delbrueckii* Biodiva.

Figure 25 shows the effect on the yeast. As seen in Figure 25, the different sugar concentration did not have an impact on the growth of the yeasts. The yeast grew from day 0 to day 4 and remained fairly constant till the end of the fermentation period.

15 Figures 26 (a) and (b) shows the pH changes and the changes in the soluble solid content over the course of the fermentation. All the samples experienced a drop in the pH after the fermentation. The soluble solid content for all samples experienced a drop throughout the fermentation. The samples with the higher starting sugar concentration (25 and 30 °Brix) still contained significant amount of sugars even after 18 days of  
20 fermentation. This indicates that the fermentation duration will need to be increase for these two set of samples in order for the yeasts to metabolise the sugar to a larger extent. Another solution to speed up sugar utilization is to use another wine yeast (such as *Saccharomyces cerevisiae*) to ferment these soy wheys that have higher starting sugar concentration.

25 Other properties of the resulting soy whey wine after fermentation is provided in Table 7.

Sample		Specific Gravity	Brix	Alcohol content (1)	Alcohol content (2)
Unfermented	5°Brix	1.0235	5.905	N.A	N.A
	15°Brix	1.0629	14.915		
	25°Brix	1.1041	24.76		
	30°Brix	1.128	29.52		
Fermented	5°Brix	1.0034	2.625	2.64	2.73
	15°Brix	0.9984	5.555	8.47	8.77
	25°Brix	1.0222	13.47	10.75	11.13
	30°Brix	1.0486	19.33	10.42	10.79
Formula				(OS-FS)*131.25	(OS-FS)*1000/7.36

N.A: Not applicable

5 **Table 7 – Specific gravity and alcohol content of the resulting soy whey wine after fermentation**

The ethanol produced was proportional to the drop in the soluble solid content (Brix). The ethanol content for 25 and 30 °Brix samples can be further increased if the sugars are utilized to a larger extent.

Claims

1. A soy whey-derived beverage comprising  $\geq 10$  mg/L free soy isoflavones.
2. The beverage according to claim 1, wherein the beverage is a fermented  
5 beverage.
3. The beverage according to claim 1 or 2, wherein the beverage has an alcohol content of  $< 0.5\%$  by volume.
- 10 4. The beverage according to claim 1 or 2, wherein the beverage has an alcohol content of  $\geq 0.5\%$  by volume.
5. The beverage according to claim 4, wherein the beverage has an alcohol content of 5-40% by volume.
- 15 6. The beverage according to any preceding claim, wherein the beverage has a total soy isoflavone content of  $\geq 20$ .
7. The beverage according to any preceding claim, wherein the beverage has a  
20 pH of 2-6.
8. The beverage according to any preceding claim, wherein the beverage has a Brix of 3-20 °Bx.
- 25 9. The beverage according to any preceding claim, wherein the beverage has an ester content of 5-10 mg/L.
10. The beverage according to any of claims 4-9, wherein the beverage is wine.
- 30 11. A method of forming a soy whey-derived beverage according to any of the preceding claims, the method comprising:
  - providing soy whey;
  - adding a microorganism to the soy whey; and

- fermenting the soy whey at a pre-determined temperature for a pre-determined period of time to form the beverage.

12. The method according to claim 11, wherein the microorganism is yeast.

5

13. The method according to claim 12, wherein the yeast is *Saccharomyces* yeast, non-*Saccharomyces* yeast, or a combination thereof.

14. The method according to claim 12 or 13, wherein the yeast is *Saccharomyces* (S.) *cerevisiae*, *Torulaspora* (T.) *delbrueckii*, *Kluyveromyces* (K.) *thermotolerans*,  
10 *Metschnikowia* (M.) *pulcherrima*, *Pichia* (P.) *kluveri*, *Williopsis* (W.) *saturnus*, or a combination thereof.

15. The method according to any of claims 11 to 14, wherein the pre-determined  
15 period of time is 2-21 days.

16. The method according to any of claims 11 to 15, wherein the pre-determined temperature is 13-35°C.

20 17. The method according to any of claims 11 to 16, further comprising adjusting the pH of the soy whey prior to the adding a microorganism to the soy whey.

18. The method according to claim 17, wherein the adjusting comprises adjusting the pH to 3-5.

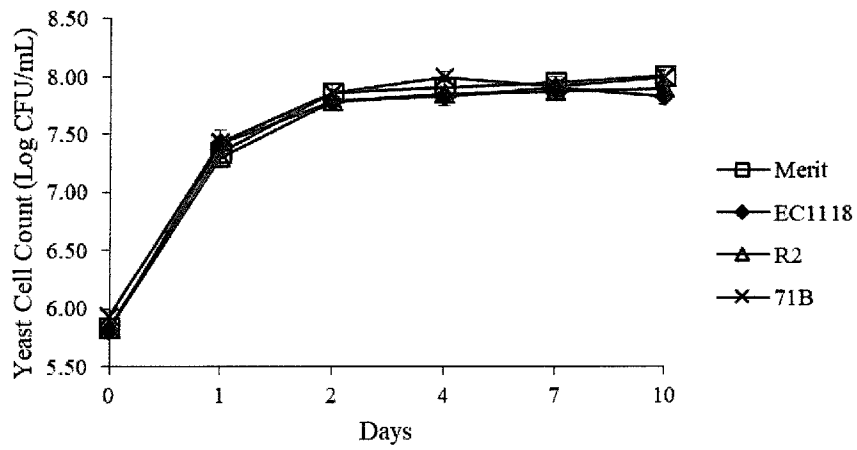
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19. The method according to any of claims 11 to 18, further comprising adding sugars to the soy whey prior to the adding a microorganism to the soy whey.

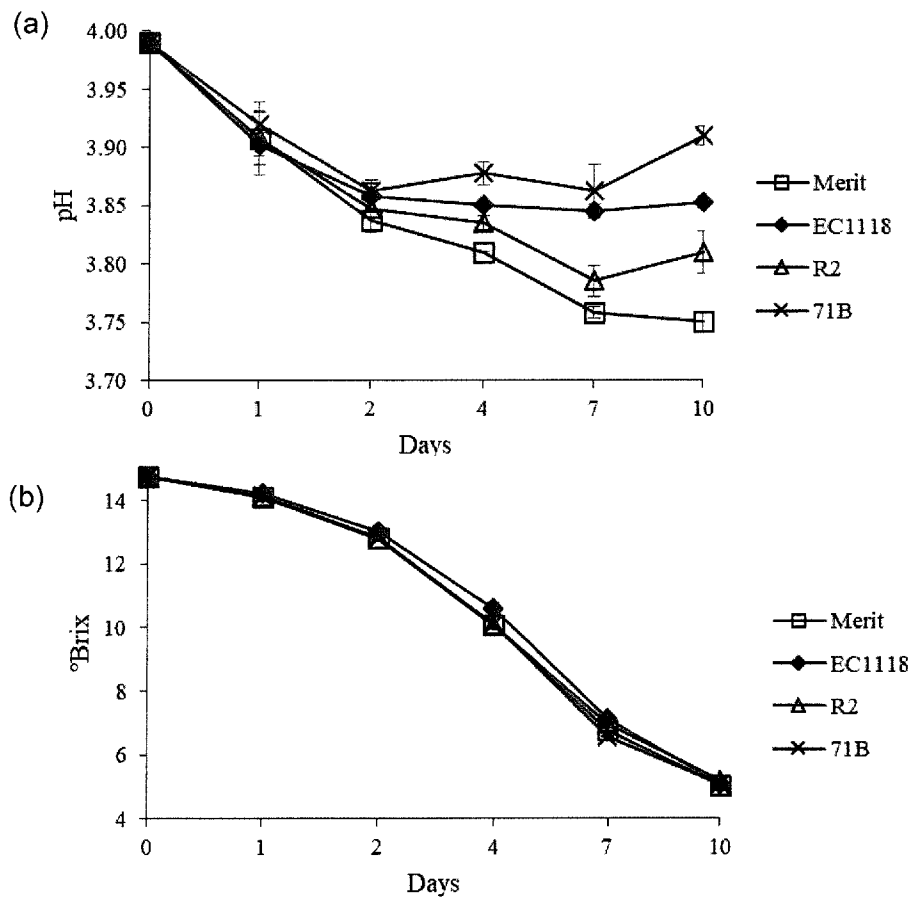
30 20. The method according to any of claims 11 to 19, further comprising heating the soy whey prior to the adding a microorganism to the soy whey.

