https://doi.org/10.17113/ftb.60.01.22.7183 original scientific paper

Rice (*Oryza sativa*) Bran and Soybean (*Glycine max*) Meal: Unconventional Supplements in the Mead Production

Running Title: Unconventional Supplements in the Mead Production

Geiza Suzart Araújo[1*], Gislane Oliveira Ribeiro[2], Sílvia Maria Almeida de Souza[1], Gervásio Paulo da Silva[3], Giovani Brandão Mafra de Carvalho[1], José Ailton Conceição Bispo[1] and Ernesto Acosta Martínez[1]

[1]Department of Technology, State University of Feira de Santana, Avenida Transnordestina, S/Nº, 44.036-900, Feira de Santana-BA, Brazil
[2]School of Agronomy, Federal University of Goiás, Av. Esperança, s/n - Chácaras de Recreio Samambaia, 74.690-900, Goiânia – GO, Brazil
[3]Department of Education, Bahia State University, Rodovia Lomanto Jr, Br. 407 Km 127, s/n,48.970-000, Senhor do Bonfim – BA, Brazil

Received: 5 February 2021
Accepted: 13 November 2021

SUMMARY

Research background. Due to the lack of nitrogen in honey, the fermentation may be limited or delayed, in addition to stimulating the production of unpleasant sensory compounds, such as sulfur derivatives. The use of natural supplements has been investigated as low-cost alternatives for mainly correcting the nutritional deficiency of nitrogen in honey must in mead production.

Experimental approach. Initially, the physicochemical characterization of the extracts was carried out. The fermentative performance of three yeasts [*Saccharomyces bayanus* Premier Blanc (*Sb*PB), *Saccharomyces cerevisiae* Montrachet (*Sc*M) and *Saccharomyces cerevisiae* Safbrew T-58 (*Sc*ST58)] in honey musts supplemented with rice bran (RBE) and soybean meal (SME) extracts was evaluated. The trials were compared with the fermentations of musts with commercial supplement (CS) and the control trials. Fermentations were carried out in Erlenmeyer flasks containing honey

*Corresponding author:
Phone: (55)75981954393
E-mail: suzart19@hotmail.com

must supplemented with RBE, SME and CS (30 g/L), inoculated with 10^6 cells/mL and incubated at 30 °C for 264 h.

Results and conclusions. There was significant difference in the evaluated properties of the extracts, with the exception of reducing sugars. The fermentations with SME reached the highest cell concentrations, as well as the largest sugar consumption of glucose and fructose and ethanol. The glycerol concentrations slightly increased when SME and CS were used. The largest concentrations of succinic and acetic acids were registered in the control trials produced by *Sb*PB, *Sc*M and *Sc*ST58. There was no production of formic and lactic acids. Results showed that the extracts can be used as low-cost alternatives for correcting the nutritional deficiency of nitrogen in honey must since they presented results similar to the synthetic supplement.

Novelty and scientific contribution: The use of low-cost, unconventional supplements such as those used in this work, in addition to reducing the cost of the process by reducing fermentation time by providing nutrients needed to improve yeast metabolism, prevents the formation of compounds undesirable in the beverage due to prolonged fermentation time. It also makes it possible to add value to industrial by-products. Unconventional supplements have still been little tested in mead production.

Key words: rice bran extract; soybean meal extract; commercial supplement; fermentation; mead

INTRODUCTION

Mead is a drink obtained from the alcoholic fermentation of honey diluted by the action of yeast (*1*), whose production is not standardized, and, therefore, winemaking techniques and ingredients are frequently used in its production (*2*). There are several studies that aim to optimize and consequently standardize the process of mead making from the selection of the type of honey (*3*), fermentation agent (*4*) cell concentration (*5*), process conditions (*6*) and supplements (*6, 7*).

The use of supplements has been the focus of numerous research studies due to the prolonged fermentation time of honey must. According to Mendes-Ferreira *et al*. (*8*), mainly due to the lack of nitrogen in honey, the fermentation may be limited or delayed, in addition to stimulating the production of unpleasant sensory compounds, such as sulfur derivatives.

Synthetic supplements have been more commonly used to correct the nutritional deficiency of must than natural ones. According to Sridee *et al*. (*14*), the use of low-cost nitrogen sources to replace supplements, such as peptone and yeast extract has been continuously investigated, however, there are still few studies in the mead production. The interest in a better use of agro-industrial by-products has increased. Rice bran and soybean meal are examples of by-products widely used as supplements to obtain compounds of industrial interest (*10*).

Rice bran contains a 14-16 % protein content, and the protein nutritional value of this bran is relatively high due to an elevated concentration of lysine (*11*). It has good levels of vitamins and minerals, such as phosphorus and manganese (*12*). In industry, it is commonly used as a raw material for oil extraction, animal feed production, and less frequently, in the preparation of dietary products, multi-mix composition, and as a nitrogen source in the production of xylitol (*10*).

Soybean meal is obtained from oil extraction and has approximately 48 % protein, 35 % carbohydrates, 10 % water, 5 % minerals and less than 1 % fat (3 to 4 % hydrolyzed fat) (*13*). There are many works in literature that address the use of bran in animal feed, but there is a high consumption of this bran as tofu in Asian countries (*14*).

Given the context, the present work aims to make use of unconventional supplements, mainly as nitrogen sources in honey must in the mead production. This work is unique, as it represents the first example of a study in which agro-industrial waste was used in the preparation of supplements for mead production.

MATERIALS AND METHODS

Raw material

Amber colored honey [pH (3.4 ± 0.0), total soluble solids (81.70 ± 0.06) °Brix; glucose (21.70 ± 0.98) % (*m*/*v*); fructose (46.45 ± 0.27) % (*m*/*v*), sucrose (7.70 ± 0.01) % (*m*/*v*); proteins (0.51 ± 0.11) % (*m*/*m*); lipids (0.39 ± 0.01) % (*m*/*m*), ash (0.07 ± 0.01) % (*m*/*m*), potassium (1062 ± 3) mg/kg, sodium (584 ± 4) mg/kg, phosphorus (168.57 ± 0.82) mg/kg, calcium (50.96 ± 0.65) mg/kg, magnesium (11.16 ± 0.15) mg/kg, iron (2.07 ± 0.16) mg/kg, manganese (1.13 ± 0.08) mg/kg, zinc (0.28 ± 0.08) mg/kg] (*4*), from Cooperative of Ribeira do Pombal, Brazil), rice bran and soybean meal (Feira de Santana, Brazil) were used.

Yeast strains

The strain of *Saccharomyces bayanus* Premier Blanc (Red Star brand, Bagstone, Belgium) and two strains of *Saccharomyces cerevisiae*: Safbrew T-58 (Fermentis, Marcq-en-Baroeul, France) and Montrachet (Red Star, Bagstone, Belgium) were tested.

Preparation of rice bran and soybean meal extracts

Within glass bottles, the solid and distilled water (150 g/L) were added. Subsequently, the mixture was autoclaved at 121 ^{o}C for 15 minutes and centrifuged at 3500 rpm for 10 minutes using Excelsa Baby I® bench centrifuge (FANEM, São Paulo, Brazil) according to Araújo *et al*. (5).

Preparation of the commercial supplement solution

The commercial supplement solution (150 g/L) was obtained from the dilution in water of the following reagents (São Paulo, Brazil): yeast extract (36.8 g/L), malt extract (36.8 g/L), peptone (73.5 g/L), magnesium chloride (0.38 g/L), ammonium sulfate (2.25 g/L) and dibasic ammonium phosphate (0.38 g/L) according to Amorim *et al*. (*7*). The solution was autoclaved at 121 °C for 15 minutes.

Physicochemical characterization of rice bran and soybean meal extracts

The titratable acidity (%) was performed by volumetry with indicator. The protein concentration (%) was obtained by the Kjeldahl method, and ashes (%), by calcination in muffle (SolidSteel, São Paulo, Brazil) (*15*).

The pH reading was performed from the digital pH meter (Instrutherm, São Paulo, Brazil); total soluble solids (°Brix), by the refractometric method, using a digital device (Reichert, São Paulo, Brazil) at 20 °C.

