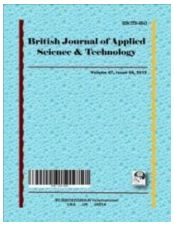

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# Production of Alcoholic Beverage from Ginger: Study of Fermentation Process and Final Product Quality

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***Authors' contributions***

*This work was jointly carried out by all the authors. Author ML designed the study. Authors LMT, ELG and TPRDS jointly managed the analyses of the study and managed the literature searches under the supervision of author ML. Author MMM was responsible for the statistical analysis of data. All authors read and approved the final manuscript.*

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## ABSTRACT

**Introduction:** In Brazil part of the production of ginger is of inadequate quality for export. The production of spirit from felt-over rhizomes is an alternative of great interest to producers of these rhizomes.
**Aim:** Aiming to increase the value of felt-over rhizomes, this work aimed to study the use of ginger as a raw material for alcoholic beverage production. It was evaluated the effect of fermentation conditions on the components of fermented alcoholic, as well as, the quality of alcoholic distilled beverage of ginger.
**Methods:** Dehydrated ginger passed by enzymatic hydrolysis-saccharification processes. The hydrolysate obtained was analyzed for sugar profile in HPLC. The alcoholic fermentation process followed the central composite rotational design for three factors: fermentation temperature (23 to 37ºC), time of fermentation (17 to 33 h) and concentration of inoculum (0.22 to 3.00%). The fermented alcoholic obtained was analyzed in HPLC for the contents of ethanol, methanol, glycerol

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and residual sugars. The distillated alcoholic beverage of ginger was analyzed for ethanol, methanol, acetaldehyde, ethyl acetate and higher alcohols in the gas chromatography (GC). In addition, copper content and acidity were analyzed

**Results:** Sugar profile of the ginger hydrolysate revealed the presence of 77.8% of glucose. Data analysis of fermentation process showed influence of temperature on ethanol and methanol content of the fermented alcoholic of ginger. Time of fermentation had effect on glycerol content. All parameters of process had influence on residual sugars contents. The HPLC analysis has shown presence of methanol, ethyl acetate, aldehyde, acids, higher alcohols and esters in distilled alcoholic beverage of ginger.

**Conclusion:** Fermented alcoholic of ginger with higher levels of ethanol can be obtained under the conditions of 1.5% w/w of inoculum, 30°C of temperature and 24 hours of fermentation time. In this condition of fermentation process the beverage of ginger had good quality.

*Keywords: Zingiber officinalle Roscoe; starch; ethanol; beverage.*

## 1. INTRODUCTION

Ginger (*Zingiber officinale* Roscoe), a plant of the *Zingiberaceae* family is originally from Southeast Asia. Nowadays, various kinds of ginger products are provided to the international market for its piquant and aromatic volatile constituents. The ginger products such as dried slices, powder, candy, flavoring tea, or condiment are very popular. Another product is ginger flavor beverages (GFBs), new kind of functional nonalcoholic beverages, which have a unique flavor with specific health care effect [1-5].

The world production of ginger in 2011 was 2.02 million tons within an area of 314,000 hectares. India is the largest producer, with 702,000 tons, followed by China (388,800 tons), Nepal (216,280 tons), Nigeria (160,000 tons) and Thailand (152,600 tons). Yet Brazil produces about 7,000 tons / year [6], and the States of Espírito Santo, São Paulo, Paraná and Santa Catarina are the main producers [7].

Ginger marketing aspects in Brazil are believed to be limiting factors for this agribusiness expansion. Contracts between farmers and purchasers, Chinese lower prices and a high number of poor quality export products are among the main handicaps.

Contemporarily, the Brazilian and the worldwide consumer market with higher purchasing power have interest in "natural" products. Handmade products have a certain commercial appeal, enabling competitiveness of micro, small and medium producers against the so-called "industrial product", being essential the quality of handmade product.

Considering these aspects, researches aiming to evaluate the use of low quality ginger rhizomes as raw material to develop high value-added products have gained great interest by producers.

