

# PRODUCTION OF GINGER VINEGAR

## Produção de vinagre de gengibre

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### ABSTRACT

Vinegar is a food of condiments group that have great use in the food industry. This study aimed to evaluate the effects of parameters of the acetic fermentation process in the production of ginger vinegar. A suspension of ginger rhizomes with 12% of starch was subjected to enzymatic hydrolysis process to obtain hydrolyzed with 85.6% of glucose. After the alcoholic fermentation the wine was obtained with 40.3% ethanol. The acetic fermentation process of ginger alcoholic solution followed a completely randomized design in a factorial for three factors at two levels. The independent variables were: temperature, nutrients and proportion of "strong vinegar" and alcoholic solution (initial acidity). Results showed variation from 2.74 to 3.70% for dry extract and 2.13 to 2.83 % for ash in vinegars. The profile of organic acids of ginger vinegars showed the presence of acetic, citric, malic and succinic acids in all treatments. The condition of 20°C, initial acidity 1:1, with addition of nutrients allow obtaining good quality vinegars and higher GK yields.

**Index terms:** *Zingiber officinale*; fermentation; ethanol; acetic acid.

### RESUMO

O vinagre, alimento do grupo de condimentos, tem seu uso bastante difundido na indústria de alimentos. Neste trabalho, objetivou-se avaliar os efeitos de parâmetros do processo de fermentação acética na obtenção de vinagre de gengibre. Uma suspensão de rizomas de gengibre, com 12% de amido, foi submetida ao processo de hidrólise enzimática, sendo obtido o hidrolisado com 85,6% de glicose. Após o processo de fermentação alcoólica, obteve-se uma calda com 40,3% de etanol. O processo de fermentação acética da calda de gengibre seguiu o delineamento inteiramente casualizado, em esquema fatorial, para três fatores em dois níveis, tendo como variáveis independentes: a temperatura, os nutrientes e a diluição da acidez inicial da calda. Os resultados obtidos mostraram uma variação de 2,74 a 3,70% de extrato seco e de 2,13 a 2,83% de cinzas nos fermentados acéticos. O perfil de ácidos orgânicos dos vinagres de gengibre evidenciou a presença de ácido acético, ácido cítrico, ácido málico e ácido succínico em todos os tratamentos. Vinagres de boa qualidade e maiores rendimentos GK podem ser obtidos nas condições de 20°C, acidez inicial 1:1 e adição de nutrientes.

**Termos para indexação:** *Zingiber officinale*; fermentação; etanol; ácido acético.

### INTRODUCTION

Vinegar is known since antiquity and its name comes from the French *vinaigre*, or sour wine. It was originally obtained from spontaneous fermentation of wine, other fermented beverages and grape fruit left in the air.

Vinegar has been used in food preparation, not only as preservative for vegetables, fish and meat but likewise as an ingredient in mustard, ketchup, mayonnaise, sauces and dressings. Vinegar also has medicinal uses by virtue of its physiological effects, such as promoting recovery from exhaustion, regulating blood glucose, blood pressure, aiding digestion, stimulating the appetite, and promoting calcium absorption (Kishi et al., 1999; Fushimi et al., 2001; Kondo et al., 2001; Xu et al., 2007; Schlepütz; Gerhards; Büchs, 2013).

Vinegar is formed by the stoichiometric conversion of ethanol with oxygen to acetic acid and water by acetic acid bacteria. Vinegar is produced by two well-defined methods: a slow surface process, in which acetic acid bacteria (AAB) are placed on the air-liquid interface in direct contact with atmospheric oxygen, and a fast submerged process, in which AAB are submerged in the acetifying liquid and a continuous strong aeration is applied to provide the necessary oxygen for acetic fermentation to take place. Generally, the surface process is employed for elaborating traditional vinegars and the submerged process is employed for the elaboration of most commercial vinegars of major consumption (Fernández-Pérez et al., 2010; Schlepütz; Gerhards; Büchs, 2013; Schlepütz; Büchs, 2014).

