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Acetic Acid Fermentation of Soybean Molasses and Characterisation of the Produced Vinegar

Running title: Parameters of Vinegar Production from Soybean Molasses

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SUMMARY

Soybean molasses is a by-product from the production of protein-concentrate soybean meals that predominantly contain sugars, with sucrose as the major component. In Brazil, soybean molasses is used for animal feed or it is discarded, although a few industries use it to produce ethanol. This study aimed to evaluate the parameters required for the acetic acid fermentation of soybean molasses, and characterise the resultant vinegar generated. To study the most suitable parameters for the acetic acid fermentation, vinegar was produced from the alcoholic fermentation of soybean molasses through eight fermentation cycles: five for adaptation and three for production. The average acidity of the acetic acid fermentation product was 50.60 g/L, with an acetic acid yield, total concentration yield and productivity of 65.01 %, 92.76 % and 0.033 g/(L·h), respectively. The vinegar produced from soybean molasses presented an acidity of 5.07 % (m/V), residual ethanol content of 0.17 % (m/V), 7.86 % (m/V) sugars, 14.67 % (m/V) dry extract, 2.27 % (m/V) ash, and a density of 1.023 g/cm³. The contents of total phenolics and isoflavones decreased after the alcoholic and acetic acid fermentations. Moreover, the isoflavones profile of the fermented product comprised only three forms: daidzein, glicitin and genistin. According to our results, 3460 L of vinegar can be produced for every tonne of soy molasses, with an acetic acid content of 40 g/L, the minimum required by
the legislation on vinegar production. Thus, these findings demonstrate that soy molasses represents a useful raw starting material for the production of vinegar.

**Key words:** vinegar, acetic acid fermentation, soybean molasses, by-product

**INTRODUCTION**

Soybean molasses is a viscous, brown and bittersweet liquid, obtained from the processing of soy protein concentrates. The soy proteins are concentrated from defatted soybean meals by ethanol washing, a procedure that promotes the extraction of soluble carbohydrates and other water-soluble compounds. Sugars are then concentrated via the evaporation of ethanol and the partial evaporation of water, resulting in the production of soybean molasses (1). Sucrose, raffinose and stachyose are the main sugars present in the molasses, which also contain proteins, lipids, minerals and isoflavones (1,2). A large portion of this by-product is used for animal feed or discarded. In Brazil, however, some soybean processing industries use soybean molasses to produce hydrated alcohol, because sucrose is the predominant sugar and it can be fully metabolized by the yeasts *Saccharomyces cerevisiae*. In contrast, raffinose and stachyose cannot be metabolized by *S. cerevisiae*, as this yeast lacks the α-galactosidase enzyme necessary to hydrolyse the α-1,6-glycosidic bonds comprising these sugars (1,3). The isoflavones are the main phenolic compounds present in soybean and its products. These compounds are distributed in aglycone forms (daidzein, glycine and genistein) and their respective β-glucoside (daidzin, genistin and glycitin), acetyl-glucoside (6"-O-acetyl-daidzin, 6"-O-acetyl-genistin and 6"-O-acetyl-glycitin) and malonyl-glucoside conjugates (6"-O-malonyl-daidzin, 6"-O-malonyl-genistin and 6"-O-malonyl-glycitin), totalling twelve different forms (4). According to Handa et al. (5), the glycosidic forms are predominant in soybeans, constituting 50–90 % of the total isoflavones. It is reported that these isoflavones, mainly the aglycones, have biological activity. Moreover, they have attracted attention because of their ability to reduce the risk of cardiovascular diseases, inhibit cancer cell growth, prevent diseases, such as osteoporosis, and alleviate the symptoms of menopause (5).

Vinegar is a food condiment produced by a double fermentation: an initial alcoholic fermentation, followed by an acetic acid fermentation, which can be performed from amylaceous or sugary starting materials (6–9). In biochemical terms, acetification is an oxidation process carried out by acetic acid bacteria (AAB). However, the process of vinegar production is commonly referred to as a fermentative process because it involves three main processes: slow, fast and submerged (8,10). The submerged process of fermentation consists
of a controlled agitation and aeration of the fermentative must in which the obligate aerobic bacteria oxidise the ethanol into acetaldehyde and then into acetic acid.