Fresh ginger chemical composition varies with geographical locations. The rhizomes have on average 84.37% moisture, 1.17% protein, 0.86% fat, 0.93% fiber, 0.96% ashes, 0.34% sugars and 11.42% starch (% wet basis) [8,9].

Concerning starch content, one possibility of increasing ginger production chain in Brazil could be the use of low quality rhizomes as raw material in fermentation processes to produce distilled beverages [10].

An alcoholic beverage is a complex mixture of components with volatile compounds, which are responsible for aroma and flavor; and fixed compounds that consist of a large variety of substances with different characteristics [11].

Considering these aspects, researches that have as objective to study the use of ginger rhizomes of low quality as raw material to high value-added products have gained great interest of producers.

The chemical composition of fresh ginger is different due to different geographical locations. Ginger rhizomes have about 84.37% of moisture, 1.17% of protein, 0.86% of fat, 0.93% of fiber, 0.96% of ash, 0,34% of sugars and 11.42% of starch (wet basis) [8,9].

Front of the content of starch present in this rhizome a possibility of increasing the production chain of ginger in Brazil would be the use of low quality ginger as raw material in fermentation

processes in order to obtain a distilled beverage [10].

An alcoholic beverage is a complex mixture of components presenting volatile compounds, responsible for aroma and flavor, and fixed compounds, consisting of a large variety of substances with different characteristics [11].

For enabling processing of ginger aiming to obtain alcoholic beverage it is necessary evaluating the technical aspects of the process and the quality of final product. In this line, this study aimed to evaluate the effects of the conditions of the alcoholic fermentation process of ginger's hydrolysate on the contents of ethanol, methanol, glycerol and residual sugars, as well as, evaluate the quality of distilled beverage of ginger obtained under the optimized conditions of the alcoholic fermentation process.

## 2. MATERIALS AND METHODS

Ginger rhizomes were dried, crushed and sieved (0.85-mm mesh) to be used as raw material. The chemical composition was 7.2% moisture, 67.41% starch, 6.44% ash, 10.29% protein, 8.69% fiber, 1.95% fatty matter, 4.73 % soluble sugars (dry basis).

It was prepared a suspension with 10 liters of water and dried ginger with 10% starch (w/ w) at pH 6.0. The process was carried out in an 18-liter stainless steel reactor (Ranazzi Ltda) under temperature control and constant stirring (60 rpm). Hydrolysis has started by adding 0.4 kg α-amylase (Termamyl 2X, Novozymes) per ton of starch in the suspension and 60 ppm of calcium. The reactor was set for gradual heating up to 105°C and kept at this temperature for one hour. After this period, temperature was adjusted to 95°C and 0.8 kg of the α-amylase / ton of starch was added. Stirring and heating remained for one more hour. Then, temperature was reduced to 60ºC and pH was adjusted to 4.5. Next, it was added glucoamylase (AMG 300L, Novozymes) at a concentration of 3.00 liters/ ton of starch. Thus, reactor remained at the same temperature for 24 hours under constant stirring. The hydrolysate material was then filtered in vacuum filter for residual fiber separation.

Sugar profile of ginger's hydrolysate was analyzed by high performance liquid chromatography on a Varian Prostar HPLC system (Varian, Sint-Kateliine-Waver, Belgium), column AMINEX HPX 42A ((Bio-Rad, Eke, Belgium), stationary phase Pb, 300 x 0.25 mm, using water as the mobile phase and sample flow of 0.6 ml.min$^{-1}$ at 80°C, which allows quantifying saccharides with degree of polymerization (DP) from 1 to 9. Then, sucrose, glucose, fructose and maltose concentrations (g L$^{-1}$) were determined from standard curves.

Aiming to evaluate the effect of fermentation process conditions on the chemical composition of alcoholic fermented extract (wine), the ginger's hydrolysate was fermented under different experimental conditions.