Carbohydrates (%) were determined according to the method proposed by Trevelyan and Harrison (*16*), and the reducing sugars (%) were quantified by dinitrosalicylic acid (DNS) spectrophotometric method with a wavelength of 540 nm according to Miller (*17*), using the spectrophotometer (SHIMADZU UV mini-1240, São Paulo, Brazil).

The assimilable nitrogen (mg/L) was determined using the titrimetric method with standardized 0.1 N NaOH solution according to Zoecklein *et al*. (*18*).

The elements Na and K (mg/100g) were quantified by atomic emission and Ca, Mg, Zn, Fe and Mn (mg/100g) by atomic absorption. The P element (mg/100g) was analyzed using the ammonium metavanadate method on a UV-VIS spectrophotometer (Femto, São Paulo, Brazil) (*19*).

Inoculum preparation

The yeasts were weighed according to the manufacturer's instructions, and transferred to different 125 mL Erlenmeyer flasks containing 50 mL of honey must (30° Brix). The mixture was subjected to shaking (Tecnal TE-420, São Paulo, Brazil) at 150 rpm, at 30 °C for 24-48h in order to reach the cell concentration of 10^7 cells/mL.

Fermentation tests

In 500 mL Erlenmeyer flasks, the honey was diluted in sterile water, and the mixture was supplemented. The pH of the mixtures was adjusted to 5.0 using calcium carbonate.

The inoculation of the supplemented must was performed according to Amorim *et al*. (7) and Araújo *et al*. (*4*). Subsequently, the Erlenmeyer flasks were sealed with a stopper coupled with an airlock valve containing 70 % ethyl alcohol. The systems were placed in a Biochemical Oxygen Demand (BOD) oven (Tecnal, São Paulo, Brazil) at 30 °C for 264 h.

The experiments were carried out in triplicate and, for each yeast strain, soybean meal and rice bran extracts (30 g/L) were evaluated and these trials were compared to the must fermentation process with commercial supplement (30 g/L) and the control trials.

Analytical Monitoring of the Fermentation Process

Cell concentration

Monitoring of fermentation was carried out every 24 hours from the collection of samples to determine the following parameters: cell concentration from counting performed in a Neubauer chamber (1/400 mm^2 x 1/10 mm) and to determine viable and non-viable cells, the International Staining Method was used (*20*).

Concentration of sugars (glucose and fructose), ethanol and organic acids

The concentrations of glucose, fructose, sucrose, ethanol, glycerol and organic acids (acetic, lactic, succinic and formic) (g/L) during fermentation were quantified by high performance liquid chromatography (HPLC) (Waters 2414, Torrance, USA), using a BIORAD AMINEX HPX-87H column (300 mm × 7.8 mm) and RID 6A refractive index detector, using H_2SO_4 0.005 M as eluent, at a flow rate of 0.6 mL/min and column temperature of 45 °C.

Statistical analysis

The analysis of variance (ANOVA) and Tukey's test were performed to identify significant differences between the means of the results obtained using the SISVAR program (5.6) (*21*). Differences between means at the 5 % level ($p<0.05$) were considered significant.

RESULTS AND DISCUSSION

Physicochemical characterization of rice bran and soybean meal extracts

As shown in Table 1, there was a significant difference ($p\leq0.05$) in all parameters evaluated for the rice bran extract (RBE) and soybean meal extract (SME), with the exception of reducing sugars.

Table 1

RBE showed a slightly lower pH (6.1) than SME (pH 6.5), and consequently, the total titratable acidity of RBE (2.21 %) was 1.55 times higher than SME (Table 1). The acidity and pH values (2.1 % and 6.5) for rice bran, respectively, were found by Feddern *et al*. (*22*). Ginger-Reverdin *et al*. (*23*) found a close pH value (6.76) and lower concentration of total titratable acidity (0.82 %) for soybean meal. Acidity and pH are parameters that indicate the conservation state of the raw material. The

increase in acidity may result from storage, which may favor the fermentation process, as well as hydrolysis of lipids (*24)*.

Regarding the concentration of total soluble solids and carbohydrates, SME presented values approximately four times higher than those found for RBE (1.6 °Brix; 1.65 %), respectively (Table 1). Carbohydrate values between 25.71 % and 32.89 % were verified by Garcia *et al*. (*25*) for rice bran. According to Malekian *et al*. (*11*), the carbohydrates present in the rice bran are hemicellulose (8.7–11.4 %), cellulose (9.0–12.8 %), starch (5–15 %) and beta-glucans (1 %). According to Choct *et al*. (*26*), soybean meal has around 35 % of carbohydrates, composed mainly of sucrose, stachyose, raffinose, pectin, cellulose and starch.

The concentrations of reducing sugars were similar (0.11 and 0.12 %) for RBE and SME, respectively (Table 1). Garcia *et al*. (*25*) analyzed rice bran that showed higher concentrations of reducing sugars (1.23–2.60 %). A concentration of approximately 0.6 % of reducing sugars in conventional soybean meal was reported by Paris (*34*).

The RBE and SME showed protein values (0.42 and 1.70 %) and assimilable nitrogen (15.86 and 69.10 mg/L), respectively (Table 1). Bhosale and Vijayalakshmi (*28*) reported a concentration of 17.5 % for stabilized rice bran and 19.25 % for probiotic rice bran. Protein concentration of 48.38 % in soybean meal was found by Gerber *et al*. (*29*). Regarding the assimilable nitrogen content, the value obtained for soybean meal was higher than the one reported by Araújo *et al*. (*4*) for cowpea extract (45.73 %), used in the mead fermentation process. Assimilable nitrogen is a fundamental component in the fermentation process of beverages, such as wine and mead. According to Iglesias *et al*. (*30*), an inadequate amount of assimilable nitrogen in fermentation can harm yeast growth, extend the fermentation and decrease ethanol productivity.

The ash content was approximately three times higher in the SME than in the RBE (0.23 %) (Table 1). Sairam *et al*. (*31*) reported a value of 7.4 % of ashes in rice bran. Ghadge *et al*. (*32*) found the content of 6.89 % in soybean meal. According to Vicentini-Polette *et al*. (*33*), the ash content is linked to the minerals present in the product.

The highest concentrations (mg/100g) were found in the SME: calcium (10.95), magnesium (27.15), zinc (0.30), iron (2.40), sodium (7.50), potassium (505.50) and phosphorus (18.50) which were 3.87, 1.84, 2.14, 2.72, 1.39, 3.67 and 8.30 times higher, respectively, than in the RBE (Table 1). It was not possible to detect the presence of manganese in both extracts. These minerals positively influence cell growth and the fermentation process (*34*). Carvalho *et al*. (*35*) also found higher concentrations of minerals in the soybean extract. The authors reported that soybean and brown rice extracts had the following mineral concentrations (mg/kg): calcium (15.75; 12.03), magnesium (28.50; 1.69), zinc (1.82, 0.18), iron (4.31, 0.77) and manganese (0.16, 0.15), respectively.

Influence of rice bran and soybean meal extracts on cell growth of Premier Blanc, Montrachet and Safbrew T-58

Figs. 1a-c shows the cell growth profile of the yeasts *Sb*PB, *Sc*M and *Sc*ST58, respectively, during the fermentation of honey must supplemented with RBE, SME and CS (30 g/L), and within the control trials.

Fig.1

The evaluated supplements (RBE, SME and CS) favored the cell growth of all yeasts when compared to the control assays, however, the highest cell concentration values were obtained in fermentations with SME during 264 h of fermentation regardless of the yeast. Thus, it can be inferred that SME provided necessary nutrients in an ideal concentration for cell growth, such as assimilable nitrogen and minerals, since it had higher concentrations of these components than RBE (Table 1). The lack or limitation of these nutrients and other growth factors compromise the development of yeast (*36*). Schwarz *et al*. (*37*) evaluated the influence of honey must supplementation with nitrogen, minerals and vitamins on the mead production and found that these components favored the production of yeast biomass, which was associated with the availability of nitrogen, showing a linear relationship between 15 and 135 mg/L.

For all yeasts, the effect of supplementation can be clearly seen throughout the fermentation process compared to the control trials (Figs. 1a-c) Almeida *et al*. (*2*) evaluated the effect of commercial nitrogen sources (diammonium phosphate and ammonium sulphate) on the fermentation of honey must (25 °Brix), inoculated with 10^6 cells/mL of S*accharomyces cerevisiae* JP14 and conducted at 25 °C for 28 days. These authors reported that up to 120h of fermentation, both supplements favored an increase in the concentration of viable cells.