In Brazil are consumed 170 million liters of vinegar and about 80% corresponds to the alcohol vinegar. The

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Southeast region accounts for 53% of consumption of the product followed by the South (23%), North-Northeast (19%) and Midwest (5%). The largest producer of vinegar, concentrating 37% of total production, is the São Paulo city region, while the interior of the state is the main market, where consumption reaches 24% of national production (Suman; Leonel, 2013).

Each type of vinegar has its own flavor because during the transformation of ethanol into acetic acid valuable aromatic substances of raw materials are preserved and also other organic acids can be formed. The production of good vinegar depends on several factors related to the microorganism, to the raw material, the conditions for development of alcoholic and acetic fermentation and also to the product, maturation, conservation, clarification, bottling and pasteurization (Tefaye et al., 2002).

Ginger (*Zingiber officinale* Rosc.) is one of the most important and extensively used spices worldwide. Ginger as an important component of traditional Asian herbal medicine, is used for management of such symptoms as the common cold, digestive disorders, rheumatism, neuralgia, colic and motion-sickness, as well as being an important spice to flavor foods and beverages (Yeh et al., 2014).

The proximate composition of ginger rhizomes is 84.16% of moisture, 97.19% carbohydrates, 0.16 of ash, 0.55 of crude fat, 1.05 of crude fiber, 1.05 of protein (g/100g dry weight). The content of organic acids (mg/g dry weight) is: 0.04 of citric acid, 0.02 of malic acid, 14.13 of oxalic acid, 0.06 of succinic acid and 23.08 of tartaric acid. Gingerols and shogaols in ginger rhizomes are responsible for the pungent taste (Yeh et al., 2014).

According to the consumers' preferences, a wide variety of ginger products deriving from different countries are known. Besides salted, pickled and juicy products, ginger is currently processed into ginger powder, oleoresin and oil, respectively (Schweiggert et al., 2008).

In Brazil, the production of ginger rhizomes aiming to export *in natura* is recent; however, this market requires quality standards that lead to considerable losses in production (about 20-30%) (Torres; Leonel, 2012; Suman; Leonel, 2013).

During the period 1996-2006 the State of São Paulo exported an average of 4,000t/year of ginger with average prices around US\$ 0.80/kg. In the same period, the State of Paraná exported on average 1,200 t year<sup>-1</sup>, for an approximate price of US\$ 0.81/kg and Santa Catarina, participated in Brazilian exports of ginger with 300 t year<sup>-1</sup>, a compatible market price around US\$ 0.70/kg (Paraná, 2012).

Considering the chemical composition of the ginger and the importance of seeking alternatives to the use of

low quality rhizomes as raw material for special products, this study aimed to produce vinegar from ginger evaluating the effect of processing conditions on the chemical characteristics of final product.

## MATERIAL AND METHODS

To obtain the ginger hydrolyzed were initially performed the processes of hydrolysis and saccharification of the suspension of dried ginger and water with 12% of starch concentration in suspension. The enzymes Termamyl 2X (1.2 kg ton<sup>-1</sup> of starch, pH 6.0, at temperatures of 105°C for one hour and 95°C for another hour, on constant stirring) and amyloglucosidase AMG 300L (3.0 L ton<sup>-1</sup> of starch, pH 4.5, 60°C, 24 hours, on constant stirring) were used. The sugar profile of the ginger hydrolyzed was 85.6% of glucose, 0.8% of maltose, 0.6% of maltotriose, 0.4% of maltotetraose and 12.48% of dextrans. In alcoholic fermentation of the ginger hydrolyzed it was used 1.5% of the yeast *Saccharomyces cerevisiae* (strain Y-904, dehydrated provided by Mauri Brazil). The temperature was 28°C and the time of fermentation was 48 hours. The process resulted in a solution with 40.3% of ethanol, 0.9% of methanol, 0.2% of glycerol and 0.5% of glucose. These processes were carried out in stainless steel reactor (18 liters) (Ranazzi; Cia Ltda), which has adjusting temperature, agitation and oxygenation.