The process of acetic acid fermentation involves several bacterial genera, such as *Acetobacter*, *Gluconobacter*, *Gluconacetobacter* and *Komagataeibacter* (10,11). In an ad-hoc-designed fermenter, the combination of agitation and aeration promotes the formation of micro-bubbles of air that facilitate the diffusion of oxygen, which increases the contact surface between bacteria and substrate, in turn, boosting the efficiency and productivity of the fermentation. The absence of aeration for even 1 min during this process can result in eight consecutive non-productive days of fermentation (12).

In the production of vinegar with a high content of acetic acid, the fermentation is typically a semi-continuous process, which operates in cycles. This process consists of starting the cycle with a total concentration (TC) ranging from 12 % to 15 % (7–10 % acetic acid and 5 % ethanol). When the ethanol concentration is between 0.2 % and 0.3 %, a relative volume of fermented broth is withdrawn, and a new volume is added to the medium (13).

In order to expand the use of soybean molasses, this work aimed to identify the best parameters to be used during the cycles of acetic acid fermentation of soybean molasses by the submerged semi-continuous process and, secondly, to characterise the vinegar obtained.

MATERIALS AND METHODS

*Material*

The ethanol from soybean molasses used for the production of vinegar was produced, according to Caldeirão et al. (1). It had an alcohol content of 60.00 mL/L (47.40 g/L) and acidity of 7.40 g/L (acetic acid). Isoflavone standards were obtained from Wako Pure Chemical Industries, Ltd. (Osaka, Japan) and Sigma–Aldrich Co. (St. Louis, MO, USA). All other reagents were of analytical grade and sourced commercially from various manufacturers.

*Inoculum activation and preparation*

As also occurs in industrial processes, pure cultures of AAB were not used for the production of vinegar in this work. The reason is that the environmental conditions, such as low pH, presence of ethanol, aeration and temperature, naturally select the best producer species. The mixed culture of AAB was obtained during the production of unfiltered white vinegar, according to Spinosa (14) with modifications. This material was quickly collected from the German fermenter of a vinegar-producing industry (Tecnologia em Saúde, Assis, Brazil). This fermenter had a 300-L total capacity and operated at 30 °C, with 1.0 % (V/V) ethanol and 4.0 % (m/V) total titratable acidity. To obtain the initial inoculum, 50 mL of white vinegar was added to a
flask containing 250 mL of mannitol–yeast (Himedia, Mumbai, India) broth and placed in a shaking incubator (Cientec CT-712 R, Belo Horizonte, Brazil) at 30 °C for 72 h. This initial inoculum was transferred to a bioreactor holding vessel containing 4 L of a broth prepared with a mixture of 2 L of unfiltered white vinegar containing 10.00 % acetic acid (m/V) and 0.4 L ethanol with 50.0 % alcohol (V/V), which contained a bacterial count equal to or greater than 8 log CFU/mL.

**Acetic acid fermentation**

The bioreactor (6 L total volume) used for soybean molasses vinegar production in submerged fermentation at the pilot scale was obtained from Rubia Basic (Biofoco, Piracicaba, Brazil). The conditions of the fermentation process in the bioreactor were 30 ± 0.5 ºC, 800 rpm agitation, and aeration flow of 0.50 L/min and 0.25 V/(V·min) (air volume per volume of culture medium per minute). During the process, the total acidity, alcohol content and aeration were managed, as the acidity and alcohol content are the determining parameters for loading and unloading the fermentation cycles. The TC of the initial broth was defined as the sum of the concentrations of ethanol (%, V/V) and acetic acid (%, m/V). Therefore, the TC of the initial broth was 10.00 % (5.00 %, V/V ethanol plus 5.00 %, m/V acetic acid). The cycles of submerged acetic acid fermentation process were conducted as a fed-batch and each one of these cycles is considered as a replicate of the experiment. When the alcohol content approached 0.5 %, a fraction of the vinegar volume was withdrawn from the reactor and the same volume of ethanol from soybean molasses was added.