From the comparison of the cell growth profile of the three yeasts in the media supplemented with SME and RBE and the assays with CS, it can be seen that the cell concentrations obtained during the fermentation were similar in the RBE and CS media and lower than those obtained in trials with SME (Figs. 1a-c).

Using SME, *Sb*PB showed higher maximum cell concentration (16.9 x 10^7 cells/mL), followed by *Sc*M (13.0 x 10^7 cells/mL) and *Sc*ST58 (10.7 x 10^7 cells/mL) in 120 h of fermentation. On the other hand, in the fermentation of must with RBE, *Sb*PB reached a concentration of 13.6 x 10^7 cells/mL, *Sc*M (10.0 x 10^7 cells/mL) in 120 h and *Sc*ST58 (8.8 x 10^7 cells/mL, 144h) (Figs.1a-c).

The maximum values of cellular concentration of the commercial supplement media were slightly higher than those obtained in the trials with RBE. *Sb*PB, *Sc*M and *Sc*ST58 reached the following maximum cell concentrations: 14.3 x 10^7 cells/mL in 120 h, 11.6 x 10^7 cells/mL, 144 h and

9.0 x 10^7 cells/mL in 144h, respectively. In the control trials, the values of the maximum cell concentrations were lower than the other trials (9.7 x 10^7 cells/mL in 144 h, 7.4 x 10^7 cells/mL and 6.1 x 10^7 cells/mL in 168 h) obtained by *Sb*PB, *Sc*M and *Sc*ST58, respectively (Figs. 1a-c). Honey lacks nitrogen, minerals and factors that stimulate yeast growth during fermentation (*1*).

Amorim *et al*. (*7*) evaluated the influence of the addition of acerola pulp (10, 15, 20, 25 and 30 %) in the fermentation of honey must (30 ºBrix) by *Saccharomyces cerevisiae* AWRI796 (10^7 cells/mL) at 30 °C, pH 5.0. The increasing concentrations of pulp progressively favored cell growth, which reached maximum concentration (2.09 x 10^8 cells/mL) after 288h of fermentation. Araújo *et al*. (*4*) supplemented the honey musts (30 °Brix) with cowpea extract (0, 5 and 30 g/L), and the media was inoculated with 10^6 cells/mL, with fermentations conducted at 30 °C, pH 5.0 for 264 h. According to these authors, cell growth was favored by the use of higher concentration of cowpea extract (30 g/L), with higher cell concentration equal to 19.0 × 10^7 cells/mL of *S. bayanus* Premier Cuvée in 168h, 11.3 x 10^7 cells/mL of *S. bayanus* Premier Blanc in 120 h and 11.1 × 10^7 cells/mL of *S. cerevisiae* Safbrew T-58 in 96 h. Pereira *et al*. (*36*) evaluated the cell growth profile of two strains of *S. cerevisiae* (QA23 and ICV D47) in honey musts (37 m/*v*), supplemented with salts, vitamins and the combination of both nutrients, which were inoculated with 10^5 cells/mL and fermented at 25 °C for 288 h. These authors found that the growth profile was influenced more by the yeast strain than by the supplements added to the must. The QA23 strain reached a maximum concentration of approximately 10^8 cells/mL, whereas the ICV D47 strain reached a concentration of 7 to 8x10^7 cells/mL after 48 h of fermentation.

Influence of rice bran and soybean meal extracts on profile of sugar (glucose and fructose) and ethanol concentrations during the fermentation honey of must *using commercial yeasts Premier Blanc Montrachet and Safbrew T-58*

From Figs. 2a-c, it is possible to observe that the consumption of glucose and fructose by *Sb*PB, *Sc*M and *Sc*ST58 occurred simultaneously both in the fermentation of supplemented musts and in the control trials (Figs. 2a-c). Similar behavior was observed by Araújo *et al*. (*4*), where in all trials performed (supplemented with cowpea extract and control), both sugars were consumed at the same time.

In general, it can be seen that regardless of the yeast, the trials with supplements showed a higher consumption of sugars and production of ethanol than the control trials (Figs. 2a-c), and it can be inferred that the rice bran and soybean meal extracts provided essential nutrients to the media in adequate concentrations for better yeast fermentation performance. According to Gibson *et al*. (*38*) and Silva *et al*. (*39*), honey has a low concentration of nitrogen, minerals and vitamins, so the

correction of the nutritional deficiencies of the must can reduce the sensitivity of the yeast to stress, improving the fermentative performance.

There was no significant difference ($p \leq 0.05$) in sugar consumption in fermentations of supplemented musts. However, it was possible to verify that with the use of SME, *Sb*PB, *Sc*M and *Sc*ST58 presented slightly higher values regarding the consumption of glucose (98.6, 97.1, 89.1 %) and fructose (93.2, 91.6 and 84.8 %), when compared to those obtained in the media containing RBE, in which glucose (97.1, 96.0, and 87.4 %) and fructose (92.0, 89.5 and 82.7 %) consumption values were obtained by *Sb*PB, *Sc*M and *Sc*ST58, respectively (Table 2).

Table 2

On the other hand, with the use of the commercial supplement (CS), the consumption of glucose and fructose were closer to those obtained in fermentation media with RBE (Table 2). In the control trials, the values of consumption of these sugars differed significantly ($p \leq 0.05$) compared to all supplemented media, presenting lower values regarding the consumption of glucose (91.9, 90.3, 83.6 %), fructose (87.5, 84.0, 74.6 %) by *Sb*PB, *Sc*M and *Sc*ST58, respectively (Table 2).

Higher values of glucose consumption (99.8 and 99.8 %) and fructose (95.5 and 85.6 %), respectively, were reported by Araújo *et al*. (*4*) when they evaluated the fermentative performance of *Sb*PB and *Sc*ST58 in the fermentation of honey must supplemented with 30 g/L of cowpea extract. Kawa-Rygielska *et al*. (*40*) used fruits and herbs as supplements in the fermentation of honey must (34 °Brix) by *S. bayanus* Safspirit Fruit, conducted at 22 °C for 16 days. These authors reported higher glucose consumption (77 %) in the trials with grape seeds, while a lower amount of glucose (60 %) was consumed in the control trials and the fructose consumption was on average 45 % in all trials.

Lower concentrations of sugars are observed in meads resulting from must supplemented with SME (glucose (1.2, 2.6 and 10.0 g/L), fructose (9.2, 11.4 and 20.51 g/L)), followed by the concentrations obtained from the trials with RBE (glucose (2.6, 3.5 and 11.2 g/L), fructose (10.5, 13.9 and 22.7 g/L)) as shown in Figs. 2a-c. Close values for glucose (2.1, 3.1 and 10.7 g/L) and fructose (10.3, 12.8 and 22.1 g/L) were obtained in the mead produced from the must containing commercial supplements.

Fig. 2a-c

On the other hand, as expected, in the control trials, the final concentrations of these sugars in the meads were higher (glucose (6.0, 6.6 and 10.7 g/L), fructose (17.1, 22.1 and 35.0 g/L). Gomes *et al*. (*41*) reported lower concentrations of glucose (2.55-5.11 g/L) and fructose (1.51, 27.6 g/L) in meads obtained from fermentations of honey must (395 g/L) conducted at 20 to 30 °C during 15 days. Lower concentrations of sugars are indicative of complete fermentation avoiding re-fermentation, which can favor contamination by undesirable microorganisms and consequently the production of

odors not characteristic of the product (*41*). Silva *et al*. (*42*) produced dry and sweet meads from the fermentation of honey must (28.2 ºBrix) at 25 ºC for 13 days and reported glucose concentration (4.9 and 50.5 g/L) and fructose (5.45 and 99.9 g/L) for dry and sweet mead, respectively.

The meads obtained can be characterized as smooth, since they had a residual sugar concentration greater than 3 g/L as recommended by the Brazilian legislation (*43*).

As shown in Figs. 2a-c, in all trials where the media was supplemented, there was ethanol production (4-14 %) from 24 to 48 h of fermentation, which is characteristic alcoholic content of mead according to the Brazilian legislation (*43*). However, in the control trials, meads containing this concentration of ethanol were solely obtained after 72 to 96 h. Hernández *et al*. (*44*) reported an ethanol concentration between 2 and 8 % from the fermentation of honey musts (24 °Brix), separately supplemented with yeast extract, pollen, pre-treated pollen and the mixture of pollen and ammonium dihydrogen phosphate, fermented by strains of *S. cerevisiae* (UVAFERM BC, FERBLANC AROM, and LALVIN QA23) in 24–36h.