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**Figure 1**

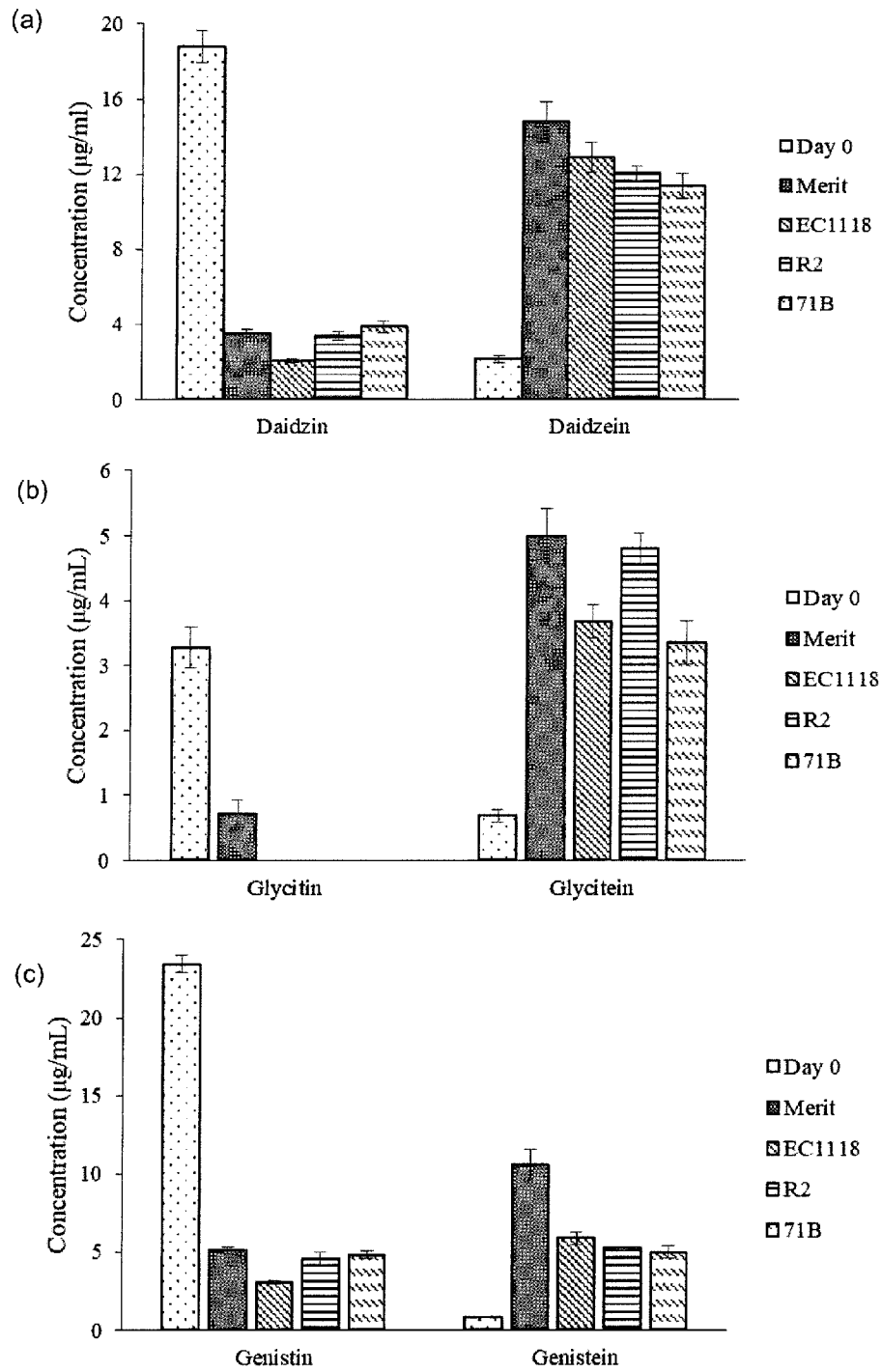


**Figure 2**

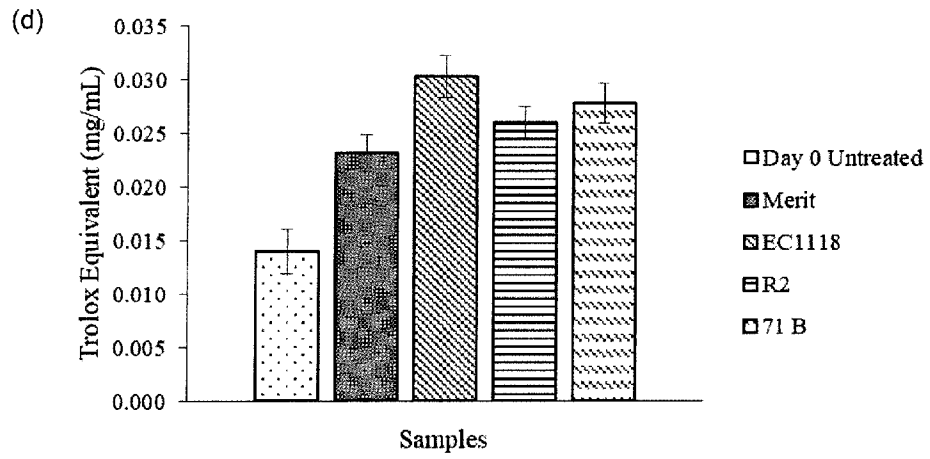


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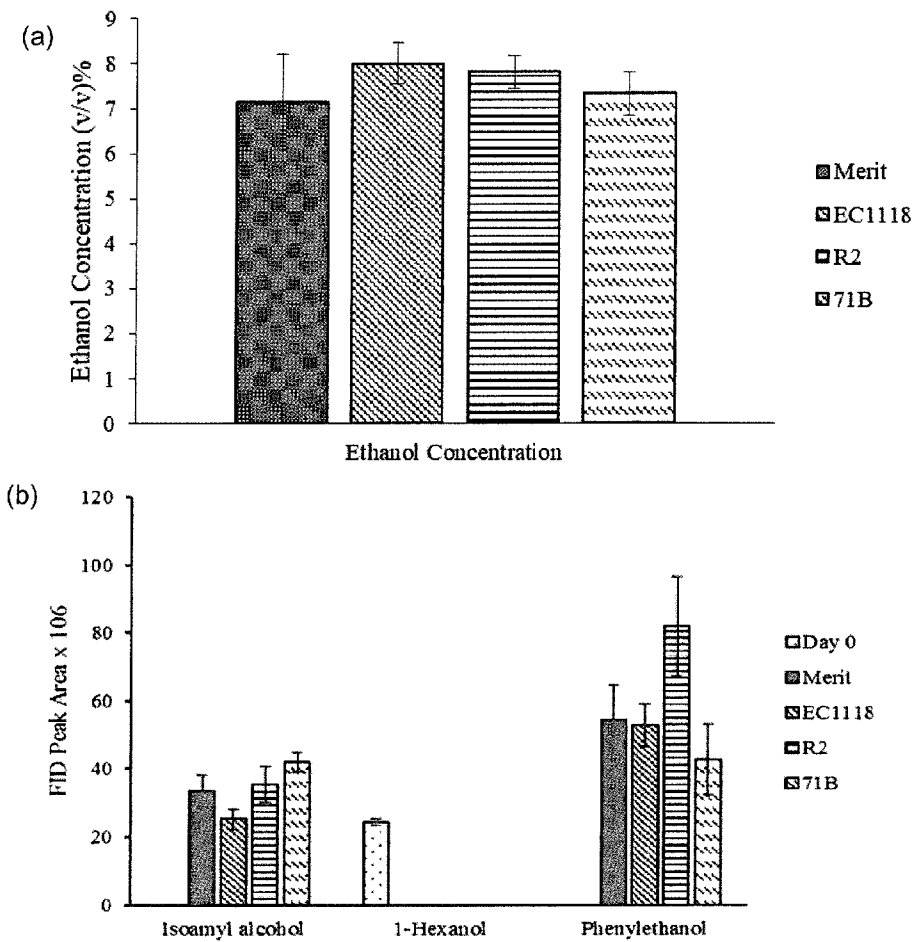
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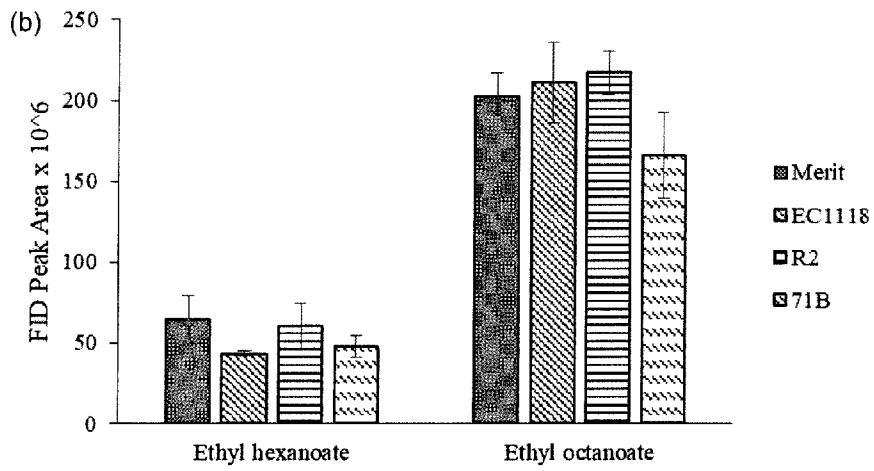
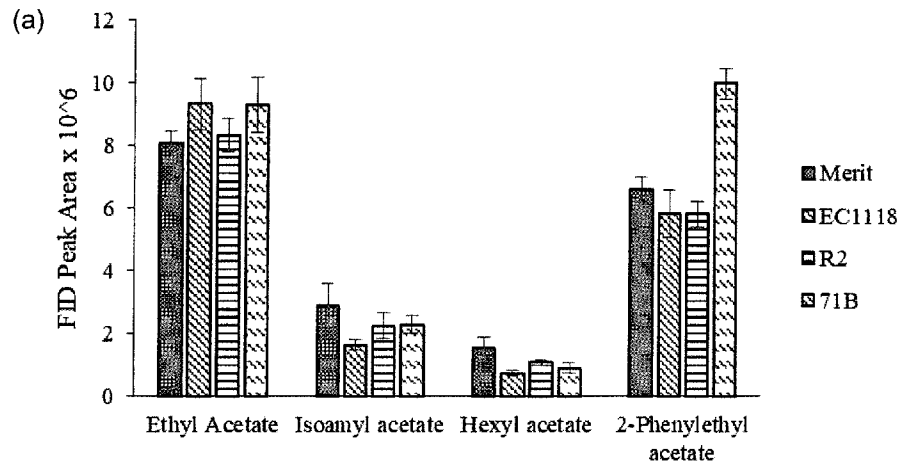
**Figure 3 (continued)**