Four liters of hydrolysate was divided into twenty parts of 200 mL and poured into 500-mL Erlenmeyer flasks. Hereupon, it was added a suspension of commercial yeast of *Saccharomyces cerevisiae* (strain Y-904, dehydrated provided by Mauri Brazil). The flasks were kept under agitation (100 rpm) and in a refrigerated incubator (Tecnal, TE-422 model, São Paulo, Brazil).

The Response Surface Modeling (RSM) is the most widely used statistical technique for bioprocess optimization, which is effective for responses to several factors and interactions. A central composite rotatable design (CCRD) was used for prediction of responses based on few sets of experimental data, in which all factors varied within a chosen range. It was adopted for fermentation process a three factor and five level experimental design (Table 1).

The RSM describes the behavior of a system in which independent variables ($X_k$) and dependent variables or responses ($Y_i$) are combined. Thus, the response depends on the levels at which the factors were combined and defined.

**Table 1. Levels of variation and variable parameters of the alcoholic fermentation process**

| Independent variables | Levels of variation | | | | |
|---|---|---|---|---|---|
| | -<alpha> | -1 | 0 | +1 | +<alpha> |
| Fermentation time (h) | 17 | 20 | 24 | 30 | 33 |
| Temperature (°C) | 23 | 26 | 30 | 34 | 37 |
| Yeast concentration (%) | 0.22 | 0.75 | 1.50 | 2.25 | 3.00 |

*<alpha>= 1.682*

Within the proposed variation ranges, i.e. within the region characterized by these levels, the behavior of each response can be predicted in a general form according to the equation:

$$Y_1 = \beta_o+\beta_1X_1+\beta_2X_2+\beta_3X_3+\beta_{11}X_1^2+\beta_{22}X_2^2+\beta_{33}X_3^2+ \beta_{12}X_1X_2+\beta_{13}X_1X_3+\beta_{23}X_2X_3+\varepsilon$$

Where in:

$Y_1$ = Dependent variable or response function;
$X_1,X_2,X_3$= Values of the independent variables;
$\beta_o$= Coefficient related to the line interception with response axis (y-coordinate);
$\beta_1,\beta_2,\beta_3$= Linear coefficients estimated by least-square method;
$\beta_{11},\beta_{22},\beta_{33}$= Coefficients of quadratic variables;
$\beta_{12},\beta_{13},\beta_{23}$= Coefficients of the interaction between independent variables;
$\varepsilon$ = Experimental error.

The model was adjusted by the stepwise procedure of SAS software; therefore, the obtained model was validated through F-test using the pure error mean square as denominator.

RSM plots were generated from adjusted models using the Statistica® 6.0 (StatSoft Inc.).

After the fermentation process the samples were centrifuged for separation of the yeast.

Analyses of residual sugars (sucrose, glucose and maltose) and ethanol, methanol and glycerol levels of the ginger's alcoholic fermented were also performed in HPLC (Varian).The column used was AMINEX HPX 87H (stationary phase H+) 300 x 0.25 mm, sulfuric acid (0.001N) as mobile phase, sample flow of 0.6 ml.min$^{-1}$ and 50ºC of temperature. Then, sucrose, glucose, fructose and maltose concentrations (g L$^{-1}$) were determined from standard curves.

After analysis of data of fermentation process it was produced a distilled beverage of ginger. For production of beverage it was prepared a suspension with 10% of starch, and the enzymatic hydrolysis-saccharification process was carried out. The fermentation was performed by adding of 1.5% of yeast (*Saccharomyces cerevisiae*) on 30ºC of temperature for 24 hours. The distillation of alcoholic fermented was carried out in copper pot still as in production of spirit from sugar cane in Brazil. Distillate was separated into three fractions according to the ethanol concentration. The heart fraction was analyzed for the content of ethanol, methanol, isopropanol, ethyl acetate, isoamyl alcohol in the gas chromatograph (GC) (Varian - model 3380), equipped with FID detector and column OHI Valley (60 mt x 0.25 mm SD), model OV 1301, 1.4 micras. The operating conditions were: temperature ramp of 35°C to 100ºC for 5 minutes and 35ºC to 1ºC.minuto$^{-1}$, nitrogen as carrier gas, flow of 40 ml. min$^{-1}$, injected volume of 1 µL and total running time of 135 minutes.