It is possible to verify that in relation to the ethanol concentration of the meads, there was also no significant difference between the supplemented trials, with the exception of the control trials, which presented lower concentrations (Table 2).

With the use of SME, ethanol concentrations were slightly higher (118.0 g/L (15 % v/v); 112.7 g/L (14.3 % v/v), 116.3 g/L (14.7 v/v) and 98.6 g/L (12.5 % v/v)) to those obtained in fermentation with RBE (115.0 g/L (14.6 % v/v); 109.7 g/L (13.9 % v/v) and 96.8 g/L (12.3 % v/v)) by *Sb*PB, *Sc*M and *Sc*ST58, respectively. In the fermentation using must supplemented with CS, the ethanol contents of the meads were close to those obtained with the use of SME and RBE (Figs. 2a-c). Lower concentrations of ethanol (10.7–11.4 %) in musts supplemented with diammonium phosphate and 10.8 % for the control trials, were obtained by Mendes-Ferreira *et al*. (*45*) in the fermentation of honey must (37g: 100 mL) by *S. cerevisiae* UCD522 at 22 °C for 25 days. Amorim *et al*. (*7*) reported that at the optimum point in the fermentation of honey must supplemented with acerola, 120.1 g/L (15.2 %) of ethanol was obtained.

Influence of rice bran and soybean meal extracts on the production of glycerol and organic acids (succinic, formic, lactic and acetic) by SbPB, ScM and ScST58

The glycerol production profile had a varied behavior depending on the yeast used (Fig. 3). *Sb*PB produced mead with higher and similar concentrations of glycerol (8.3, 8.2 and 7.5 g/L) from the fermentation of the must supplemented with SME and CS than the trials with RBE, respectively.

Fig. 3

Araújo *et al*. (*4*) reported that the highest concentrations of glycerol (10.08 and 8.49 g/L) obtained by *Sb*PB and *Sc*ST58, were observed in trials with a higher concentration of supplement (30

g/L). Similar to the results obtained in this study, they reported that lower concentrations of this by-product were observed in the control trials. Gomes *et al*. (*41*) reported glycerol contents between 5.42 and 6.89 g/L for meads obtained from the fermentation of honey must supplemented with different concentrations of nutrient salts at different temperatures.

Glycerol is a by-product of fermentation that contributes to sensory characteristics, being generally found in the range of 7 to 10 % of the ethanol produced (*46*). According to Adamenko *et al*. (*47*), the glycerol concentration can be affected by the presence of organic acids, such as succinic, formic and acetic. Organic acids have an influence on the sensory characteristics and stability of alcoholic beverages (*48*). Formic, lactic acids were not detected in the trials (Fig. 3).

In fermentations of honey must supplemented with SME, the concentration of succinic (1.56; 2.04 and 2.32 g/L) and acetic (0.47; 0.52 and 0.61 g/L) acids in the meads were slightly lower than the following concentrations observed in the trials with RBE: succinic (2.20, 2.40 and 2.45 g/L) and acetic (0.50; 0.60 and 0.65 g/L) acids after fermentation by *Sb*PB, *Sc*M and *Sc*ST58, respectively. On the other hand, with the use of SC, yeasts produced lower values of succinic than those obtained in the trials with SME and RBE and higher concentrations of acetic acid were verified (Fig. 3). In trials without addition of supplements, higher values of succinic (2.51, 2.83 and 2.56 g/L) and acetic (0.58, 0.72 and 0.76 g/L) acids produced by *Sb*PB, *Sc*M and *Sc*ST58, respectively, when compared to trials in which the must was supplemented. The values found for succinic acid are in the range (0.34 and 3.98 g/L) reported by Švecová *et al*. (*49*), but some values are below the minimum limit of the range 0.62-16.61 g/L for acetic acid. Lower concentrations (0.2 and 1.0 g/L) of succinic and acetic acids, respectively, were reported by Sroka and Satora (*50*) in the mead obtained from honey must (1:3).

CONCLUSIONS

Rice bran and soybean extracts obtained from low-cost raw materials as well as commercial supplements provided the honey wort with the nutrients needed for better yeast performance, thus reducing the time of the fermentation process and consequently the cost of the final product. Premier Blanc and Montrachet yeasts showed better fermentation performances than Safbrew T-58, regardless of the supplements used. Thus, the tested extracts have the potential to be used as innovative supplements in the mead production. Furthermore, it is possible to add value to industrial by-products.

ACKNOWLEDGEMENTS

We thank the Graduate Program in Biotechnology (PPGBiotec) of the State University of Feira de Santana (UEFS).

FUNDING

This work was supported by Coordenação de Aperfeiçoamento de Pessoal de Nível Superior – Brazil (CAPES), Financial Code – 001, and Fundação de Amparo à Pesquisa do Estado de Bahia (FAPESB), Brazil.

CONFLICT OF INTEREST

The authors declare that they have no conflict of interest.

AUTHORS' CONTRIBUTION

GSA carried out the experiments and collected the data. GOR and GPS assisted in the execution of physicochemical analysis and fermentative tests. SMAS, GBMC, JACD and EAM contributed to the treatment of data, creation of tables and figures. The writing of the manuscript had the collaboration of all authors.

ORCID ID

G. S. Araújo https://orcid.org/0000-0003-1479-1459
G. O. Ribeiro https://orcid.org/0000-0002-1244-8385
S. M. A. de Souza https://orcid.org/0000-0002-3683-9276
G. P. da Silva https://orcid.org/0000-0003-1076-9115
G. B. M. de Carvalho https://orcid.org/0000-0002-6635-9775
J. A. C. Bispo https://orcid.org/0000-0002-7623-9806
E. A. Martínez https://orcid.org/0000-0002-5262-2878

REFERENCES

1. Akalin H, Bayram M, Anli RE. Determination of some individual phenolic compounds and antioxidant capacity of mead produced from different types of honey. J Inst Brew. 2017;123:167-74. https://doi.org/10.1002/jib.396

2. Almeida EL, Silva GM, Vassali IA, Silva MS, Santana WC, da Silva PHA, *et al.* Effects of nitrogen supplementation on *Saccharomyces cerevisiae* JP14 fermentation for mead production. Food Sci Technol. 2020;40:336-43.
https://doi.org/10.1590/fst.11219

3. Araújo GS, Sampaio KF, Bastos TS, Oliveira PP, Carvalho GBM, Souza SMA, Martínez EA. Biochemical, physicochemical and melissopalynological analyses of two multifloral honey types from Brazil and their influence on mead production. J. Apic Res. 2021;60:784-96.

https://doi.org/10.1080/00218839.2020.1828236

4. Araújo GS, Gutiérrez MP, Sampaio KF, de Souza SMA, Rodrigues, RCLB, Martínez EA. Mead production by *Saccharomyces cerevisiae* Safbrew T-58 and *Saccharomyces bayanus* (Premier Blanc and Premier Cuvée): effect of cowpea (*Vigna unguiculata* L. Walp) extract concentration. Appl Biochem Biotech. 2020;191:212-25.
https://doi.org/10.1007/s12010-020-03267-0

5. Erten H, Tanguler H, Cabaroglu T, Canbas A. The Influence of Inoculum Level on Fermentation and Flavour Compounds of White Wines Made from cv. Emir. J Inst Brew. 2006;112:232-36.
https://doi.org/10.1002/j.2050-0416.2006.tb00718.x

6. Navrátil M, Šturdik E, Gemeiner P. Batch and continuous mead production with pectate immobilised, ethanol-tolerant yeast. Biotechnol Lett. 2001;23:977-82.
https://doi.org/10.1023/A:1010571208324

7. Amorim TS, Lopes SB, Bispo JAC, Bonafe CFS, de Carvalho GBM, Martínez EA. Influence of acerola pulp concentration on mead production by *Saccharomyces cerevisiae* AWRI 796. LWT Food Sci Technol.2018;97:561-69.
https://doi.org/10.1016/j.lwt.2018.07.009

8. Mendes-Ferreira A, Mendes-Faia A, Leão C. Growth and fermentation patterns of *Saccharomyces cerevisiae* under different ammonium concentrations and its implications in winemaking industry. J Appl Microbiol. 2004;97:540-45.
https://doi.org/10.1111/j.1365-2672.2004.02331.x

9. Sridee W, Laopaiboon L, Prasit J, Pattana L. The use of dried spent yeast as a low-cost nitrogen supplement in ethanol fermentation from sweet sorghum juice under very high gravity conditions. Electron J Biotechnol. 2011;14:1-15.
https://doi.org/10.2225/vol14-issue6-fulltext-5

10. Martínez EA, Santos JAF. Influence of the use of rice bran extract as a source of nutrients on xylitol production. Ciênc Tecnol Aliment. 2012;32:308-13.
https://doi.org/ 10.1590/S0101-20612012005000036

11. Malekian F, Rao RM, Prinyawiwatkul W, Marshall WE, Windhauser M, Ahmedna M. Lipase and lipoxygenase activity, functionality, and nutrient losses in rice bran during storage. Bulletin of the Louisiana Agricultural Experiment Station. 2000; 870:1-68.