The process of acetic fermentation followed a completely randomized factorial design with three factors at two levels (2<sup>3</sup>), totaling eight treatments with four replications. The slow process of acetic fermentation was conducted in a glass bottle with a capacity of 1 liter with holes for air intake and outlet of vinegar. The variable process parameters were: fermentation temperature (20°C or 27°C), with or without addition of nutrients and proportion of "strong" vinegar and alcoholic solution (initial acidity 1:1 or 2:1) (Table 1). For acetic fermentation takes place in good conditions there needs to be certain acidity in alcoholic solution being common to add "strong" vinegar. The vinegar used was the undiluted and unpasteurized ginger vinegar from a previous fermentation containing high concentrations of acetic bacteria.

Ammonium sulfate (0.1 g L<sup>-1</sup>), ammonium phosphate (0.5 g L<sup>-1</sup>), potassium phosphate (0.1 g L<sup>-1</sup>), magnesium sulphate (0.1 g L<sup>-1</sup>), glucose (1 g L<sup>-1</sup>), potassium citrate (0.1 g L<sup>-1</sup>) and calcium pantothenate (0.001 g L<sup>-1</sup>) were added in alcoholic solutions in the treatments with addition of nutrients.

During the acetic fermentation process samples were collected for analysis of acidity and alcohol content. In the fourth week the acetic fermented were removed and these were centrifuged and pasteurized at 65°C for 15 minutes.

**Table 1:** Experimental treatments of the acetic fermentation process of ginger.

Treatments	Independent variables					
	Codified			Real		
	X1	X2	X3	T	N	A
T1	-1	-1	-1	20	With	1:1
T2	-1	-1	1	20	With	2:1
T3	-1	1	-1	20	Without	1:1
T4	-1	1	1	20	Without	2:1
T5	1	-1	-1	27	With	1:1
T6	1	-1	1	27	With	2:1
T7	1	1	-1	27	Without	1:1
T8	1	1	1	27	Without	2:1

X1=T=temperature (°C); X2=N= nutrients; X3=A= dilution of initial acidity.

The acetic fermented from ginger were analyzed for ash content, dry extract, total acidity in acetic acid, alcohol content (Instituto Adolfo Lutz, 1985) and organic acids profile.

The profile of organic acids was determined by analysis of liquid chromatography. The sample was centrifuged at 12,000 rpm for 8 minutes and then filtered through a PVDF membrane with a porosity of 0.22 microns. For this analysis it was used Varian Pro Star HPLC with autosampler, refractive index detector, BIORAD column (Aminex HPX87H), mobile phase H2SP4 (0.005M), flow of 0.6 mL min<sup>-1</sup> and temperature of 65°C.

Based on the results obtained in acetic fermentation the efficiency was calculated by GK (Gesammte Konzentration), extensively used in the vinegar industry, in which efficiency is expressed in terms of the sum of the concentrations of ethanol (% v/v) and acetic acid (% w/v) at the beginning and end of the fermentation.

The results of dry extract, ash and organic acids of final vinegars of ginger were subjected to analysis of variance and then the comparison between means of treatment by Tukey test. The variations of total acidity, alcohol content and GK throughout the time of fermentation for each treatment were performed by analysis of variance model with “treatment”, “time” and interaction ‘treatment x time’. After checking the interactions between regressions and treatments were determined regression equations and the graphs were constructed. The level of significance was 5%.

## RESULTS AND DISCUSSION

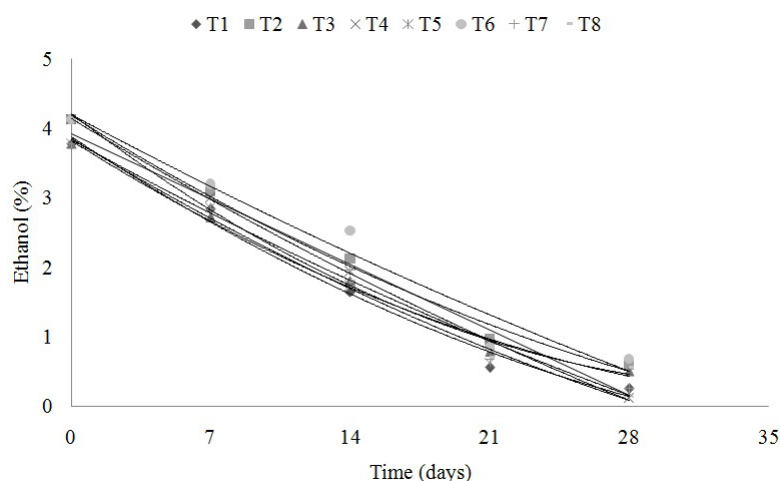
The alcohol content of ginger vinegars ranged over the time for all treatments with the level in final vinegars lower than 1.0%. Brazilian legislation does not determine