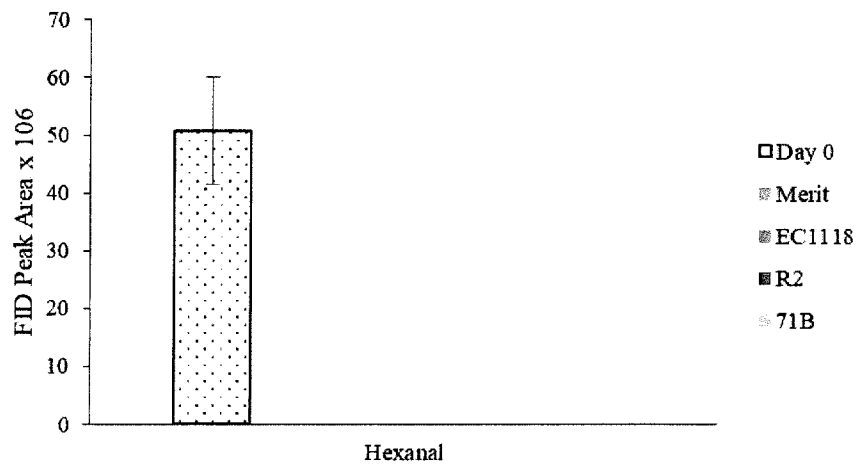
**Figure 4**



**Figure 5**

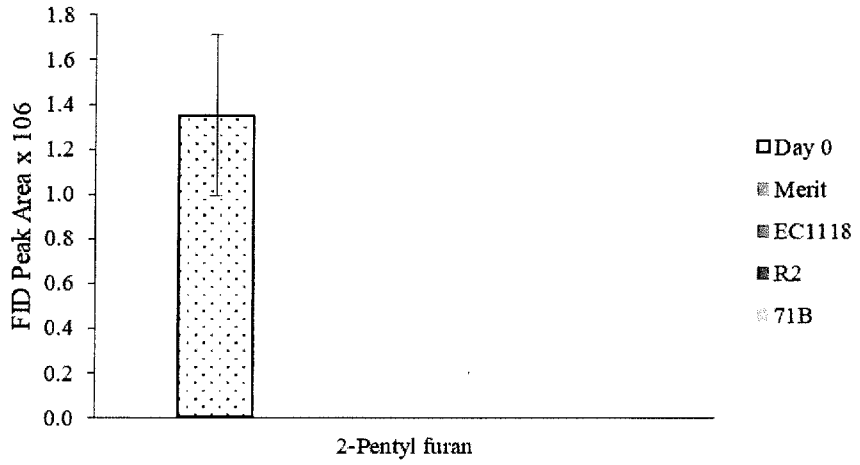


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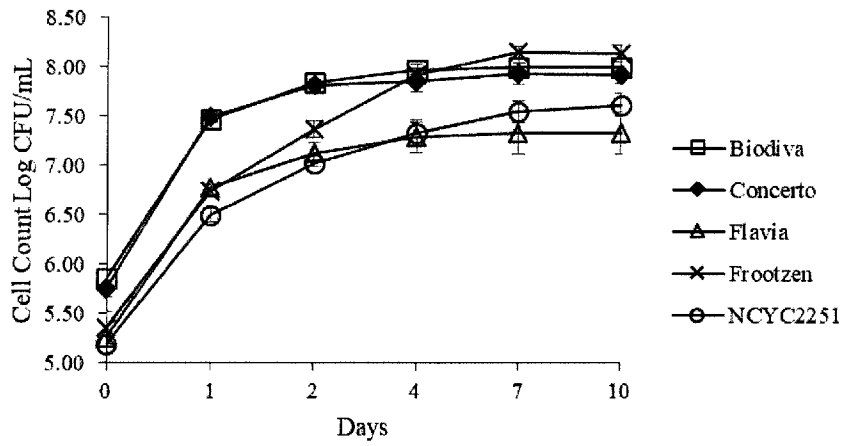


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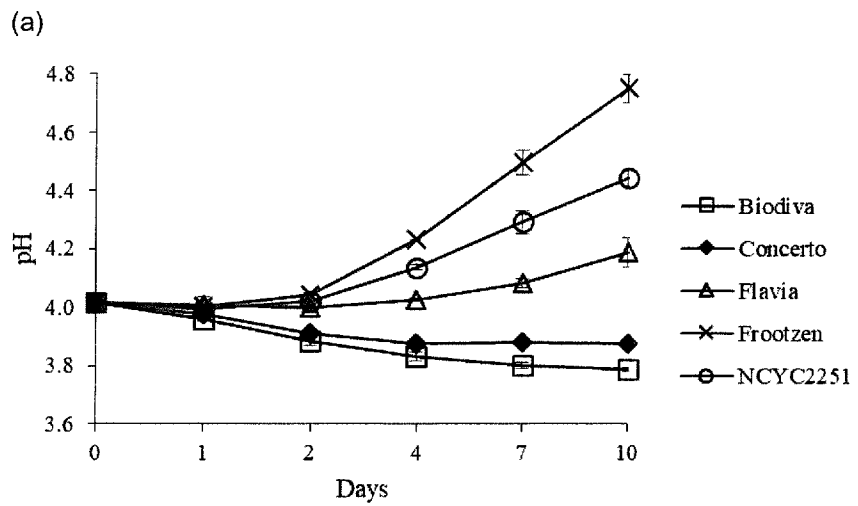
**Figure 7**



**Figure 8**

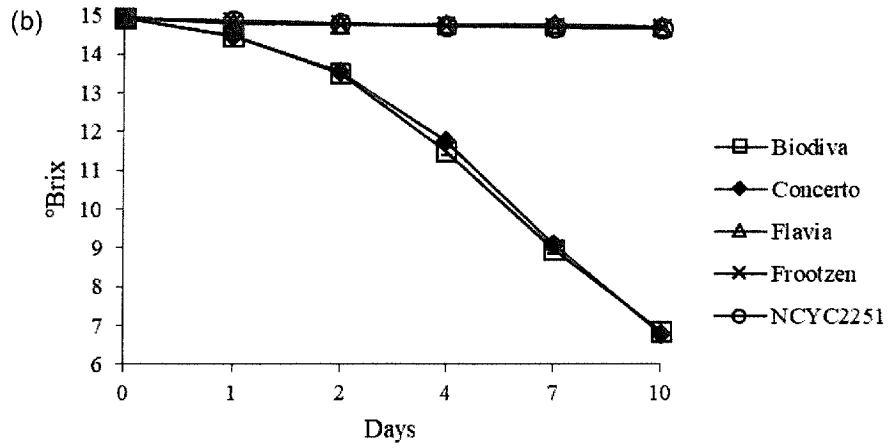


**Figure 9**

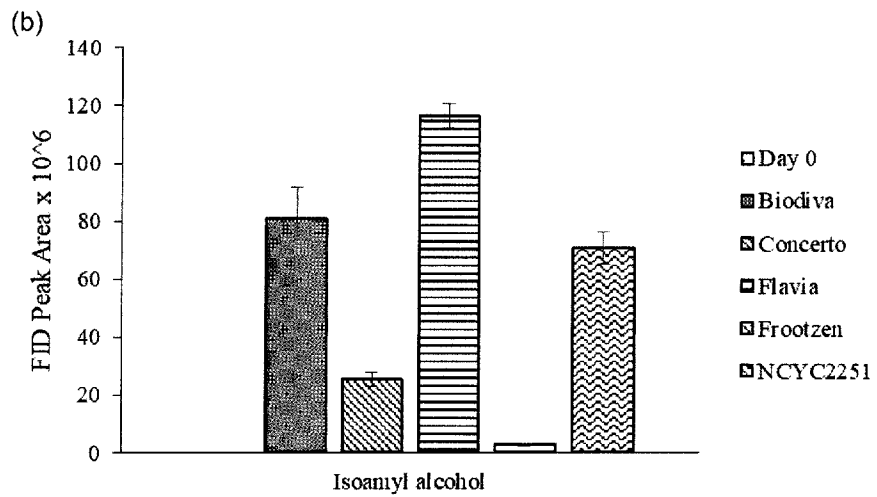
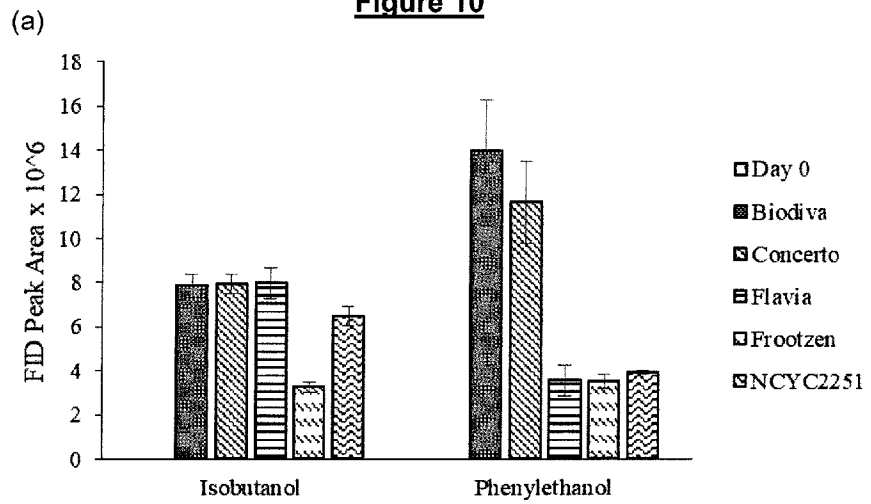