Distillate samples were also derivatized with 1 ml dinitrophenyl-hydrazine in 4 mL distillate, followed by addition of 20-µl phosphoric acid (PA). The solution was filtered through a 0.22-micron membrane, and the content of aldehydes was achieved by HPLC technique using a methanol and water mixture as mobile phase through a 200-mm C18 HP column.

Distillate acidity was measured according AOAC method [12]. The copper content was determined by atomic absorption spectrophotometer (Perkin-Elmer) with air-acetylene flame and lamp hollow cathode to 324 nm.

## 3. RESULTS AND DISCUSSION

The analysis of sugar profile of the ginger hydrolyzed revealed the presence of 77.8% of glucose, 15.3% of maltose, 5.58% of sucrose and 0.33% of dextrin, showing the effective action of amylases.

Results showed that the main sugar of ginger hydrolyzed was glucose followed by maltose and sucrose. Very low content of dextrin was observed indicating efficiency of hydrolysis process. In the process of starch hydrolyzing to obtain sugars, starch granules dispersed in water are heated, gelatinization occurs and by the action of α-amylase, the breakage of 1-4 glucose bonds occurs, resulting in the liquefaction of medium. In a second step, by the action of a debranching enzyme, α-1.6 links of starch are broken, occurs the saccharification, ultimately resulting in a glucose-rich solution.

In a study performing ginger starch hydrolysis (saccharifying activity), it was observed the presence of glucose (79.87 g L$^{-1}$ to 109.06 g L$^{-1}$) and dextrin (0.179 g L$^{-1}$ to 1.432 g L$^{-1}$) at different concentrations of amylolytic enzymes [13]. The authors reported that the higher concentration of amyloglucosidase (AMG 300L, Novozymes) reduced hydrolyzed extract dextrin content.

Several physical (temperature, osmotic pressure), chemical (pH, oxygenation, minerals nutrients and organic inhibitors) and microbiological factors (kind and concentration of yeast strain, bacterial contamination) affect the fermentation efficiency and the efficiency of conversion of sugar to ethanol [14].

Chromatographic analysis of the fermented alcoholic of ginger showed the presence of ethanol, methanol, glycerol and residual sugars in all treatments. It was observed that temperature influenced ethanol and methanol contents. Moreover, fermentation time had effect on glycerol content (Table 2).

Ethanol content analysis of the fermented alcoholic ranged from 10.54 g $L^{-1}$ to 35.61 g $L^{-1}$. Regression analysis showed a linear effect of fermentation temperature on the ethanol content (Table 2). By the results, it can be stated that ethanol content was lower in treatments at higher temperatures (Fig. 1a). These results match findings of other authors that evaluating the effect of temperature, sugar concentration and inoculum percentage on ethanol production and they observed a raise in ethanol production with increasing temperature up to 32°C, and subsequent decrease because of negative effects on cell viability [15,16].

Temperature is one of the most important parameters that affects fermentation by influencing yeast metabolism and producing volatile compounds. Nevertheless, an optimal temperature range is still a divergent aspect. Low temperatures show as initial effect the lag-phase prolongation, as well as other metabolite formation such as glycerol [17]. Temperatures above 35°C benefit bacteria multiplication, yeast viability reduction and acidity increase. Industrial strains of *S. cerevisiae* are typically resistant to high temperatures; however, it may interfere with cell viability when in synergy with the presence of ethanol or a low pH [18].

Results obtained for the analysis of glycerol content in the fermented alcoholic of ginger ranged from 3.56 g.$l^{-1}$ to 6.52 g.$l^{-1}$. It was observed linear effect of fermentation time on the content of this compound in the fermented alcoholic (Table 2). Lower concentrations of glycerol were observed in treatments with lower fermentation time (Fig. 1b). In studies of fermentation using *S. cerevisiae*, in addition to biomass and carbon dioxide are produced various others products, including glycerol and organic acids [19]. Generally, the high production of glycerol in the process results in lower ethanol production and low yield. High time of fermentation can contribute to the proliferation of bacteria that modify the pH of the medium and generate conditions that lead to excretion of glycerol.