a minimum value for alcohol content in vinegars, only determines the maximum value of 1.0% by volume at 20°C (Brasil, 2012). It is desirable that there are small amounts of residual alcohol in the vinegar produced because this will affect the flavor of vinegars favorably by forming the bouquet especially if the product is stored for a few months.

Regression analysis for varying ethanol content throughout the time showed that the polynomial model of second degree fitted well to the data of all treatments (Figure 1).

Different types of vinegar have the same main components, water and acid. Vinegar contains many nutrients, which contains more acids, such as acetic acid, lactic acid, pyruvic acid, formic acid, malic acid, citric acid, oxaloacetate, and succinate. These amount nutrients are connecting with the total acidity content (TAC). That is, TAC is highly connecting with the quality of vinegar (Ji-Yong et al., 2013).

Results of total acidity of vinegars ranged for all treatments over the time of acetic fermentation (2.27 to 4.82%). Brazilian legislation and the States Food and Drug Administration (FDA) require that any product called “vinegar” contain at least 4% acidity. So, regardless of the experimental condition were obtained vinegars with total acidity in acetic acid within the minimum established. However in disagree with the limits established by the Codex standard that proposed a minimum of 6% for wine vinegar and 5% for others because the percent of acetic acid present in the product varies according to what they are made from (Moros et al., 2008; Brasil, 2012; Ji-Yong et al., 2013).



$$T1: Y=0.002x^2-0.1895x+3.8691$$

$$R^2=0.9877$$

$$T5: Y=0.0014x^2-0.1737x+3.8493$$

$$R^2=0.9914$$

$$T2: Y=0.0017x^2-0.1784x+4.1735$$

$$R^2=0.9913$$

$$T6: Y=0.0008x^2-0.153x+4.1902$$

$$R^2=0.9600$$

$$T3: Y=0.0021x^2-0.1801x+3.8142$$

$$R^2=0.9929$$

$$T7: Y=0.0009x^2-0.1580x+3.8279$$

$$R^2=0.9871$$

$$T4: Y=0.001x^2-0.174x+4.1387$$

$$R^2=0.9993$$

$$T8: Y=0.0034x^2-0.2200x+4.2100$$

$$R^2=0.9884$$

**Figure 1:** Ranges of ethanol concentration over time of acetic fermentation.

The acidity of commercial vinegar is quite variable. Schmoeller and Balbi (2010) evaluating the quality of commercial vinegars observed values of total acidity ranging from 4.42 to 7.53 g 100 mL<sup>-1</sup> of acetic acid.

The analysis of variance for total acidity throughout the fermentation times showed significant effects of “treatments”, “time” and “treatments x times”. The variation in total acidity differed according to the treatment.

The polynomial model of second degree fitted well to the data of all treatments, except for the treatment 6 (Figure 2). There was an increase in total acidity for treatments in the first 21 days of acetic fermentation. After this period the total acidity were decreasing.