**Parameters for the cycles of acetic acid fermentation of ethanol from soybean molasses**

After the conditions had stabilised, 0.680 L ethanol from soybean molasses (60.00 mL/L ethanol), previously centrifuged at 12,000 ×g, 4 °C for 15 min, was added to the bioreactor. Hence, the initial concentration of ethanol reached 15.20 g/L. Once the fermentation cycles had started, the vinegar collection was carried out when the ethanol concentration in the vinegar reached a content lower or equal to 0.50 % (V/V). In order to maintain the TC of the broth, an adequate volume of the ethanol had to be replaced. To evaluate the most suitable parameters for the acetic acid fermentation of ethanol from soybean molasses, eight fermentation cycles were performed. The first five cycles were performed to facilitate culture adaptation, whereas, during the last three cycles, the reactor only contained the acetic acid and the alcoholic-fermented soybean molasses. For each cycle, the following parameters were determined: Y_{AA} (acetic acid fermentation yield expressed as acetic acidity), TC (total concentration of the broth expressed as the percentage of acetic acid), Y_{TC} (total
concentration yield of the broth) and $P_{AA}$ (productivity of acetic acid). These parameters were calculated using the following expressions, respectively:

\[
Y_{AA} = \gamma_f \cdot 0.77 \cdot 100 / V_R \quad /1/
\]

\[
Y_{TC} = TC_f \cdot 100 / TC_i \quad /2/
\]

\[
P_{AA} = (V_i \cdot \gamma_f / t \cdot V_R) \cdot 10 \quad /3/
\]

where $\gamma_f$ is the final acetic acid content (g(acetic acid)/100 mL), 0.77 is the stoichiometric yield for conversion of ethanol to acetic acid, $V_R$ is the volume of the reactor, $TC_f$ is the total concentration (%) of the broth at the end of the cycle, $TC_i$ is the total concentration (%) of the broth at the beginning of the cycle, $V_i$ is the volume (L) of vinegar removed and $t$ is the time (h) of a cycle.

**Characterisation of soybean molasses vinegar**

The soybean molasses vinegar produced was distilled using an alcohol micro-distiller (TE-012, Tecnal, Piracicaba, Brazil), and the dry extract and ash contents were determined gravimetrically using an oven (Odontobrás EL 1.5, Brazil) at 105 °C and a muffle (Quimis, Diadema, Brazil) at 550 °C, respectively. The acidity of the distilled vinegar was determined by titration with 0.1 M NaOH (Química Moderna, Barueri, Brazil), using 1.0 % phenolphthalein (Inlab, Brazil) as an indicator, and it was expressed as grams(acetic acid)/100 mL (solution). The ethanol content and relative density of the distilled vinegar were determined using a digital densimeter (Rudolph Research Analytical DDM 2909, USA) at 20 °C.

The total sugars content of the vinegar was determined, according to DuBois et al. (15), using glucose (Synth, Diadema, Brazil) as a standard. The total phenolic content was determined, as described by Singleton et al. (16), and the results were expressed as milligrams of gallic acid equivalents/100 mL. To separate the different isoflavone forms present in the vinegar, we used a 1:1:1 volume ratio of ultrapure water (Merck Millipore, EUA), acetone (Anidrol, Diadema, Brazil) and ethanol (Anidrol, Diadema, Brazil). The extracts were homogenized every 15 min for 1 h on a tube shaker, followed by sonication for 15 min, and centrifugation at 794 x g for 15 min at 4 °C. The supernatants were filtered with a 0.22 μm PVDF membrane (Millipore Millex, Ireland) and analyzed by ultra-performance liquid chromatography (Acquity UPLC System, Waters, USA), as described by Handa et al. (5).
Cell count
The AAB used in the soybean molasses vinegar process were quantified by plate counting after allowing them to grow on a double layer of mannitol, yeast and peptone agar (Himedia, Mumbai, India) (0.500 % and 1.00 % agar in the lower and upper layer, respectively) at 30 °C for 48 h. The counts (CFU/mL) were expressed as described by Spinosa (14).