Glycerol is one of the most extensively excreted by-products during fermentation with *S. cerevisiae.* Nonetheless, it is known that the lactic acid bacteria can metabolize glycerol to produce acrolein, resulting in negative sensory product characteristics [20].

The results obtained in the analysis of methanol in fermented alcoholic of ginger showed small amounts of this component with a range from 0 to 0.165 g.$l^{-1}$. It was observed by statistical analysis that the time and temperature of fermentation influenced methanol content (Table 2). In high temperature conditions and intermediate conditions of fermentation time the concentration of methanol was higher (Fig. 1c).

Interestingly, all parameters of the process had influence on residual sugars (Table 2). Residual sugar or fibers presence could form undesirable compounds catalyzed by increased temperatures and acidity.

The analysis of the residual sucrose showed values from 3.84 to 4.16%. The results showed that there was an interaction between time and temperature on the sucrose content and also the interaction of inoculum concentration and temperature (Table 2). The lower sucrose concentrations were obtained in conditions of low temperature and fermentation time (Fig. 1d) and under high percentage of inoculum and low temperatures (Fig. 1 e).

For the content of maltose the values ranged from 8.99 g.$l^{-1}$ to 13.92 g.$l^{-1}$. The analysis of the regression coefficients showed the occurrence of the linear and quadratic effects of the percentage of inoculated yeast, and quadratic effects of time and temperature of fermentation (Table 2). The response surface plotted shows that the lowest levels of this residual sugar were obtained in intermediate conditions of time and temperature (Fig. 1f). With fermentation time in the intermediate condition, the lowest levels of maltose occurred under conditions of low percentage of inoculum and intermediate temperature (Fig. 1g).