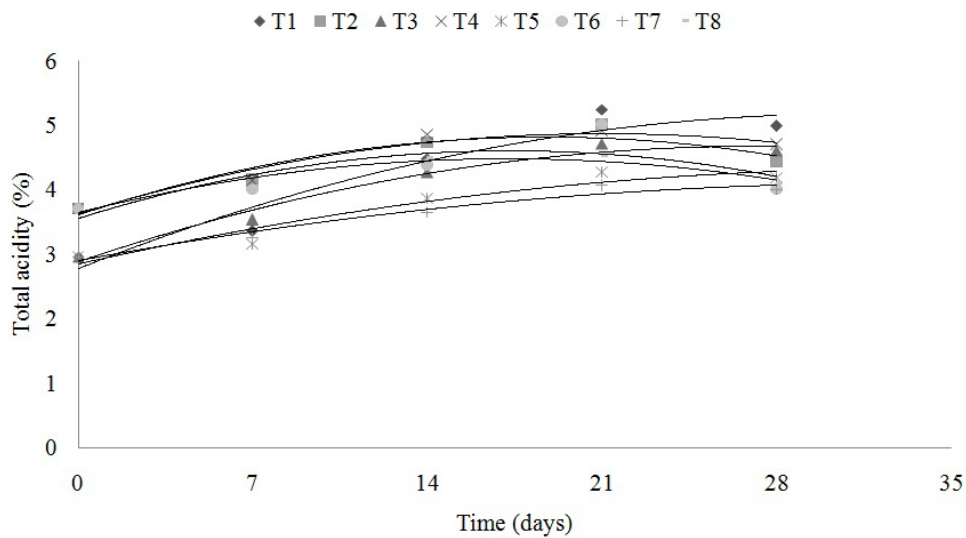
Acetic acid bacteria exhibit three growth phases; first growth proceeds by completely oxidizing ethanol to acetic acid (ethanol oxidation-phase), then growth stops after consuming the ethanol as the respiratory substrate and the viable cell number gradually decreases in this stationary phase (acetic acid resistance-phase). However, when the viable cell number decreases to some threshold cell growth starts again by utilizing acetic acid accumulated (acetate overoxidation-phase) (Matsutani et al., 2013).

Data analysis of GK throughout the time showed higher values in the first 15 days of fermentation for all treatments, after this, the GK decreased. Better GK value was observed in treatment T1 (Figure 3).

The decrease of GK value over the time may be due to evaporation of volatile acids, with more pronounced effects with longer fermentation time. In the absence of losses through evaporation, over oxidation and conversion to biomass, the GK value should remain constant throughout the time.

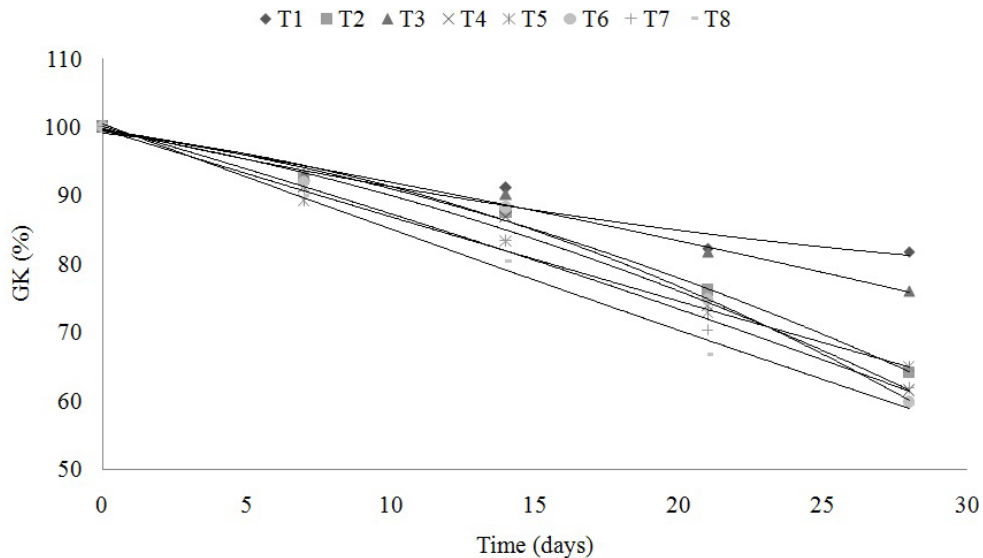
In acetic acid fermentation the conversion from ethanol to acetic acid results in a relatively high enthalpy change which causes heat generation. The treatments with 27°C of temperature showed lower GK when compared with treatments at 20°C probably due to interference of the temperature on microbial growth.