Statistical analysis
Three independent assays were performed, and the average values determined. Data are expressed as mean value ± standard deviation using the statistical software R (17).

RESULTS AND DISCUSSION
Evaluation of the parameters used for the acetic acid fermentation of ethanol from soybean molasses
The conversion rate of acetic acid (Table 1 and Fig. 1) decreased with the addition of the alcoholic fermentation product from soybean molasses into the fermenter. The same was also observed for the productivity ($P_{AA}$), which decreased considerably in the fermentation time after the fourth cycle, starting with 0.106 g/(L·h) in the first cycle and decreasing to 0.023 g/(L·h) in the final cycle. This decrease can be related to the fact that soybean molasses ethanol contained considerable concentrations of residual sugars (Table 2). In some AAB species, two main metabolic pathways are present: the pentose phosphate cycle for carbohydrate oxidation and the tricarboxylic acid cycle for the oxidation of organic acids (18). Due to the considerable concentration of sugars in soybean molasses ethanol, a change in the metabolic pathway by the AAB might have occurred, which affected the productivity results. In this case, the microbiota could have prioritised the carbohydrate metabolism pathway, producing less acetic acid (product of ethanol metabolism) and more carbohydrate metabolism products, such as gluconic acid or exopolysaccharides (cellulose, levan, dextran or acetan) (19). In the conversion of ethanol into acetic acid, various intermediate compounds, including acetaldehyde, ethyl acetate and other alcohols, esters and acids, are also formed. The nature and the quantity produced depend on the characteristics of the raw material (wine) and these compounds are responsible for the flavour of the vinegar (14).

The average time required for the acetification of soybean molasses (mean of the last three cycles) was 73 h. Among these three cycles (which only contained the fermented broth ethanol from soybean molasses), the shortest fermentation time was 50 h (sixth cycle) and the longest was 91 h (seventh cycle). The highest acidity level and $Y_{AA}$ (52.0 g/L and 66.7 %, respectively) were reached after 77 h (eighth cycle). Spinosa et al. (8) applied a submerged method for the
production of vinegar from rice wine and evaluated 10 fermentation cycles. During this process, the wine from alcoholic fermentation contained 62.80 g/L ethanol, which produced a vinegar with an acetic acid content of 65.80 g/L, 86.00 % \( Y_{AA} \), and a 0.70 g/L yield. In another work that aimed to obtain a vinegar from banana pulp, Tanaka et al. (20) recorded a maximal concentration of acetic acid ranging from 59.0 to 40.0 g/L, with an average of 49.7 g/L. At the end of our fermentation, the acidity, \( Y_{AA} \) and productivity were 50.70 g/L, 65.02 % and 0.033 g/(L·h), respectively, on average, over the last three cycles.

The acetic acid production by the oxidation of ethanol is an exothermic reaction that requires oxygen. Consequently, the fermentation efficiency is highly related to the aeration and the air dispersion rates inside the reactor. The low productivity (\( P_{AA} \)) observed in this work may also be associated with the non-homogeneous air distribution inside the fermenter. The same occurrence was observed by other authors (8,12). Another problem that can cause a low acetic acid yield is the evaporation of ethanol. This loss occurs during the fermentation process and can reach up to 10–30 % of the stoichiometric yield, depending on the temperature. In a study of open, semi-open and closed acetic acid fermentation, it was concluded that closed acetic acid fermentation is more appropriate for industry because it presents the loss by evaporation is less (14).