12. Giacometti RA, Teixeira AS, Rodrigues PB, de Freitas RTF, Bertechini AG, Fialho ET, *et al*. Energetic values of brown rice bran supplemented with enzyme complexes for broilers. Ciênc Agrotec. 2003;27:703-07 (in Portuguese).
https://doi.org/10.1590/S1413-70542003000300028

13. USDA - United States Department of Agriculture. National Nutrient Database for Standard Reference Release 18; 2009. Available from: http://www.nal.usda.gov/fnic/foodcomp/Data/SR18/sr18.html.

14. Yin Y, Fatufe AA, Blachier F. Soya bean meal and its extensive use in livestock feeding and nutrition. In: El-Shemy H, editor. Soybean and Nutrition. Rijeka: InTech; 2011. pp.369-84.

15. Instituto Adolfo Lutz (IAL). Physical-chemical methods for food analysis. São Paulo: Institute Adolfo Lutz; 2008.

16. Trevelyan WE, Harrison TS. Dosage of total carbohydrates by the anthrone method. J. Biochem. 1952;50:292 (in Portuguese).

17. Miller GL. Use of de dinitrosalicylic acid reagent for determination of reducing sugar. Anal. Chem. 1959;31:426-28.

18. Zoecklein BW, Fulgelsang KC, Gump BH, Nury FS. Wine production and analysis. Zaragoza: Acribia; 2001 (in Spanish).

19. Official Methods of Analysis (AOAC). USA: AOAC International; 1980.

20. American Society of Brewing Chemists (ASBC). Methods of analysis of American society of brewing chemists. Minnesota: Technical Committee and the Editorial Committe of the ASBC; 1996.

21. Ferreira DF, editor. Sisvar® (Software estatístico): Analysis of variance system for balanced data, version 5.6, Lavras: DEX/UFLA; 2011(in Portuguese).

22. Feddern V, Furlong EB, Soares LAS. Fermentation effects on the physicochemical and nutritional properties of rice bran. Ciênc Tecnol Aliment. 2007;27:800-4 (in Portuguese). https://doi.org/10.1590/S0101-20612007000400020

23. Ginger-Reverdin S, Duvaux-Ponter C, Sauvant D, Martin O, do Prado IN, Müller R. Intrinsic buffering capacity of feedstuffs. Anim Feed Sci Tech. 2002;96:83-102. https://doi.org/10.1016/S0377-8401(01)00330-3

24. Ortoland F, Hecktheuer LH, Miranda MZ. Effect of storage at low temperature (-4 °C) in the color and acidity content of wheat flour. Cienc Tecnol Alimen. 2010;30:55-9. https://doi.org/10.1590/S0101-20612010005000009

25. Garcia MC, Benassi M T, Soares Júnior MS. Physicochemical and sensory profile of rice bran roasted in microwave. Cienc Tecnol Alimen. 2012;32:754-61. https://doi.org/10.1590/S0101-20612012005000097

26. Choct M, Dersjant-Li Y, Mcleish J, Peisker M. Soy oligosaccharides and soluble non-starch polysaccharides: a review of digestion, nutritive and anti-nutritive effects in pigs and poultry. Asian-Australas J Anim Sci. 2010;23:1386-98. https://doi.org/10.5713/ajas.2010.90222

27. Paris LD. Production of fungal enzymes by solid state fermentation of organic, transgenic and conventional soybeans [MSc Thesis]. Paraná: Universidade Estadual do Oeste do Pará; 2008.

28. Bhosale S, Vijayalakshmi D. Processing and nutritional composition of rice bran. Curr Res Nutr Food Sci. 2015;3:74-80.
https://doi.org/10.12944/CRNFSJ.3.1.08

29. Gerber LFP, Júnior AMP, Ribeiro AML. Effect of soybean meal composition on broiler performance and metabolism. Rev Bras Zootec. 2006;35:1359-65 (in Portuguese).
https://doi.org/10.1590/S1516-35982006000500015

30. Iglesias A, Pascoal A, Choupina AB, Carvalho CA, Féas X, Estevinho LM. Developments in the fermentation process and quality improvement strategies for mead production. Molecules. 2014;19:12577- 90.
https://doi.org/10.3390/molecules190812577

31. Sairam S, Krishna AG, Urooj A. Physico-chemical characteristics of defatted rice bran and its utilization in a bakery product. J Food Sci Technol. 2011;48:478-83. https://doi.org/10.1007/s13197-011-0262-y

32. Ghadge VN, Upase BT, Patil PV. Effect of replacing groundnut cake by soybean meal on performance of broilers. Vet World. 2009;5:183-4.
https://doi.org/0.5455/vetworld.2009.183-84

33. Vicentini-Polette CM, Belé JSAHS, Borges MTMR, Spoto MHF, Varruma-Bernardi MR. Physicochemical and sensorial characterization of commercial sugarcane syrups. Rev Bras Ciênc Agrár. 2004;42:808-16.
https://doi.org/10.19084/rca.17279

34. Stehlik-Tomas V, Zetic VG, Stanzer D, Grba S, Vahcic. Zinc, copper and manganese enrichment in yeast *Saccharomyces cerevisiae*. Food Technol Biotechnol. 2004; 42:115-20.

35. Carvalho WT, Reis RC, Velasco P, Júnior MSS, Bassinelo PZ, Caliari M. Physico-chemical characteristics of brown rice, rice and soybean extracts. Pesq. Agropec Trop. 2011;41:422-29 (in Portuguese).

36. Pereira AP, Mendes-Ferreira A, Estevinho LM, Mendes-Faia A. Improvement of mead fermentation by honey-must supplementation. J I Brewing. 2015;121:405-410.
https://doi.org/10.1002/jib.239

37. Schwarz LV, Marcon AR, Delamare APL, Echeverrigaray S. Influence of nitrogen, minerals and vitamins supplementation on honey wine production using response surface methodology. J Apic Res.2020;60.
https://doi.org/10.1080/00218839.2020.1793277

38. Gibson BR. 125th Anniversary Review: Improvement of higher gravity brewery fermentation via wort enrichment and supplementation. J. Inst. Brew. 2011;117:268-84.
https://doi.org/10.1002/j.2050-0416.2011.tb00472.x

39. Silva IL, Pagliarini MA, Chagas TE, Frühauf M, Kempka AP, Dornelles RCP. Effect of supplementation with pollen and brewer's yeast in the fermentation and in the physicochemical properties of honey spirits. Int Food Res J. 2017; 24:1124-34.

40. Kawa-Rygielska J, Adamenko K, Kucharska AZ, Szatkowska K. Fruit and herbal meads – Chemical composition and antioxidant properties. Food Chem. 2019;283:19-27.
https://doi.org/10.1016/j.foodchem.2019.01.040

41. Gomes T, Barradas C, Dias T, Verdial J, Morais JS, Ramalhosa E, Estevinho LM. Optimization of mead production using response surface methodology. Food Chem Toxicol.2013;59: 680-86.
https://doi.org/10.1016/j.fct.2013.06.034

42. Silva SMPC, Carvalho CAL, Sodré GS, Estevinho LM. Production and characterization of mead from the honey of *Melipona scutellaris* stingless bees. J. Inst. Brew.2018;124:194-200.
https://doi.org/10.1002/jib.485

43. BRASIL. Ministry of Agriculture, Livestock and Supply. Normative Instruction No. 34, of November 29, 2012. Approves the technical regulation of identity and quality of fermented beverages: fermented fruit; liqueur fruit fermented; fermented fruit compound; cider; mead; cane fermented; sake or sake. Official Gazette of the Federative Republic of Brazil, Brasília, DF, 23 nov. 2012. Seção 1, p. 3 (in Portguese).