It is well-known that ginger essential oil and oleoresin contain considerable amounts of phenolic compounds (eugenol, shogaols, zingerone, gingerdiols, gingerols, etc.), which are responsible for antimicrobial effect (Bellik, 2014). However, the yields obtained in this study for both the alcoholic fermentation and for acetic fermentation may indicate that the microorganisms involved in these processes are little affected by these components.



T1: $Y = -0.0025x^2 + 0.154x + 2.7686$	$R^2 = 0.9237$	T5: $Y = -0.0013x^2 + 0.0871x + 2.8403$	$R^2 = 0.9257$
T2: $Y = -0.0034x^2 + 0.1286x + 3.6043$	$R^2 = 0.9050$	T6: $Y = -0.0034x^2 + 0.1192x + 3.5589$	$R^2 = 0.6531$
T3: $Y = -0.0024x^2 + 0.1318x + 2.8769$	$R^2 = 0.9775$	T7: $Y = -0.0013x^2 + 0.0761x + 2.8926$	$R^2 = 0.9446$
T4: $Y = -0.0017x^2 + 0.1183x + 3.6263$	$R^2 = 0.9483$	T8: $Y = -0.0028x^2 + 0.0977x + 3.6394$	$R^2 = 0.9308$

**Figure 2:** Ranges of total acidity over time of acetic fermentation.



T1: $Y = 0.0102x^2 - 0.9466x + 99.66$	$R^2 = 0.9399$	T5: $Y = 0.0018x^2 - 1.2895x + 99.55$	$R^2 = 0.9944$
T2: $Y = -0.0223x^2 - 0.6362x + 99.46$	$R^2 = 0.9949$	T6: $Y = -0.0343x^2 - 0.4322x + 99.09$	$R^2 = 0.9895$
T3: $Y = -0.0045x^2 - 0.7193x + 99.57$	$R^2 = 0.9858$	T7: $Y = -0.0059x^2 - 1.2178x + 100$	$R^2 = 0.9945$
T4: $Y = -0.0227x^2 - 0.7114x + 99.30$	$R^2 = 0.9907$	T8: $Y = 0.0028x^2 - 1.5635x + 100$	$R^2 = 0.9939$

**Figure 3:** Ranges of GK values over time of acetic fermentation.

The final acetic fermented of the different treatments showed changes from 2.74 to 3.70% for dry extract and 2.13 to 2.83% for ash (Table 2). These results are higher than those obtained by Bortolini, Sant'anna and Torres (2001) in their study about production of vinegar kiwi where the authors observed levels of dry extract ranging from 1.15 to 1.29% and ash ranging from 0.11 to 0.17%. The ash contents obtained in final ginger vinegars are in accordance with the limits established by Brazilian legislation (Brasil, 2012).

Marques et al. (2010) analyzing vinegars from sugar cane, mixture of sugar cane and corn, kiwi, orange, mixture of orange and honey, apple, rice, mango, passion fruit, corn, mandarin, mixture of tangerine and corn, red wine vinegar and white wine vinegar observed levels of dry extract ranging from 5.3 to 48.8 g L<sup>-1</sup> and ash contents ranging from 0.72 to 5.14 g L<sup>-1</sup>.

The differences in levels of dry extracts and ash of vinegars may be due to the raw materials used and the methods of filtration during manufacture.

Data analysis revealed significant effects of variables parameters of process on dry extract and ash, as well as, their interaction ( $p < 0.001$ ). The condition of 27°C of temperature with added nutrients and 1:1 of dilution of the initial acidity allows obtaining the highest levels of dry extract and ash in ginger vinegar (T5). Keeping the temperature at 20°C it was not observed influence of the addition of nutrients and the initial acidity on these parameters.

The results obtained for the organic acids in final ginger vinegars (28 days) showed the presence of acetic, citric, malic and succinic acids in all treatments (Table 3). The levels observed for these acids are close to those reported in literature.