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**Figure 9 (continued)**

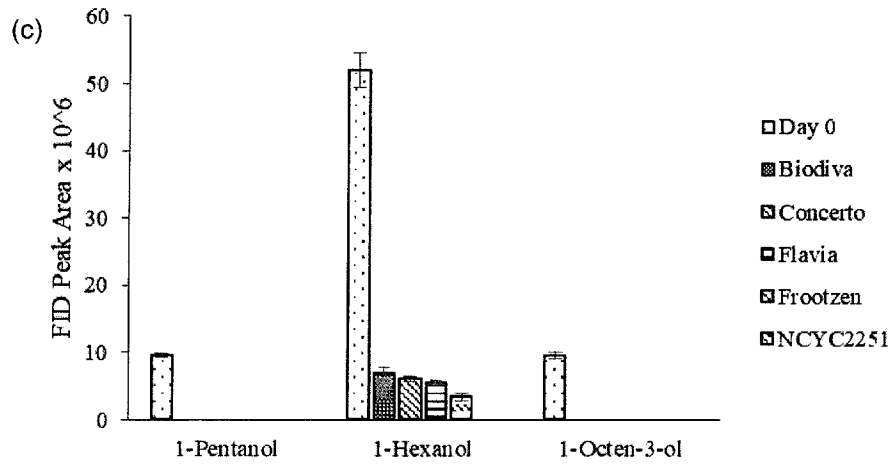


**Figure 10**

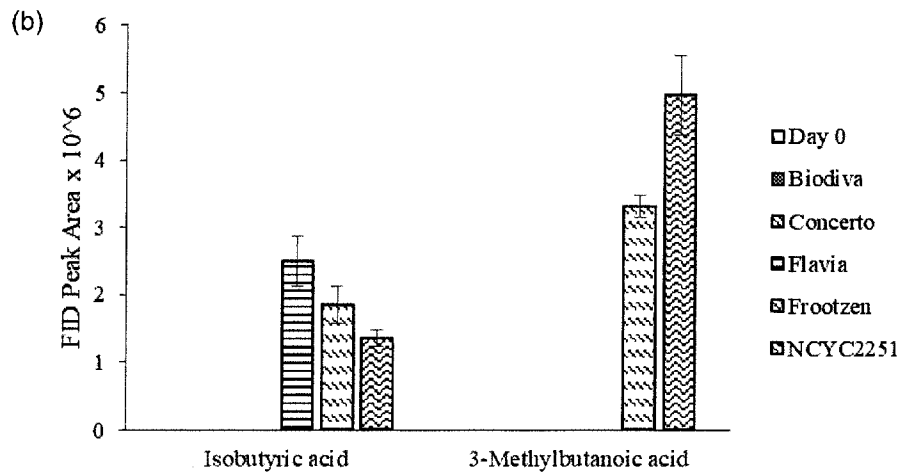
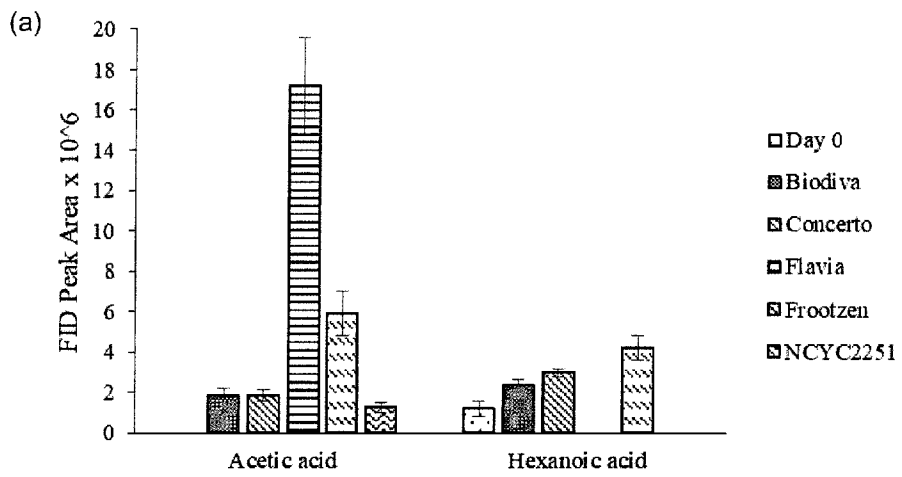


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**Figure 10 (continued)**

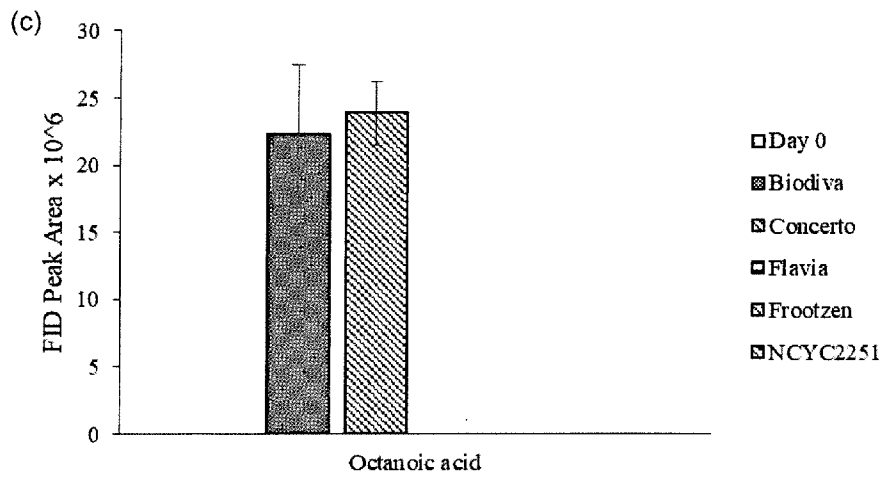


**Figure 11**

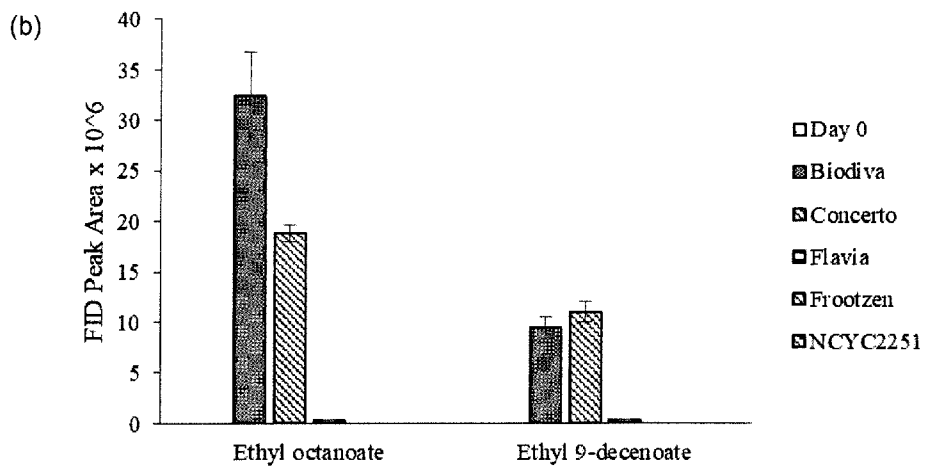
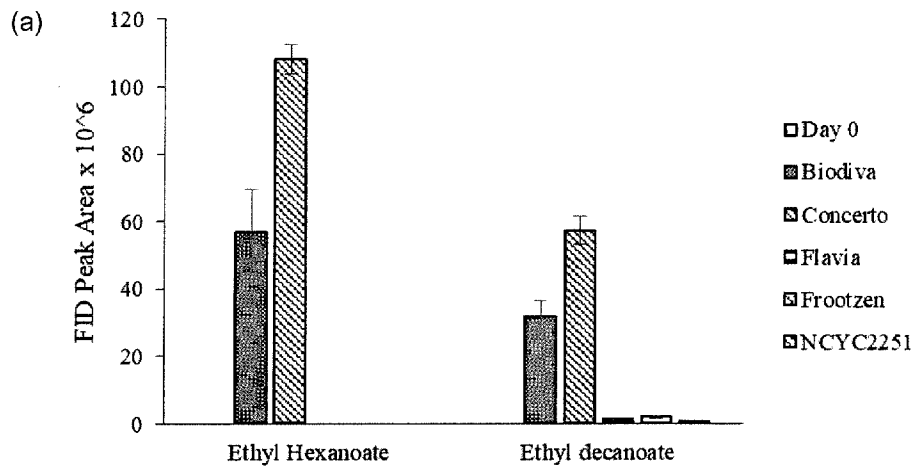