44. Hernández CY, Serrato JC, Quicazan MC. Evaluation of physicochemical and sensory aspects of mead, produced by different nitrogen sources and commercial yeast. Chem Eng Trans. 2015;43:1-6.
https://doi.org/10.3303/CET1543001

45. Mendes-Ferreira A, Cosme F, Barbosa C, Falco V, Inês A, Mendes-Faia A. Optimization of honey-must preparation and alcoholic fermentation by *Saccharomyces cerevisiae* for mead production. Int J Food Microbiol.2010;144:193-8.
https://doi.org/10.1016/j.ijfoodmicro.2010.09.016

46. Felipe ALD, Souza CO, Santos LF, Cestari A. Synthesis and characterization of mead: from the past to the future and development of a new fermentative route. J Food Sci Technol.2019;56:4966-71.
https://doi.org/10.1007/s13197-019-03968-3

47. Adamenko K, Kawa-Rygielska J, Kucharska AZ, Piórecki N. Characteristics of biologically active compounds in Cornelian cherry meads. Molecules. 2018; 23:1–13.
https://doi.org/10.3390/molecules23082024

48. Chitarrini G, Debiasi L, Stuffer M, Ueberegger E, Zehetner E, Jaeger H, Robatscher P, Conterno L. Volatile profile of mead fermenting blossom honey and honeydew honey with or without *Ribes nigrum*. Molecules. 2020;25:1-15.
https://doi.org/10.3390/molecules25081818

49. Švecová B, Bordovská M, Kalvachová D, Hájek T. Analysis of Czech meads: sugar content, organic acids content and selected phenolic compounds content. J Food Compost Anal. 2015;38:80-8.
https://doi.org/10.1016/j.jfca.2014.11.002

50. Sroka P, Satora P. The influence of hydrocolloids on mead wort fermentation. Food Hydrocolloids. 2017;63:233-39.
https://doi.org/10.1016/j.foodhyd.2016.08.044

List of tables

Table 1. Physicochemical characteristics of rice bran and soybean meal extracts

Table 2. Consumption of glucose and fructose and final concentration of ethanol (g/L) in the fermentation of honey must with 30 g/L of rice bran and soybean meal extracts, commercial supplement and without supplements using S*accharomyces cerevisiae* Montrachet (*Sc*M), S*accharomyces cerevisiae* Safbrew T-58 (*Sc*ST58) e *Saccharomyces bayanus* Premier Blanc (*Sb*PB)

List of Figures

Fig. 1. Cell concentration profile (cells/mL) during fermentation of honey must supplemented with 30 g/L of soybean meal extract at (circles), bran extract (squares), commercial supplements (upside triangle) and without supplements (upside down triangle) using commercial yeast: a) *Saccharomyces bayanus* Premier Blanc, b) S*accharomyces cerevisiae* Montrachet and c) S*accharomyces cerevisiae* Safbrew T-58

Fig. 2. Profile of sugars (glucose and fructose) and ethanol concentrations, during the fermentation process of honey must supplemented with 30 g/L of rice bran and soybean meal extracts, commercial supplement and without supplements using commercial yeast: a) *Saccharomyces bayanus* Premier Blanc, b) S*accharomyces cerevisiae* Montrachet and c) S*accharomyces cerevisiae* Safbrew T-58

Fig. 3. Profile of glycerol and organic acids (acetic, formic, lactic, acetic e succinic) during the fermentation process of honey must supplemented with 30 g/L of rice bran and soybean meal

extracts, commercial supplements and without supplements using commercial yeast: a) *Saccharomyces bayanus* Premier Blanc, b) *Saccharomyces cerevisiae* Montrachet and c) *Saccharomyces cerevisiae* Safbrew T-58

Table 1

Parameters	Rice bran extract	Soybean meal extract
pH	(6.10 ± 0.00)^{b}	(6.50 ± 0.00)^{a}
TTA/%	(2.21 ± 0.12)^{b}	(1.43 ± 0.25)^{a}
TTS (^{o}Brix)	(1.60 ± 0.06)^{b}	(6.70^{b} ± 0.00)^{a}
w(reducing sugars)/%	(0.11 ± 0.00)^{a}	(0.12 ± 0.00)^{a}
w(total carbohydrates)/%	(1.60 ± 0.03)^{b}	(6.52 ± 0.05)^{a}
w(Proteins)/%	(0.42 ± 0.03)^{b}	(1.70 ± 0.07)^{a}
γ(assimilable nitrogen (mg/L)	(16.86 ± 1.62)^{b}	(69.10 ± 0.06)^{a}
w(ash)/%	(0.23 ± 0.01)^{b}	(0.72 ± 0.01)^{a}
w(minerals) (mg/100g)		
Calcium	(2.83 ± 0.00)^{b}	(10.95 ± 0.15)^{b}
Magnesium	(14.78 ± 0.09)^{b}	(27.15 ± 0.21)^{a}
Zinc	(0.14 ± 0.03)^{b}	(0.30 ± 0.01)^{a}
Iron	(0.88 ± 0.02)^{b}	(2.40 ± 0.01)^{a}
Sodium	(5.40 ± 0.03)^{b}	(7.50 ± 0.12)^{a}
Potassium	(137.70 ± 2.21)^{b}	(505.50 ± 9.86)^{a}
Phosphor	(2.23 ± 0.21)^{b}	(18.50 ± 0.71)^{a}
Manganese	n.d	n.d

Note: n.d (not detected), TTA: total titratable acidity, TTS: total soluble solids

Table 2

Yeasts	Supplements	*w* (consumed glucose) / %	*w* (consumed fructose) / %	*γ* (ethanol)/ g/L
*Sb*PB	SME	(98.6 ± 1.20)^{a}	(93.2 ± 1.06)^{a}	(118.1 ± 2.71)^{a}
	RBE	(97.1 ± 2.01)^{a}	(92.0 ± 1.23)^{a}	(115.0 ± 2.23)^{a}
	CS	(97.7 ± 2.12)^{a}	(92.3 ± 0.98)^{a}	(116.3 ± 1.95)^{a}
	Control	(91.9 ± 0.65)^{b}	(87.5 ± 0.55)^{c}	(94.1 ± 2.24)^{c}
*Sc*M	SME	(97.1 ± 0.85)^{a}	(91.6 ± 1.34)^{a}	(112.7 ± 4.85)^{a}
	RBE	(96.0 ± 1.84)^{a}	(89.5 ± 0.87)^{a}	(109.0 ± 2.34)^{a}
	CS	(96.5 ± 1.78)^{a}	(90.4 ± 1.21)^{a}	(110.3 ± 3.65)^{a}
	Control	(90.3 ± 0.51)^{b}	(84.0 ± 1.56)^{b}	(90.3 ± 1.32)^{b}
*Sc*ST58	SME	(89.1 ± 0.94)^{a}	(84.8 ± 1.00)^{a}	(98.6 ± 2.05)^{a}
	RBE	(87.4 ± 0.98)^{a}	(82.7 ± 1.17)^{a}	(96.8 ± 1.98)^{a}
	CS	(88.0 ± 1.93)^{a}	(83.5 ± 2.10)^{a}	(98.4 ± 1.05)^{a}
	Control	(83.6 ± 1.02)^{b}	(74.6 ± 1.07)^{b}	(76.1 ± 1.00)^{b}