Sáiz-Abajo, González-Sáiz and Pizarro (2005) evaluating sixty-three vinegar samples of different origins collected from the industry and several supermarkets from the north of Spain observed that acetic acid concentration ranged from 48.63 to 69.30 g L<sup>-1</sup>, and the main acids observed were lactic acid (0.14 to 4.66 g L<sup>-1</sup>), L-malic acid (0.10 to 2.38 g L<sup>-1</sup>), D-malic acid (0.10 to 2.69 g L<sup>-1</sup>), citric acid (0.33 to 1.53 g L<sup>-1</sup>), L- proline (0.46 to 24.87 g L<sup>-1</sup>) and tartaric acid (0.16 to 1.58 g L<sup>-1</sup>).

Caligiani et al. (2007) analyzing 105 samples of vinegar (44 traditional balsamic vinegars (TBV) of different ages, 31 balsamic vinegars (BV) and 30 common vinegars, in particular wine vinegar (WV), apple vinegar (AV), rice vinegar (RV), malt vinegar (MV) and tomato vinegar (TV)) observed that acetic acid ranged from 10.6 g L<sup>-1</sup> (TBV 5 years) to 74.3 g L<sup>-1</sup>(RV), citric acid ranged from 0.02 g L<sup>-1</sup> (AV) to 18.6 g L<sup>-1</sup> (TV), succinic acid ranged from 0.27 g L<sup>-1</sup> (AV) to 1.04 g L<sup>-1</sup> (TBV 11 years) and malic acid ranged from 0.66 g L<sup>-1</sup> (WV) to 14.7 g L<sup>-1</sup>(TBV 12 years).

Data analysis showed that the condition of 20°C of fermentation temperature with nutrient and acidity (1:1) allowed obtaining vinegar with a higher content of acetic acid differing significantly from the other conditions tested. The fermentation temperature increase led to the production of citric, malic and succinic acid in higher concentrations (Table 3).

**Table 2:** Results of dry extract and ash content of ginger vinegars.

Treatments	Independent variables			Dependent variables	
	T	N	A	% Dry extract	% Ash
T1	20	With	1:1	2.83±0.02cd	2.19±0.03cd
T2	20	With	2:1	2.86±0.06cd	2.23±0.05bcd
T3	20	Without	1:1	2.80±0.04cd	2.14±0.04d
T4	20	Without	2:1	2.79±0.03cd	2.13±0.05d
T5	27	With	1:1	3.70±0.06a	2.83±0.04a
T6	27	With	2:1	3.14±0.09b	2.35±0.06b
T7	27	Without	1:1	2.90±0.05c	2.29±0.11bc
T8	27	Without	2:1	2.74±0.11d	2.29±0.05bc

T (Temperature) (°C); N (Nutrients); A (Dilution of Initial Acidity). Means followed by different letters in a column are different (Tukey test  $p < 0.05$ ).

**Table 3:** Organic acids (g L<sup>-1</sup>) of ginger vinegars.

Treatments	Independent variables			Dependent variables			
	T	N	A	Acetic acid	Citric acid	Malic acid	Succinic acid
T1	20	With	1:1	65.12a	0.21d	0.34b	0.41cd
T2	20	With	2:1	50.19b	0.33c	0.37b	0.40cd
T3	20	Without	1:1	57.78b	0.30cd	0.36b	0.40cd
T4	20	Without	2:1	41.55c	0.27cd	0.45b	0.46bc
T5	27	With	1:1	42.85c	0.65a	0.85a	0.57a
T6	27	With	2:1	32.85de	0.50b	0.90a	0.46bc
T7	27	Without	1:1	35.55d	0.41b	0.37b	0.51ab
T8	27	Without	2:1	30.58e	0.50b	0.51b	0.49b

T (Temperature) (°C); N (Nutrients); A (Dilution of initial acidity). Means followed by different letters in a column are different (Tukey test  $p < 0.05$ ).

### CONCLUSIONS

The results obtained show the influence of temperature, nutrient addition and proportion of undiluted and unpasteurized vinegar on main quality parameters of vinegar and also on GK values.

The condition of 20°C with nutrients and initial acidity 1:1 allow obtaining good quality ginger vinegars with higher yield.

Ginger rhizomes with low quality, inadequate for commerce “in natura” and to export, can be used as raw material for the production of special vinegar.

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