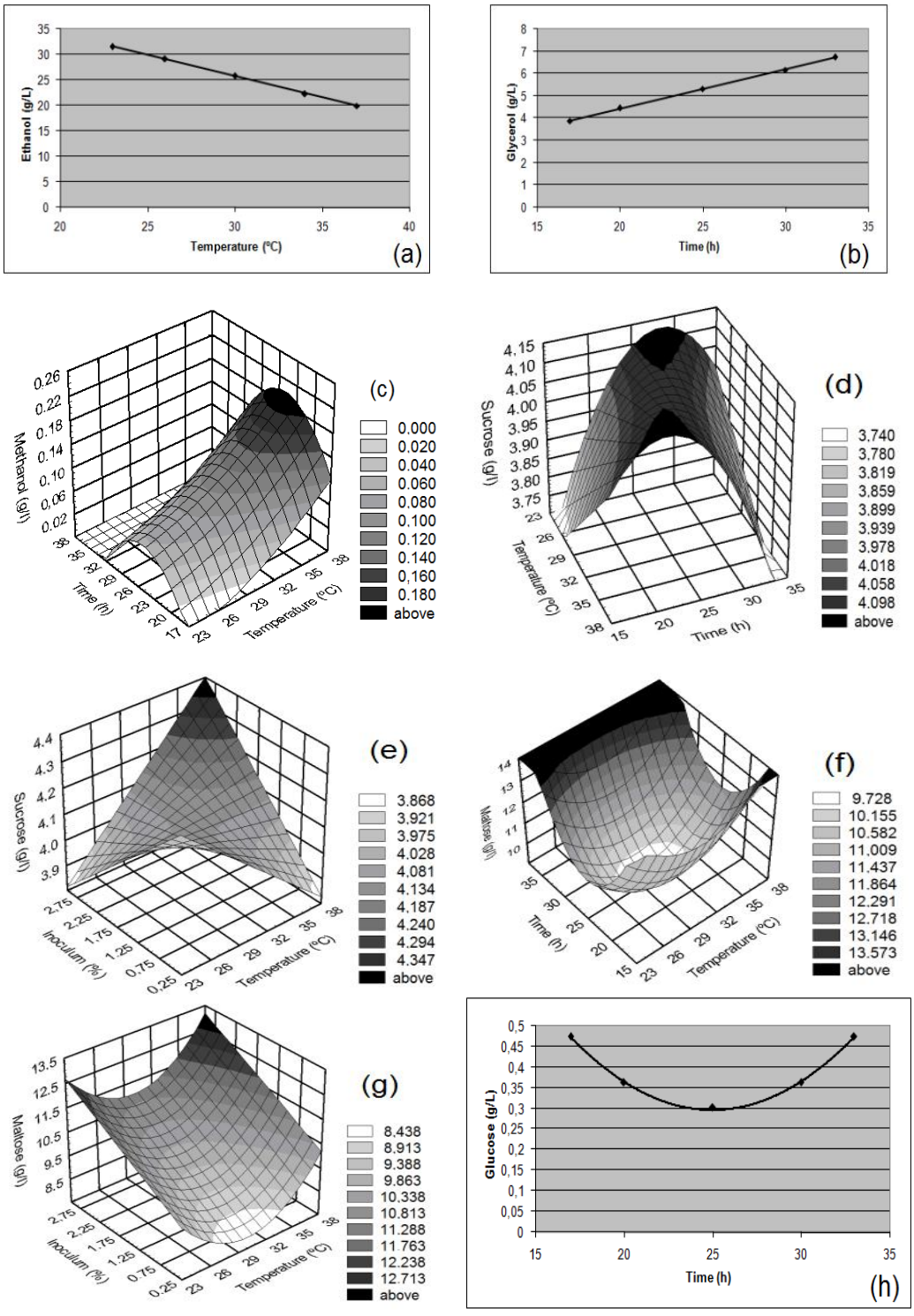


**Fig. 1. Effects of fermentation parameters on levels of ethanol (a), glycerol (b), methanol (c), sucrose (d,e) maltose (f,g), glucose (h) in the fermented alcoholic of ginger**

**Table 2. Regression equation coefficients (*= p<0.05 **=p<0.01 *** =p<0.001)**

| | Ethanol | Glycerol | Methanol | Maltose | Glucose | Sucrose |
|---|---|---|---|---|---|---|
| Intercepto | 29,07 | 5,44 | 0,15 | 9,31 | 0,02 | 4,08 |
| Time (L) | 0,65 | 0,08** | 0,03 | 0,10 | -0,02 | 0,003 |
| Temperature (L) | -3,44** | -0,18 | 0,05** | 0,39 | -0,003 | 0,02 |
| Inoculum (L) | 0,42 | 0,19 | -0,03 | 0,83* | -0,01 | -0,01 |
| Time (Q) | -0,22 | 0,16 | -0,05** | 0,80* | 0,06*** | -0,07** |
| Temperature (Q) | -2,14 | -0,08 | -0,02 | 0,61 | 0,02* | 0,01 |
| Inoculum (Q) | -2,50 | -0,26 | -0,02 | 1,34*** | 0,01 | -0,004 |
| Time x Temperature | -2,76 | -0,12 | 0,05* | 0,70 | 0,001 | -0,06 |
| Time x Inoculum | 1,50 | 0,06 | -0,05* | -0,21 | 0,02 | 0,006 |
| Temperature x Inoculum | -0,12 | -0,37 | -0,05* | -0,43 | -0,05** | 0,08* |
| $R^2$ | 0,71 | 0,70 | 0,81 | 0,75 | 0,77 | 0,69 |

*(L) = linear effect, (Q) =quadratic effect, $R^2$= determination coefficient*

Residual glucose ranged from 0.239 $g.l^{-1}$ to 0.461 $g.l^{-1}$. Regression analysis showed a quadratic effect of fermentation time (Table 2). In extreme conditions of fermentation time the residual glucose was higher (Fig. 1h).