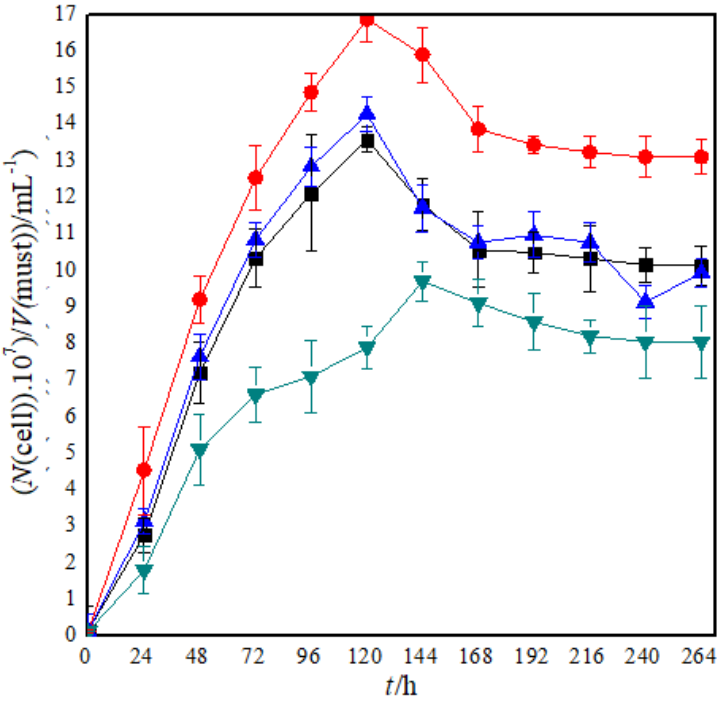

Fig 1a

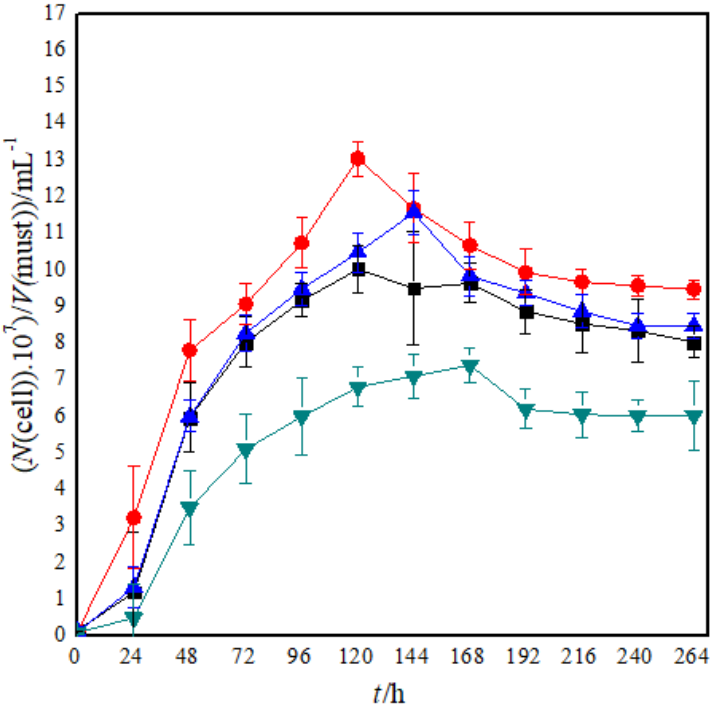

Fig 1b

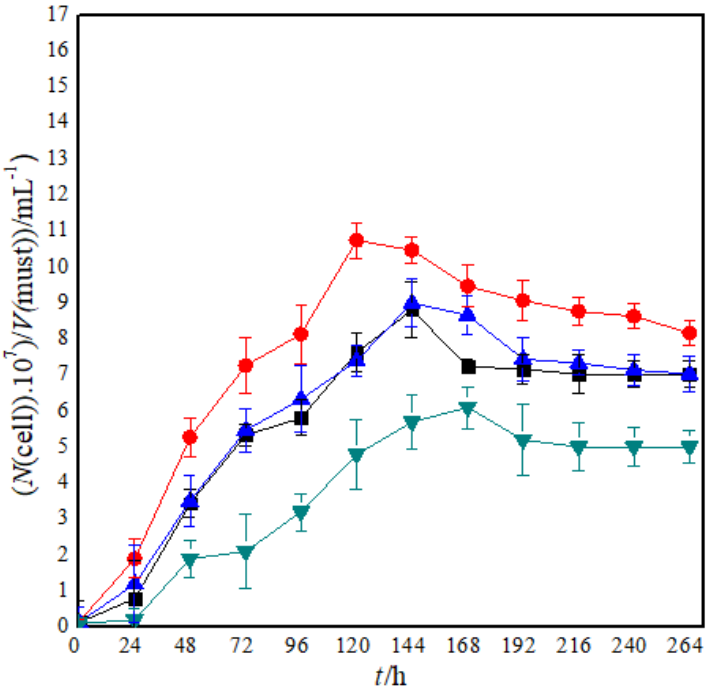

Fig 1c

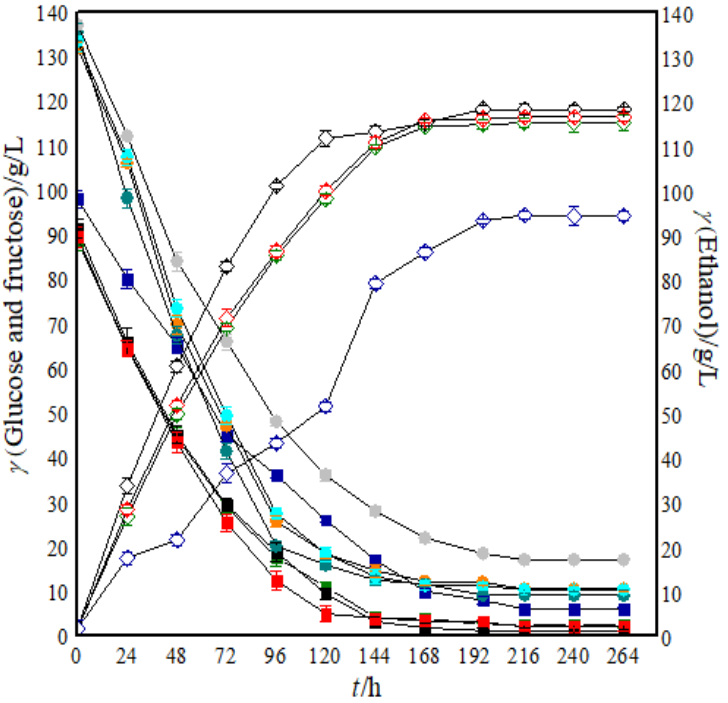

Fig 2a

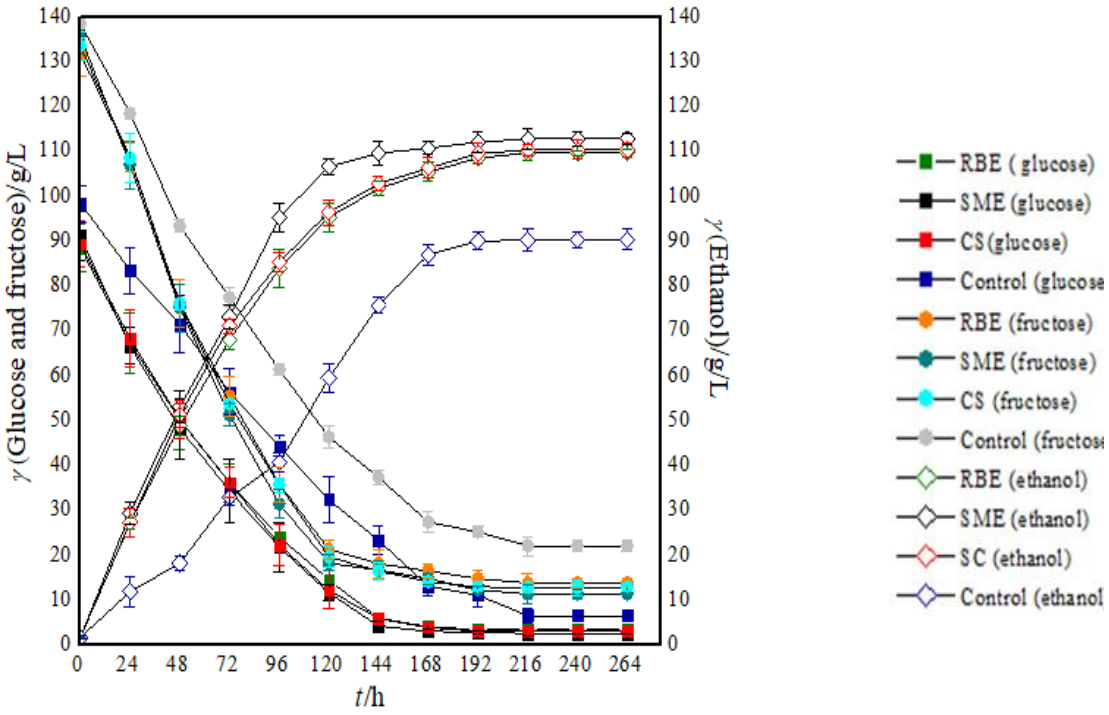