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**Figure 11 (continued)**

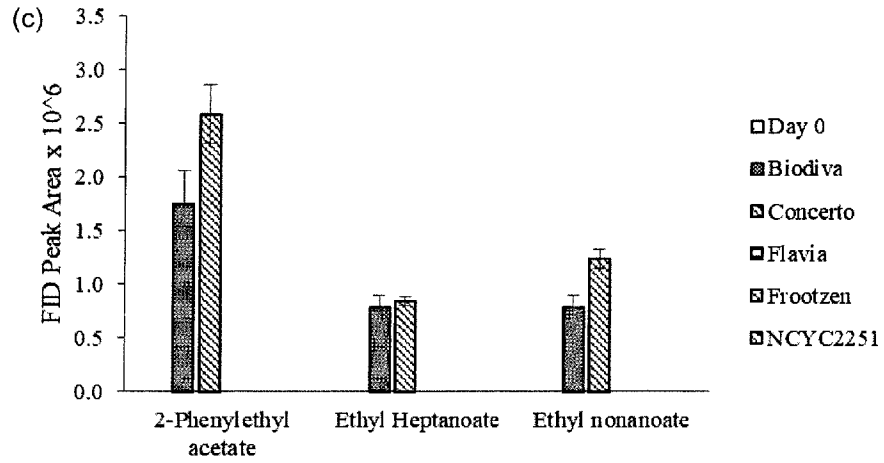


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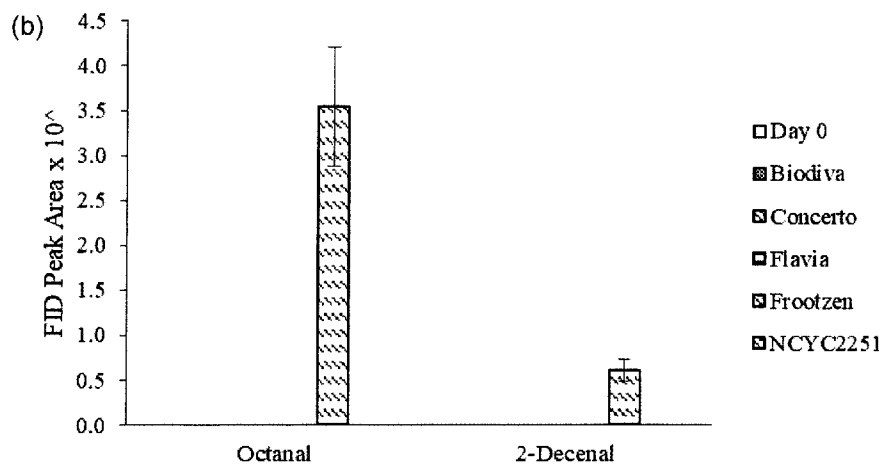
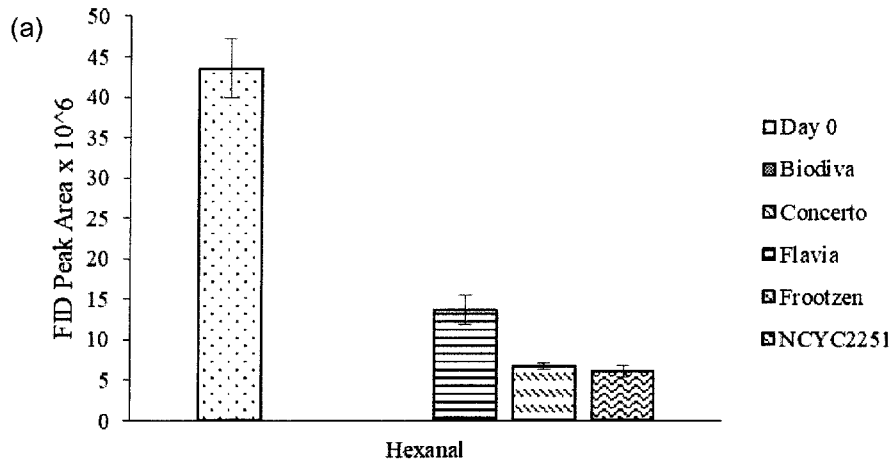


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**Figure 12 (continued)**

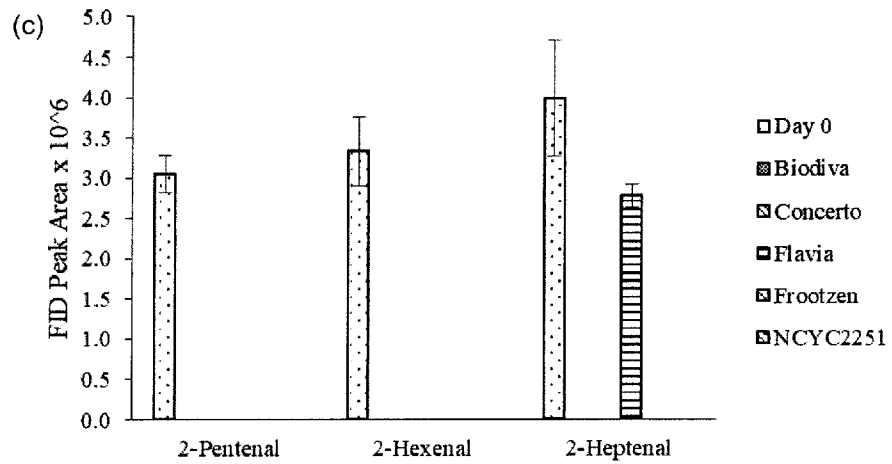


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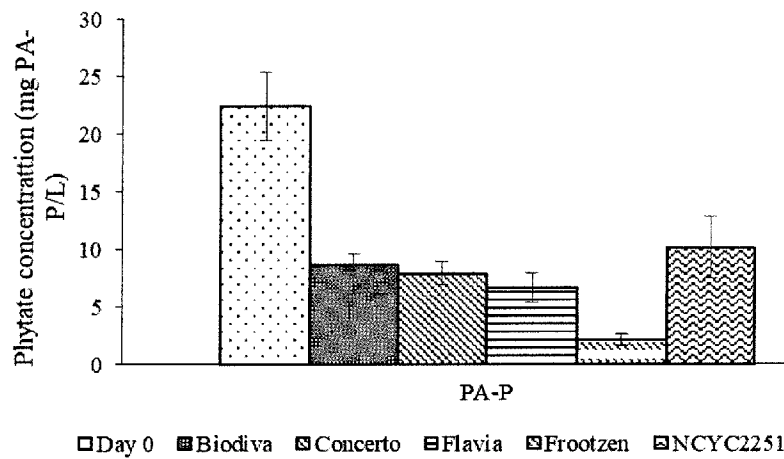


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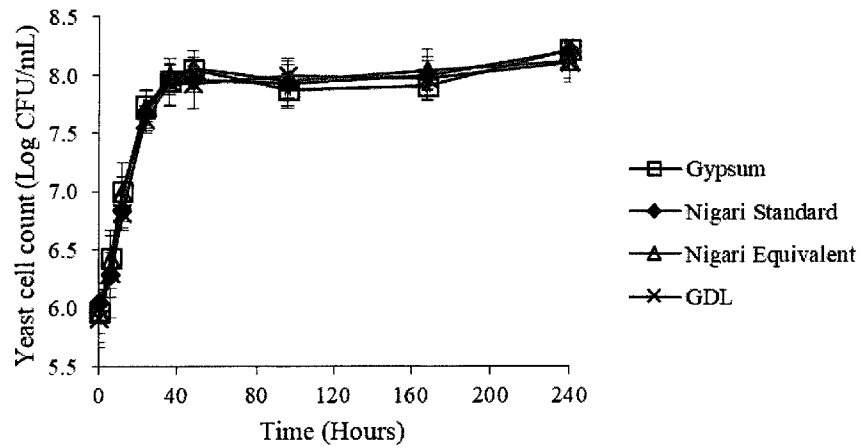
**Figure 13 (continued)**