When temperature increases, bacterial contamination is favored and yeast becomes more sensitive to ethanol toxicity [18]. This situation leads to higher levels of residual sugars in fermented and hence ethanol yield decreases.

The relationship of fermentation time and residual sugars may be due to a possible maltodextrin hydrolysis and longer stay time in the reactor, as well as the yeast stress caused by process conditions. During ethanol fermentation, yeast cells suffer from various stresses. Some are environmental such as nutrient deficiency, high temperature and contamination, while others are from the yeast cell metabolism such as ethanol accumulation and its corresponding inhibition on yeast cell growth and ethanol production, especially under very high gravity conditions. Many of than are synergistic affecting yeast cells more severely than any single one, leading to reduced yeast viability and the vigor as well as lower ethanol yield [21].

Distilled alcoholic beverage from ginger showed presence of methanol, ethyl acetate, aldehyde, acids, higher alcohols and esters (Table 3). Analyzing the results, it was observed that ginger's beverage parameters met the limits established by MAPA (Normative Instruction #15), which sets lower limit at 200 $mg.100\ ml^{-1}$ and upper limit at 650 $mg.100\ ml^{-1}$ for congeners coefficient. This standard also establishes maximum limits for volatile acidity (150 $mg.100\ ml^{-1}$ - expressed as acetic acid), esters (200 $mg.100\ ml^{-1}$ - expressed in ethyl acetate), total aldehyde (30 $mg.100\ ml^{-1}$ - expressed as acetaldehyde), furfural + hydroxyl-methyl-furfural (5 $mg.100\ ml^{-1}$) and higher alcohols (360 $mg.100\ ml^{-1}$ - expressed as the sum of n-propyl alcohol, isobutyl and isoamyl) [22].

**Table 3. Characteristics of distilled beverage of ginger**

| Components | Heart fraction |
|---|---|
| Alcoholic content | 40ºGL |
| Acetaldehyde | $7.12\ mg.100\ ml^{-1}$ |
| Ethyl acetate | $40\ mg.100\ ml^{-1}$ |
| Methanol | $5.9\ mg.100\ ml^{-1}$ |
| Isopropyl alcohol | $126\ mg.100\ ml^{-1}$ |
| Isoamyl alcohol | $144\ mg.100\ ml^{-1}$ |
| Volatile acidity | $30\ mg.100\ ml^{-1}$ |
| Higher alcohols | $270\ mg.100\ ml^{-1}$ |
| Congeners | $317.42\ mg.l^{-1}$ |
| Copper | 5.0 ppm |

In addition to that, the analysis exhibited that higher alcohols were the most abundant group of volatile compounds. Among the esters, ethyl acetate had the highest concentration. This ester higher concentration indicates non-achievement of perfect anaerobic conditions. Such chemical has a significant effect on the organoleptic characteristics of distillates [23]. At small amounts, it is responsible for a pleasant, fruity aroma. In contrast, a large quantity of it confers an undesirable flavor [24].

Fermentation process quality can be checked through acidity level. Quantitatively, organic acids are expressed in volatile, fixed and total acidity, being the latter the sum of the previous two values. Volatile organic acids are common in distilled sugarcane beverage (spirit). Organic acids are important indicators of undesirable fermentation process, as their formation is due to alcohol oxidation by lactic or acetic bacteria.

Methanol and copper contents were observed in the distillate. Methanol is an undesirable alcohol in beverages and comes from pectin degradation. Copper comes from building material of pot still.

## 4. CONCLUSION

Among the studied parameters, fermentation time and temperature have the most pronounced effects on ginger alcoholic fermented extract composition. The inoculum concentration affected residual sugars. Under intermediate conditions of time, temperature and yeast concentration (24 hours, 30°C and 1.5% w/w), it is possible to produce a ginger alcoholic fermented with higher ethanol levels and minor of the other components. At such fermentation process conditions, the produced ginger beverage showed a chemical composition that meets the main quality standards required by Brazilian legislation.

## COMPETING INTERESTS

Authors have declared that no competing interests exist.

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