Table 1

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Acidity</td>
<td>50.70 g/L</td>
</tr>
<tr>
<td>( Y_{AA} )</td>
<td>65.02 %</td>
</tr>
<tr>
<td>Productivity</td>
<td>0.033 g/(L·h)</td>
</tr>
</tbody>
</table>

Table 2

<table>
<thead>
<tr>
<th>Alcohol Fermentation Products</th>
<th>Characteristics</th>
</tr>
</thead>
<tbody>
<tr>
<td>Soybean molasses</td>
<td>5.07 % (m/V) acetic acid, 0.17 % (m/V) residual ethanol, 7.86 % (m/V) dry extract, 2.27 % (m/V) ash, and had a density of 1.023 g/cm³.</td>
</tr>
</tbody>
</table>
(ethanol) and acetic acid (vinegar) fermented products obtained from soybean molasses (Table 3). High concentrations of total phenolic compounds were observed in soybean molasses, ethanol and vinegar that were 1.52-, 61.0- and 53.9-fold higher than the total isoflavones content, respectively. With respect to soybean molasses, the alcoholic (ethanol) and acetic acid (vinegar) fermentation products exhibited a decrease in total phenolic content by 3.49 % and 35.69 %, respectively. However, when the alcoholic fermentation was extended into the acetic acid fermentation, the total phenolic content decreased to 37.86 %. The high concentration of total phenolic compounds of ethanol and vinegars derived from soybean molasses might have been caused by the retention of some of these compounds during the fermentation process. Specifically, for the alcoholic fermentation portion, new compounds could have been generated as a result of the presence of microorganisms (21).

Table 3

The total isoflavones content of soybean molasses (Table 3) was high, whereas, during the alcoholic and the acetic acid fermentation processes, the content decreased to 38.8 % and 55.64 %, respectively. The total isoflavone content decreased only 1.42 times as the alcoholic fermentation progressed into the acetic acid fermentation. In soybean molasses, the predominant forms of isoflavones were β-glycosides, aglycones and malonylglycosides at 83.01 %, 11.55 % and 5.43 % of the total, respectively. No acetylglycosides were detected. This result is interesting because these values report a very different isoflavone profile compared with soybeans grain. Moreover, the absence of acetylglycoside isoflavones indicates that soybean molasses had not undergone any high thermal treatment for its production.

The predominant isoflavone forms of the 18 soybean cultivars investigated by Ribeiro et al. (22) were malonyldaidzin and malonylgenistin and together accounted for 67.0 % of the total isoflavones content. For the same study, β-glycosides and aglycones corresponded to 31.0 % and 2.0 % of total isoflavones, respectively, whereas the acetylated form was not detected. When heat-treated in an oven at 200 °C for 20 min, whole soybean meal showed an altered isoflavones content and a high conversion rate of malonylglycosides to acetylglycosides, β-glycosides and aglycones (23). Malonylgenistin has been described as the predominant isoflavone in soybean, and it can be converted to acetylgenestin or genistin after a drying or hot-water extraction process, respectively. Humid heat is more effective than dry heat to convert and degrade the isoflavones mentioned above (24). The contents of 6′′-O-malonyldaidzin and 6′′-O-malonyl-genistin were low in soybean molasses (Table 3), and after the
alcoholic and acetic acid fermentation process, these two forms were no longer detectable. Moreover, no β-glycosides (daidzin) or aglycones (genistein) could be detected in the ethanol and vinegar produced during our study. Instead, β-glycosides (genistin and glycitin) and aglycones (daidzein) were the only forms of isoflavones present in both fermented products, at low concentrations. The low amounts or lack of detection of isoflavones in ethanol and vinegar was probably caused by the presence of yeasts, which may utilise certain cell wall phenolic compounds, as observed by Razmkhab et al. (25). The defatted soybean meal fermented in the presence of Monascus purpureus or Aspergillus oryzae at an adequate water:ethanol ratio was considered the ideal method to simultaneously extract phenolic compounds and isoflavones with high antioxidant activities (26).