**Figure 14**

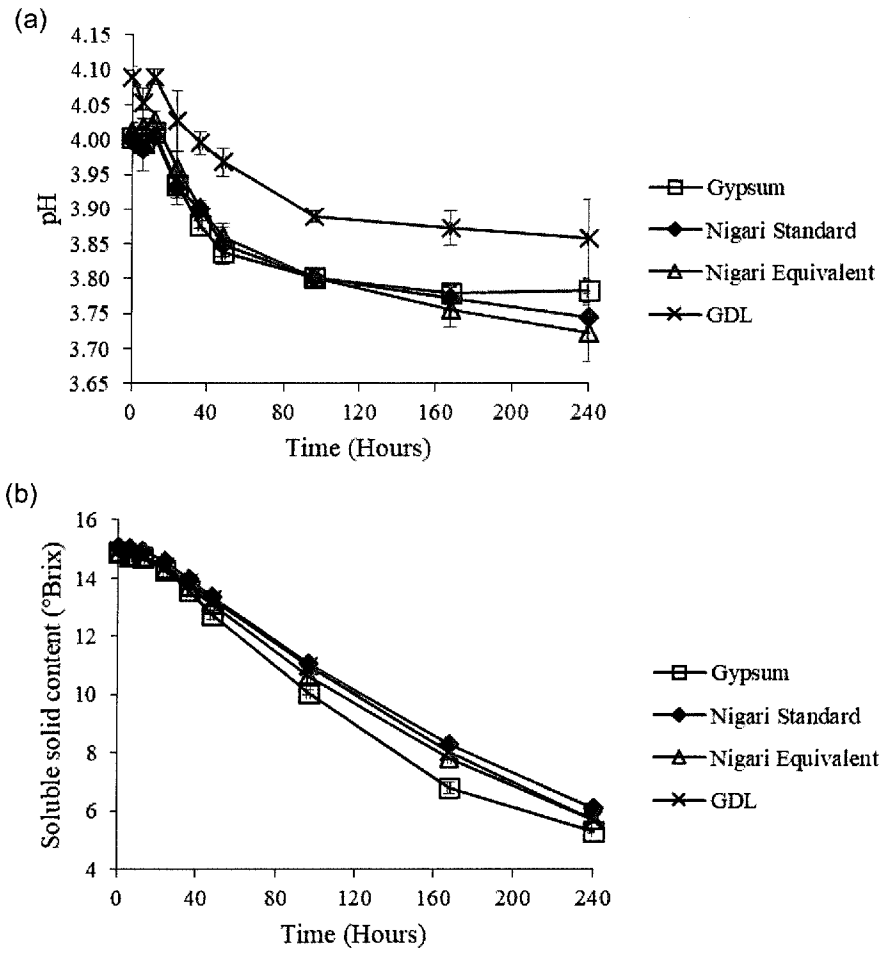


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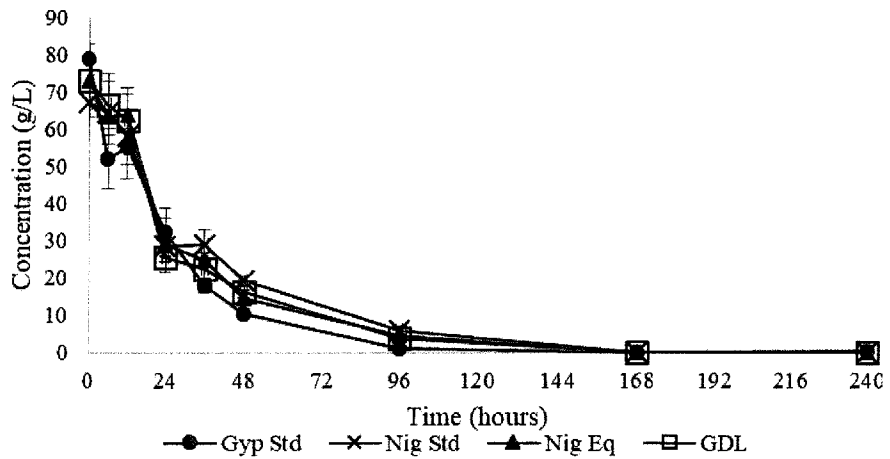


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**Figure 16**

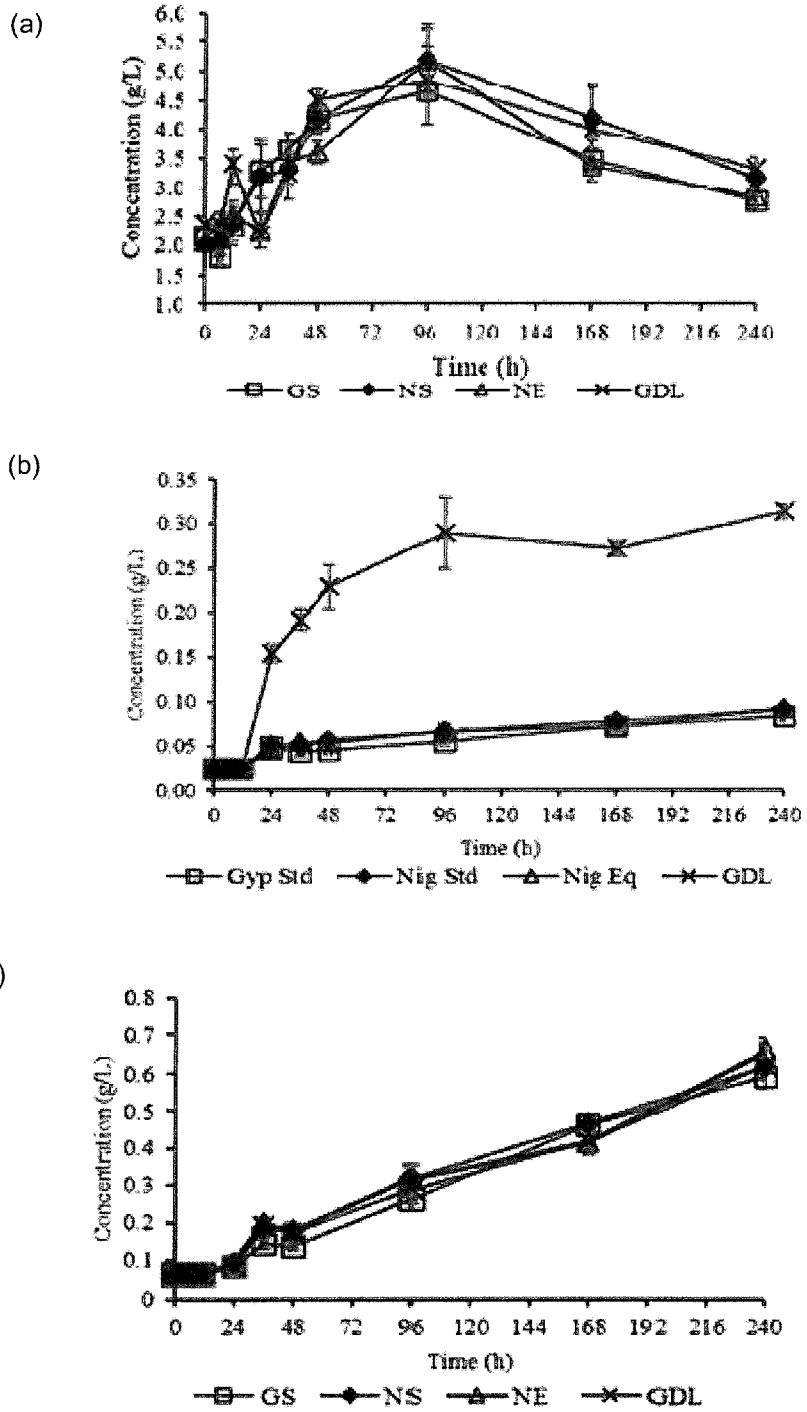


**Figure 17**

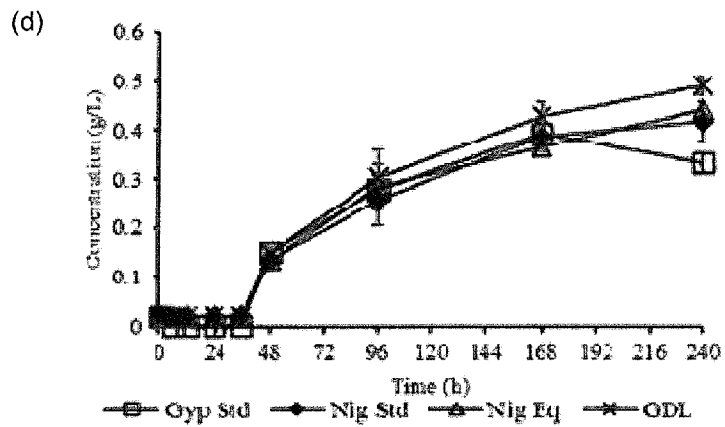


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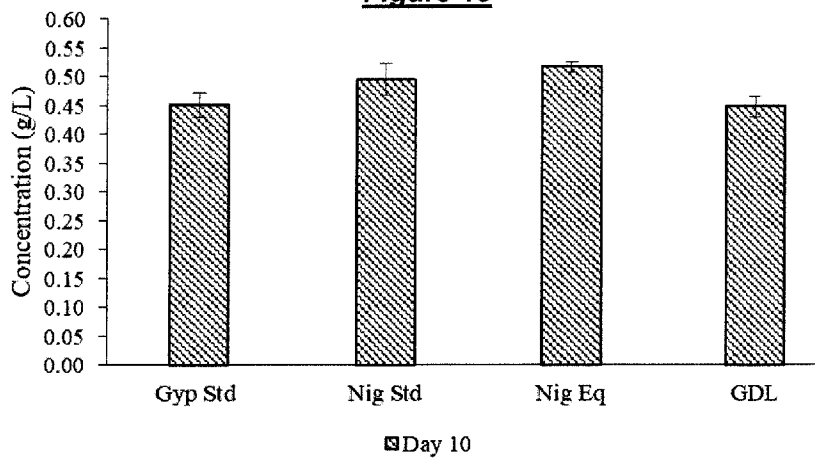
**Figure 18**