Fig 2b

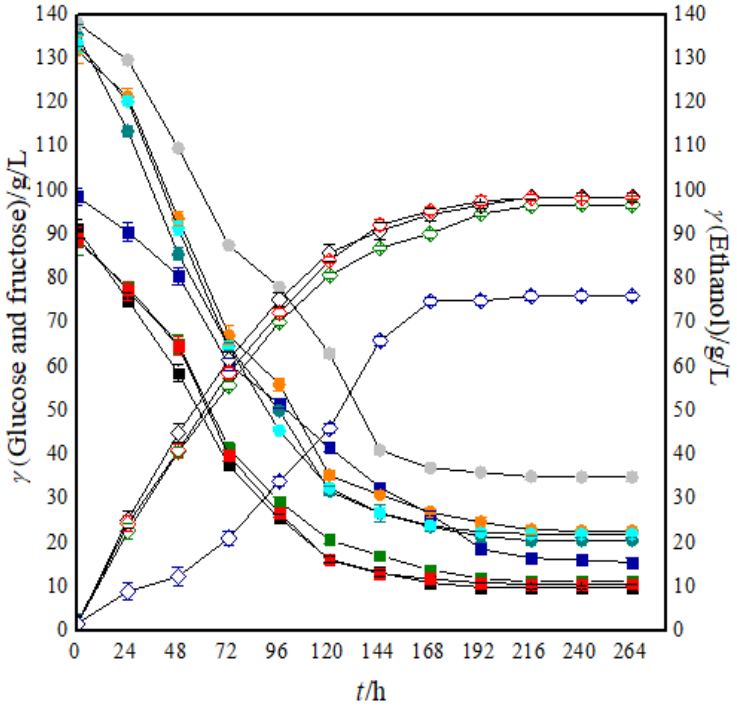

Fig 2c

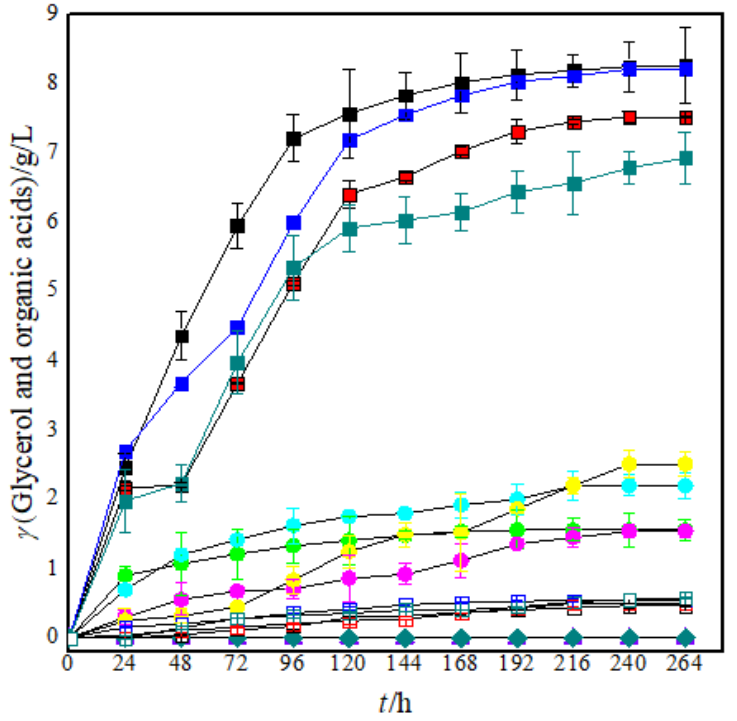

Fig 3a

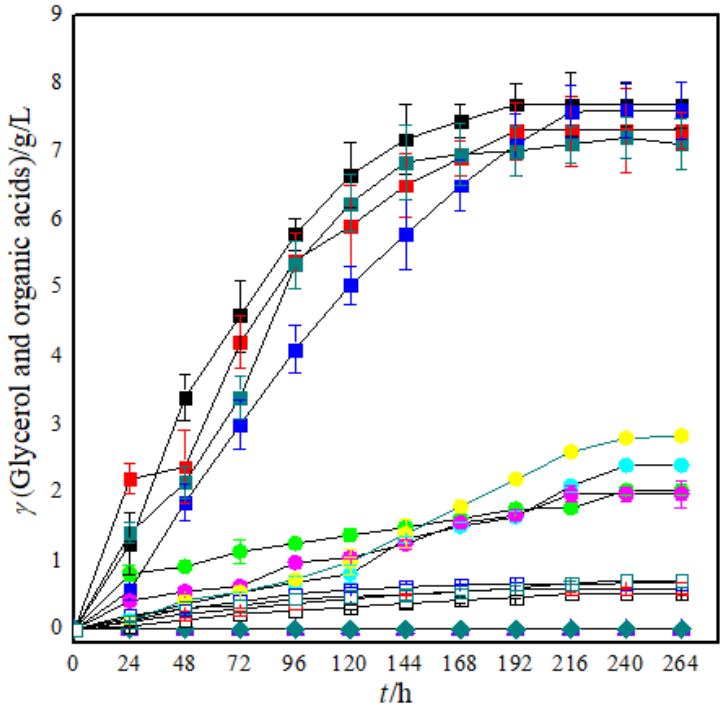

Fig 3b

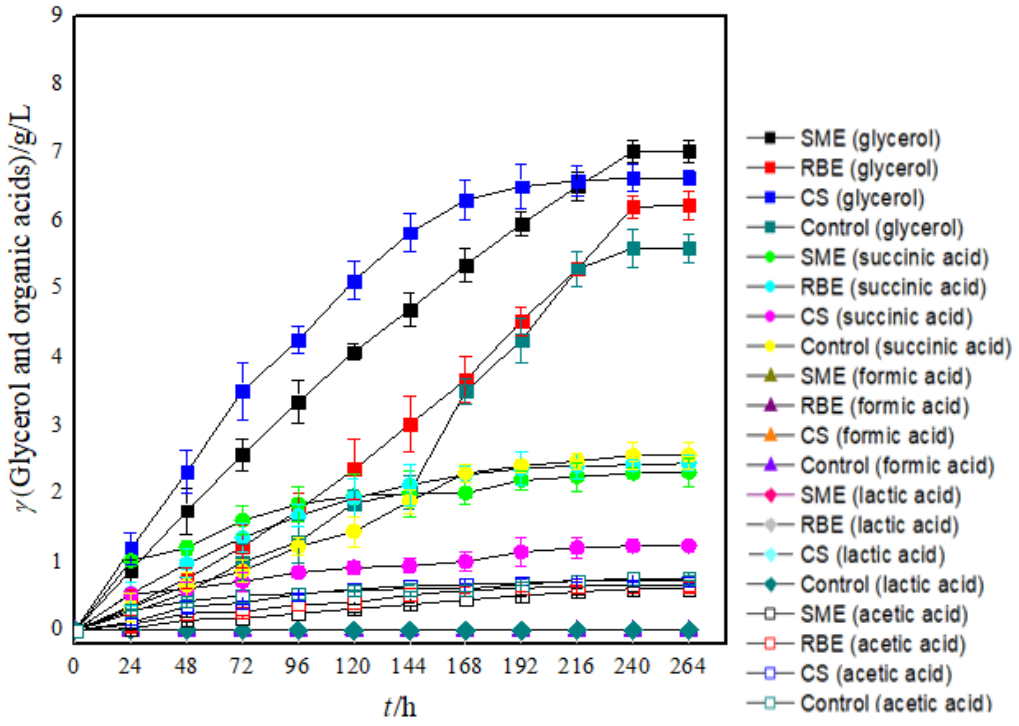

Fig 3c