There is a growing interest among consumers and industries for the production of antioxidant-rich soy products (27). In this study, we obtained alcoholic and acetic acid fermented products from soybean molasses with a unique chemical composition of total phenolic compounds and isoflavones. In particular, the presence of aglycones (daidzein) in our products is especially remarkable, as these compounds are deemed highly beneficial to human health.

CONCLUSION

The current study demonstrated that soybean molasses could be used to produce vinegar. Moreover, based on our results, each tonne of soybean molasses can produce an estimated 3460 L of vinegar, with an acetic acid concentration of 40 g/L, which is equivalent to 139 kg of acetic acid and satisfies the minimum concentration required by the legislation on vinegar production.

Additional studies should be carried out to improve the fermentation conditions and thereby increase productivity, while simultaneously reducing the extent of the acetic acid fermentation. Furthermore, for soybean molasses to be proposed for human consumption, a profile of the anti-nutritional factors and some sensorial studies still need to be performed.

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CONFLICT OF INTEREST

The authors have no conflicts of interest to declare.

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**Table 1.** Cycles and parameters of acetic fermentation of soybean molasses

<table>
<thead>
<tr>
<th>Cycles</th>
<th>$t$ (h)</th>
<th>$V_B$/L</th>
<th>$\phi_i$ (g/L)</th>
<th>$\gamma_i$ (%)</th>
<th>TC$_i$ (g/L)</th>
<th>$V_A$/L</th>
<th>V$_R$/L</th>
<th>$\gamma_f$ (%)</th>
<th>TC$_f$ (g/L)</th>
<th>$Y_{AA}$ (%)</th>
<th>$Y_{TC}$ (%)</th>
<th>$P_{AA}$ (g/L·h)</th>
</tr>
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<tbody>
<tr>
<td>1</td>
<td>49</td>
<td>4.30</td>
<td>15.20</td>
<td>56.20</td>
<td>7.14</td>
<td>0.68</td>
<td>0.50</td>
<td>62.40</td>
<td>3.00</td>
<td>6.54</td>
<td>80.08</td>
<td>91.60</td>
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<tr>
<td>2</td>
<td>48</td>
<td>4.18</td>
<td>14.80</td>
<td>53.10</td>
<td>6.79</td>
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<td>0.50</td>
<td>58.00</td>
<td>5.00</td>
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<td>74.43</td>
<td>92.78</td>
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<td>3</td>
<td>48</td>
<td>3.98</td>
<td>15.00</td>
<td>55.60</td>
<td>7.06</td>
<td>0.50</td>
<td>0.50</td>
<td>59.00</td>
<td>5.00</td>
<td>6.40</td>
<td>74.72</td>
<td>90.65</td>
</tr>
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<td>4</td>
<td>54</td>
<td>3.88</td>
<td>14.10</td>
<td>50.60</td>
<td>6.47</td>
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<td>0.50</td>
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<td>2.00</td>
<td>6.00</td>
<td>73.43</td>
<td>92.74</td>
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<tr>
<td>5</td>
<td>110</td>
<td>4.43</td>
<td>11.60</td>
<td>50.00</td>
<td>6.16</td>
<td>0.80</td>
<td>0.80</td>
<td>55.00</td>
<td>3.00</td>
<td>5.80</td>
<td>70.58</td>
<td>94.16</td>
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<td>6</td>
<td>50</td>
<td>4.40</td>
<td>17.40</td>
<td>42.60</td>
<td>6.00</td>
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<td>0.30</td>
<td>51.00</td>
<td>7.00</td>
<td>5.80</td>
<td>65.45</td>
<td>96.67</td>
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<tr>
<td>7</td>
<td>91</td>
<td>4.30</td>
<td>18.60</td>
<td>45.00</td>
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<td>0.30</td>
<td>49.00</td>
<td>4.08</td>
<td>5.38</td>
<td>62.88</td>
<td>84.59</td>
</tr>
<tr>
<td>8</td>
<td>77</td>
<td>3.80</td>
<td>11.80</td>
<td>43.50</td>
<td>5.53</td>
<td>0.20</td>
<td>0.20</td>
<td>52.00</td>
<td>5.00</td>
<td>5.70</td>
<td>66.73</td>
<td>97.92</td>
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<tr>
<td>Average*</td>
<td>73</td>
<td>4.17</td>
<td>15.90</td>
<td>43.70</td>
<td>5.96</td>
<td>-</td>
<td>-</td>
<td>50.70</td>
<td>5.60</td>
<td>5.63</td>
<td>65.02</td>
<td>92.76</td>
</tr>
</tbody>
</table>