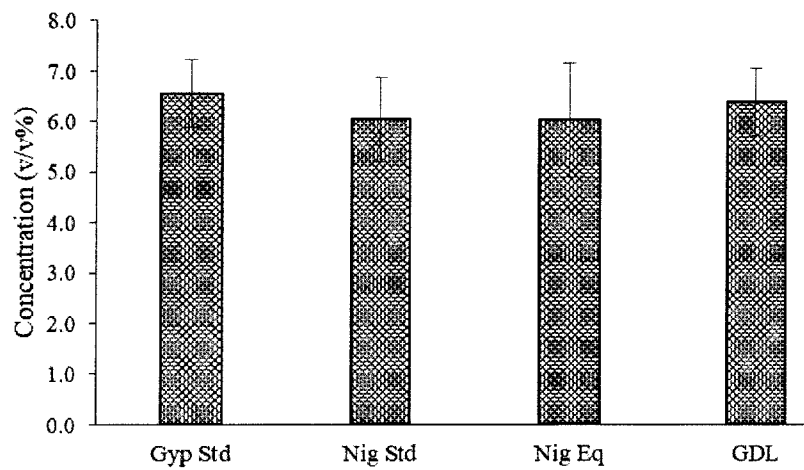
**Figure 18 (continued)**



**Figure 19**

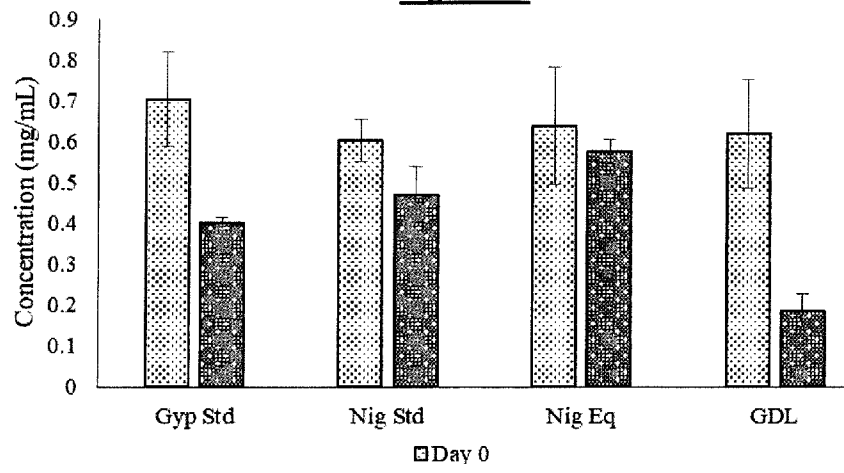


**Figure 20**



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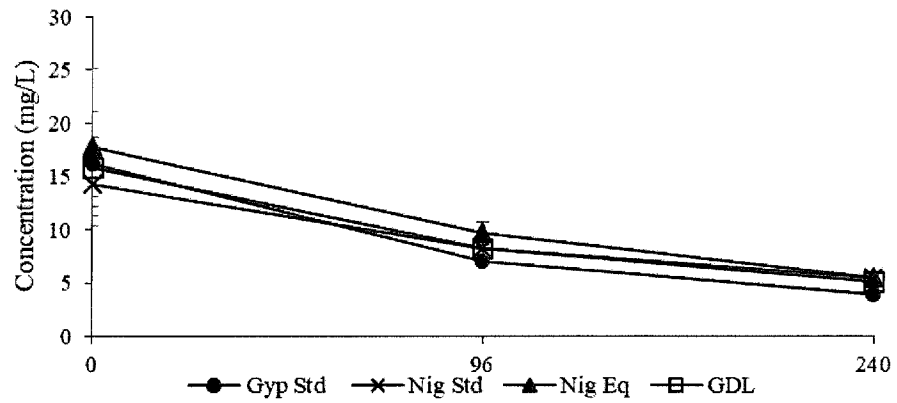
**Figure 21**



**Figure 22**

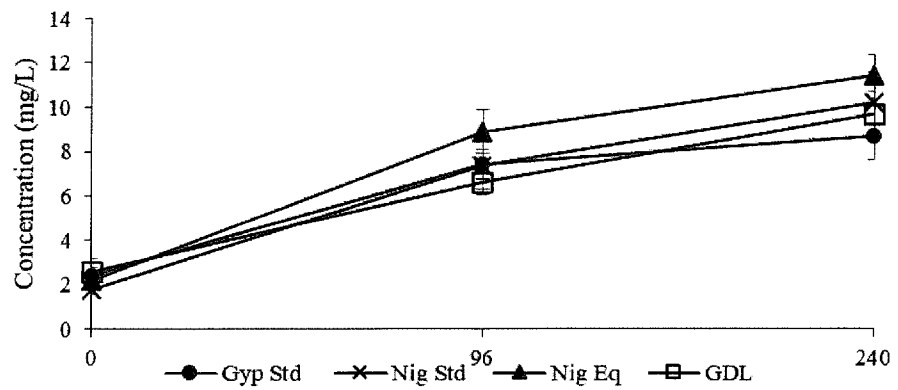
(a)

Daidzin



(b)

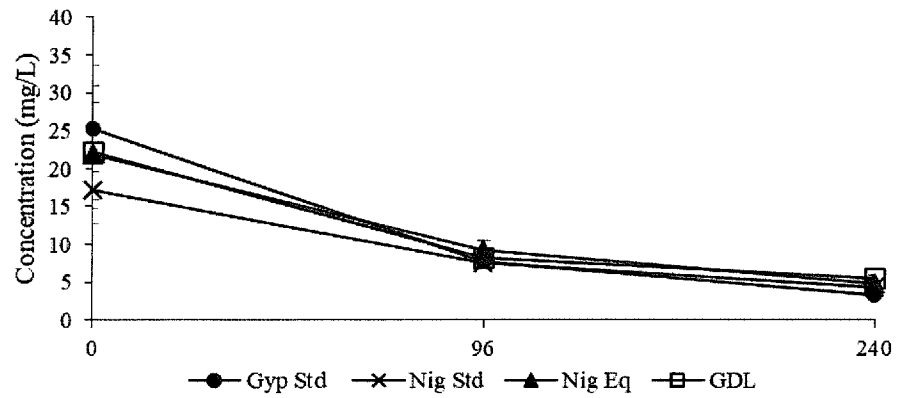
Daidzein



**Figure 22 (continued)**

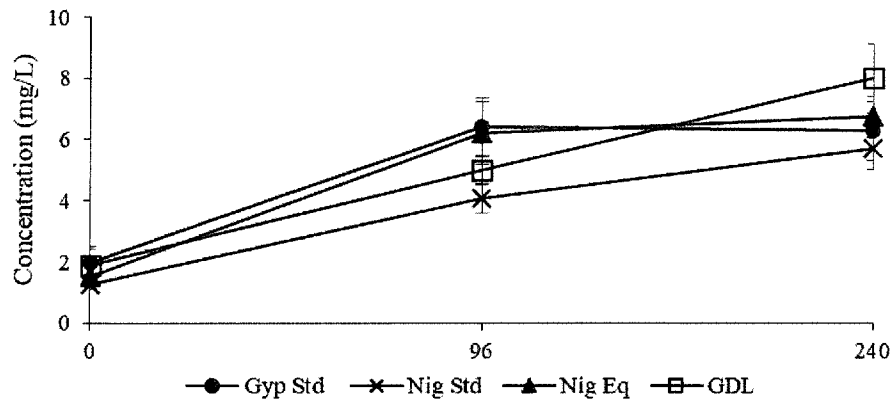
**Genistin**

(c)



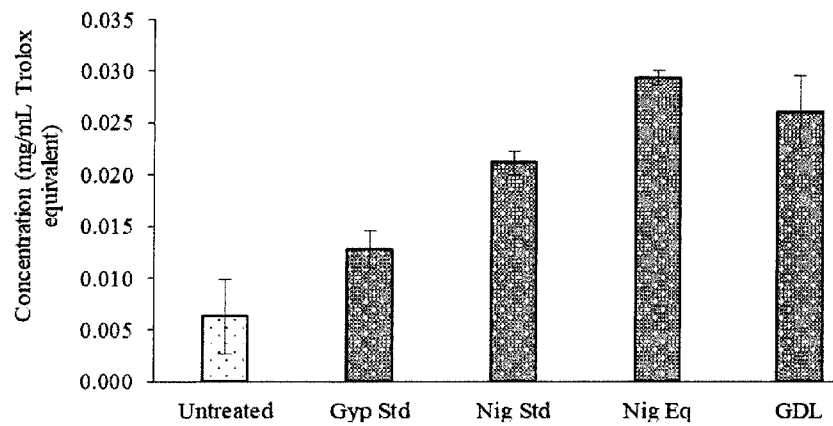
**Genistein**

(d)



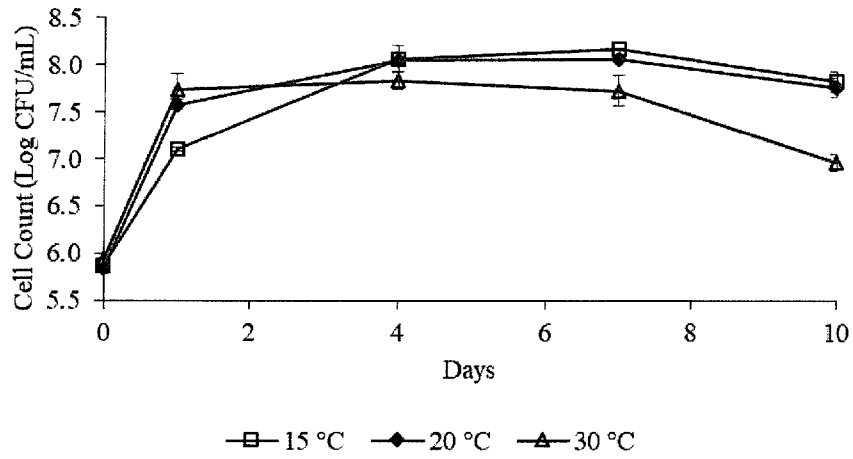
(e)

**Antioxidant Activity**

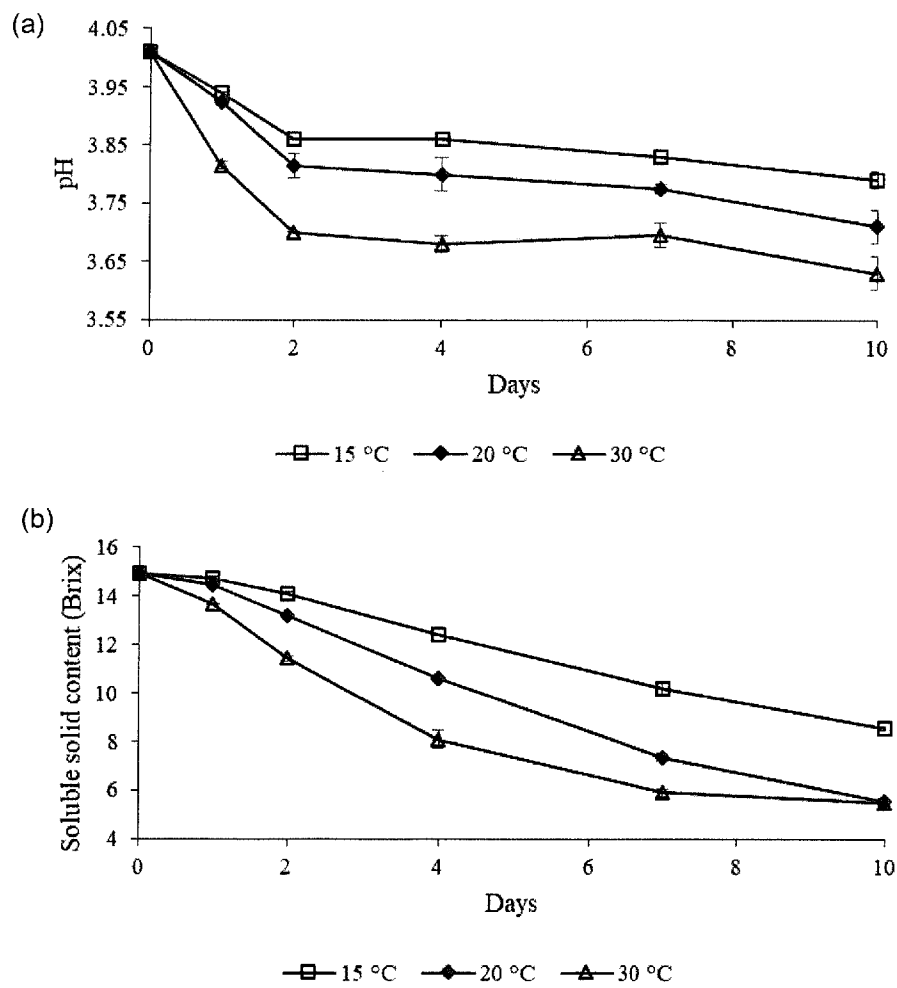


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**Figure 23**

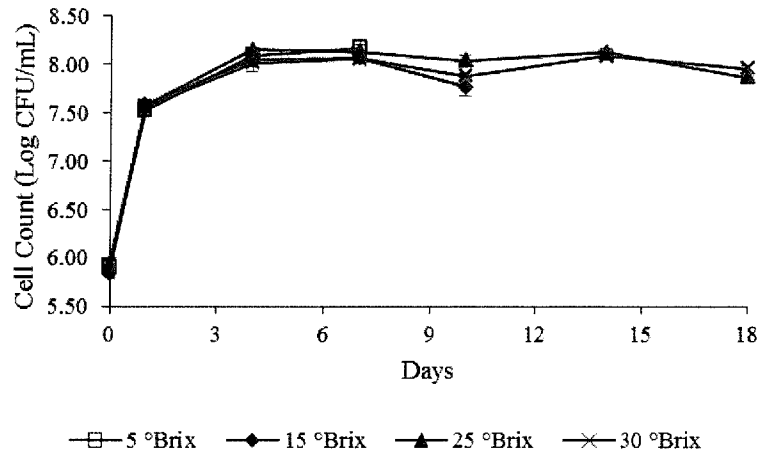


**Figure 24**

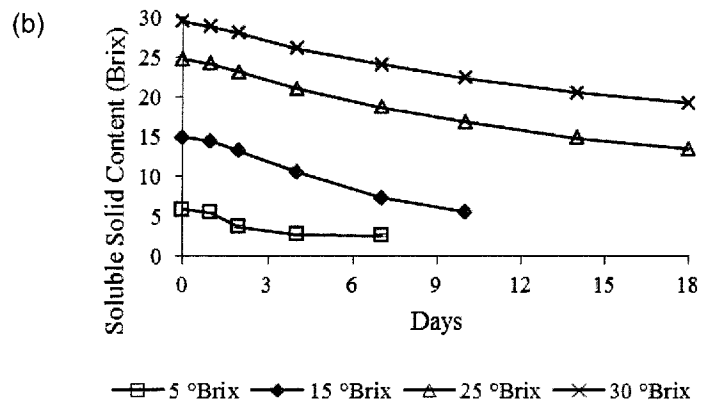
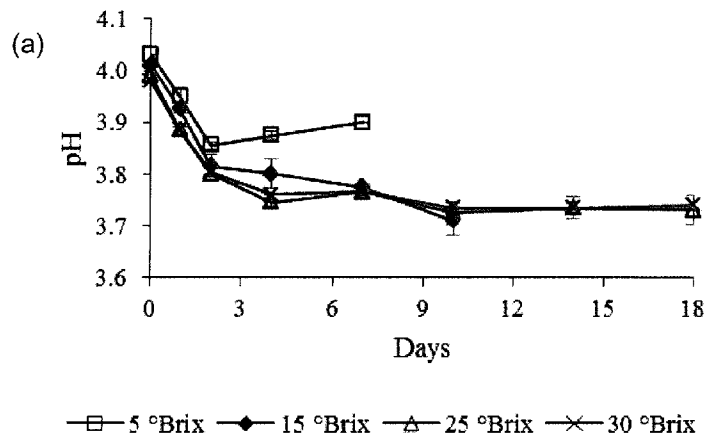


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**Figure 25**



**Figure 26**



# INTERNATIONAL SEARCH REPORT

International application No.

**PCT/SG2018/050193**

## A. CLASSIFICATION OF SUBJECT MATTER

**A23L 11/30 (2016.01) A23C 11/02 (2006.01) C12G 3/02 (2006.01)**

According to International Patent Classification (IPC)

## B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)

A23C A23L C12G

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

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

FAMPAT, INPADOC, CABA, FSTA, BIOSIS, MEDLINE: Soy whey, isoflavones, beverage, yeast, fermentation, alcohol, aglycone, daidzin, genistein, glycitein and related terms

## C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	CN 103351969 A (ANHUI QILIANG ECO-AGRICULTURE TECHNOLOGY CO., LTD) 16 October 2013 Example 1, Para [0010] of the original non-English language document (a machine translation of the Abstract is enclosed <b>only</b> for your reference)	1 - 20
X	CN 105077446 A (ANHUI RENRENFU BEAN INDUSTRY CO., LTD) 25 November 2015 Para [0005], [0009] of the original non-English language document (a machine translation of the Abstract is enclosed <b>only</b> for your reference)	1 - 20
X	EP 2014182 A1 (SCHIEFTHALER-NAIRZ U. & NAIRZ K.) 14 January 2009 Para [0015], [0023], [0036]-[0048] and Example 1 of the machine translation	1 - 20
X	JP 2011097865 A (HABUTAETOUFU CO., LTD) 19 May 2011 Examples of the machine translation	1 - 20

Further documents are listed in the continuation of Box C.

See patent family annex.

\*Special categories of cited documents:

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

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

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

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

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

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


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

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

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Date of the actual completion of the international search  
19/06/2018 (day/month/year)

Date of mailing of the international search report  
09/07/2018 (day/month/year)

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**Intellectual Property Office of Singapore**  
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Authorized officer  
  
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IPOS Customer Service Tel. No.: (+65) 6339 8616

## INTERNATIONAL SEARCH REPORT

International application No.

PCT/SG2018/050193

C (Continuation). DOCUMENTS CONSIDERED TO BE RELEVANT		
Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	XIAO Y. ET AL., Enhancement of the antioxidant capacity of soy whey by fermentation with <i>Lactobacillus plantarum</i> B1-6. <i>J. Funct. Food</i> , 19 November 2014, Vol. 12, pages 33 - 44 [Retrieved on 2018-05-15] <DOI: 10.1016/J.JFF.2014.10.033> Table 1, Sections 2.1 – 2.3, 3.2	1 - 11 and 15 - 20
Y	US 2001/0010930 A1 (KIKKOMAN CORPORATION) 2 August 2001 Entire document, particularly [0010] – [0022]	1 - 11 and 15 - 20
P,X	CHUA J.-Y. ET AL., Biotransformation of soy whey into soy alcoholic beverage by four commercial strains of <i>Saccharomyces cerevisiae</i> . <i>Int. J. Food Microbiol.</i> , 13 September 2017, Vol. 262, pages 14 - 22 [Retrieved on 2018-05-15] <DOI: 10.1016/J.IJFOODMICRO.2017.09.007> Entire document	1 - 20
P,X	CN 107751389 A (SOUTH CHINA UNIVERSITY OF TECHNOLOGY) 6 March 2018 Entire document of the machine translation	1 - 20

**INTERNATIONAL SEARCH REPORT**

Information on patent family members

International application No.

**PCT/SG2018/050193**

*Note: This Annex lists known patent family members relating to the patent documents cited in this International Search Report. This Authority is in no way liable for these particulars which are merely given for the purpose of information.*

<b>Patent document cited in search report</b>	<b>Publication date</b>	<b>Patent family member(s)</b>	<b>Publication date</b>
CN 103351969 A	16/10/2013	NONE	
CN 105077446 A	25/11/2015	NONE	
EP 2014182 A1	14/01/2009	NONE	
JP 2011097865 A	19/05/2011	NONE	
US 2001/0010930 A1	02/08/2001	JP 2001204486 A	31/07/2001
CN 107751389 A	06/03/2018	NONE	