$t$=fermentation time (h); $V_B$=volume of the bioreactor (L); $\phi_i$=initial ethanol content; $\gamma_i$=initial acidity; TC$_i$=initial total concentration; $V_A$=volume of alcoholic fermented added (L); $V_R$=volume of acetic acid removed (L); $\gamma_f$=final acidity; $\phi_f$=final ethanol content; TC$_f$=final total concentration; $Y_{AA}$=yield(acetic acid); $Y_{TC}$=yield(total concentration); $P_{AA}$=productivity.

*average of the last three cycles.
Table 2. Physico-chemical characterisation of the soybean molasses vinegar

<table>
<thead>
<tr>
<th>Component</th>
<th>% (g/100 mL)</th>
</tr>
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<tbody>
<tr>
<td>Acidity</td>
<td>5.07 ± 0.06</td>
</tr>
<tr>
<td>Residual ethanol</td>
<td>0.34 ± 0.02</td>
</tr>
<tr>
<td>Residual sugar</td>
<td>7.86 ± 0.12</td>
</tr>
<tr>
<td>Dry extract</td>
<td>14.67 ± 0.07</td>
</tr>
<tr>
<td>Ash</td>
<td>2.27 ± 0.10</td>
</tr>
<tr>
<td>Relative density*</td>
<td>1.023 ± 0.00</td>
</tr>
</tbody>
</table>

*in g/cm³

Table 3. Contents of total phenolics* and isoflavones** in soybean molasses, wine and vinegar

<table>
<thead>
<tr>
<th></th>
<th>Soybean molasses</th>
<th>Wine (alcoholic fermented)</th>
<th>Vinegar (acetic fermented)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Total phenolics</td>
<td>625.51 ± 59.29</td>
<td>647.39 ± 10.63</td>
<td>402.24 ± 4.88</td>
</tr>
<tr>
<td>6‴-O-Malonyl-daidzin</td>
<td>11.95 ± 0.55</td>
<td>nd</td>
<td>nd</td>
</tr>
<tr>
<td>6‴-O-Malonyl-genistin</td>
<td>10.43 ± 0.29</td>
<td>nd</td>
<td>nd</td>
</tr>
<tr>
<td>Daidzin</td>
<td>125.83 ± 2.05</td>
<td>nd</td>
<td>nd</td>
</tr>
<tr>
<td>Genistin</td>
<td>186.24 ± 9.31</td>
<td>3.77 ± 0.43</td>
<td>2.98 ± 0.18</td>
</tr>
<tr>
<td>Glycitin</td>
<td>29.76 ± 0.97</td>
<td>4.59 ± 1.02</td>
<td>3.18 ± 0.22</td>
</tr>
<tr>
<td>Daidzein</td>
<td>22.37 ± 1.11</td>
<td>2.17 ± 0.40</td>
<td>1.29 ± 0.06</td>
</tr>
<tr>
<td>Genistein</td>
<td>25.19 ± 0.63</td>
<td>nd</td>
<td>nd</td>
</tr>
<tr>
<td>Total isoflavones</td>
<td>411.77 ± 14.02</td>
<td>10.59 ± 1.84</td>
<td>7.46 ± 0.47</td>
</tr>
</tbody>
</table>

*milligrams of gallic acid equivalents/100 mL and
**mg/100 mL on a wet basis.

nd=not detected
Fig. 1. Parameters and productivity of the fermentative cycles for acetic fermentation.